Robin Holliday

Aging: The Paradox of Life

Why We Age

Springer

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Robin Holliday is a Fellow of the Royal Society of London, and the Australian and Indian Academies of Science. He has worked in several different biological fields. The first was the repair and genetic recombination of genes, chromosomes and DNA molecules. He devised an important DNA intermediate in genetic recombination, now known as the "Holliday structure" (or Holliday junction). He later initiated extensive research on the mechanisms of cellular ageing of normal human cells, and how they differ from immortal cancer cells.

This interest in ageing broadened to other biological systems and in 1995 he published the book *Understanding Ageing*. He was also a pioneer in the field of epigenetics, which is the study of the mechanisms for the unfolding of the genetic programme for development. Apart from the basic DNA code, there is another way heritable information is superimposed on DNA, and this has very important effects on the control of gene expression.

He obtained his Ph.D. at the University of Cambridge, England, and carried out his research at the John Innes Institute, Hertford, England, the National Institute for Medical Research, Mill Hill, London (where he was Head of the Genetics Division), and finally at CSIRO laboratories in Sydney, Australia. He has travelled extensively to international scientific conferences in the USA, Japan, Canada. Australia, India, and many European countries. He has himself organised such conferences in France, Italy and the UK. He has published over 250 scientific papers and several books.

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Genes, Proteins and Cellular Ageing
Understanding Ageing
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Origins and Outcomes

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Preface

At the end of the 20th century a remarkable scientific discovery emerged. It was not a single discovery in the usual sense, because it was based on a series of important interconnected insights over quite a long period of time. These insights made it possible for the very first time to understand the biological reasons for ageing in animals and man. This book explains what these reasons are in non-technical language.

For centuries people have been puzzled by the inevitability of human ageing. It has often been referred to as a mystery, or an unsolved biological problem. Indeed, the famous zoologist Peter Medawar, who was to become a Nobel prizewinner in 1960, delivered in 1951 an important lecture - his inaugural lecture after being appointed Professor of Zoology at University College, London. The title was "**An unsolved problem in biology**," The unsolved problem was ageing, and when it was published the following year it had a strong strong influence on the scientific study of ageing. The German zoologist, August Weismann, in the late nineteenth century had suggested that ageing was essential so that generation could follow generation, and this allowed Darwinian evolution to occur. Medawar showed that Weismann's reasoning was false, because he explained – for the first time – that animals in their natural environments die mainly from predation, disease and starvation, and rarely reach natural old age. Such ageing is seen only in protected environments, for example, in animals which are domesticated, or kept in zoos, where they are well fed and looked after. Darwin also realised that many more offspring are produced that can survive to adulthood, and amongst these offspring there is competition in a hostile environment, which results in survival of the fittest.

There had been many scientific studies of ageing before Medawar, and many more were to follow. Much of this work could be called descriptive, for example many comparisons were made between young and old animals. Innumerable differences were documented, but these were often hard to interpret. Many experimental systems have been and still are being used. These include nematode roundworms, fruit flies, mice and rats, and even yeast and fungi. There were many theories of ageing that seemed to be competing with each other. A particular proponent of a theory often claimed it could explain everything. One of the most puzzling features of the ageing of mammals was the fact

that similar changes occurred in old animals, but at very different rates in separate species. For example, mice and rats become old or senescent after about three years of adult life. Domestic dogs and cats do so around the middle of their second decade. Humans show body changes comparable to old rodents, cats and dogs after 70-80 years, although we all know that a minority reach a lifespan of 100 years.

Until quite recently this was not understood. For most of the second half of the twentieth century ageing remained something of a biological mystery. Thousands of scientific papers were published, many long reviews and several weighty books. Yet they came to no firm conclusions about the biological reasons for ageing, or why animals age at different rates. It was no accident that three books with positive affirmative titles were published in the 1990s. The first was by Leonard Hayflick "**How and Why We Age**" (1994); then my own "**Understanding Ageing**" (1995), and finally Steven Austad's "**Why We Age**" (1997). In addition, there was a book written in more popular style: Tom Kirkwood's "**Time of Our Lives**" (1999). These books are by no means the same, but they come to a similar conclusion, namely, that ***ageing is no longer an unsolved problem in biology***.

In science, some discoveries immediately make their mark. A very good example of that was the momentous discovery of the structure of DNA by Jim Watson and Francis Crick, which earned them the Nobel Prize in 1962. Other discoveries are ignored, or even ridiculed. Gregor Mendel's breeding experiments with peas resulted in the elucidation of the laws of inheritance, which were published in 1865. This was ignored and then "re-discovered" at the beginning of the 20th century. The German scientist Alfred Wegener proposed the theory of continental drift in 1915, and based it on very sound arguments. Most geophysicists, geologists and geographers dismissed it out of hand. Yet today it is accepted.

It can already be said that the many observations and insights that explain ageing will not be accepted as established knowledge for a long time. The field is still full of scientists, and non-scientists, who are just happy to go on speculating about the "mystery" of ageing. As I have just said, many propose new theories of ageing, as if *their* theory will by itself explain everything. In fact, of the various theories of ageing that have been proposed over the years, several undoubtedly have a degree of truth. What we need now is refinement of existing ideas, not entirely different ones. Also, there are many clinicians and

scientists who talk about “anti-ageing medicine”. Many seem to regard ageing as a disease that can be cured like any other. I will discuss the myth of excessive prolongation of life in Chapter 9. The myths are largely due to complete ignorance of the reasons for ageing. The aim of this book is to dispel ignorance.

Acknowledgements

My interest in the biology of ageing began in the 1960s. Many thanks are due to all those colleagues who in one way or another, by experiment or discussion, contributed to my present knowledge and interpretations of the field. They are too many to list here, but their names will be found in my earlier book *Understanding Ageing*. More recently, I have appreciated many discussions with Leonard Hayflick and our agreement that ageing is no longer an unsolved problem of biology. I also thank Jenny Young who typed many early draft chapters, and my daughter Mira Holliday for her help with the figures and final compilation of the manuscript.

Author's Note

This book is largely written in non-technical language, with very few scientific references. These references are to be found in my earlier book *Understanding Ageing*, which was written for scientific readers. Some use of scientific and medical terminology is unavoidable, and I have provided at the end a glossary explaining or defining particular words. The words ageing and senescence are used more or less interchangeably. At various times 'the process of ageing' is referred to, and at other times 'the processes of ageing'. Both are legitimate, but the latter is more scientifically correct. Gerontology is the scientific study of all aspects of ageing, including social, demographic, and so on. Biogerontology is the study of the biology of ageing. Geriatricians are physicians with expertise in the care of old people.

Chapter 1. Longevity

Today, almost every society contains old individuals, and children soon learn, through language and family, that they have elderly relatives, who one day will die. This was not always the case, because when humans first found themselves in natural environments, lifespans were very much shorter. The major causes of death were disease, attack by predators and shortage of food or water. A human population can sustain itself if infant mortality is about 25 per cent (as it is today in the great apes in their natural environments) and annual mortality is about 7 per cent thereafter. Under these circumstances the expectation of life at birth is less than 20 years. Females who reached reproductive maturity would expect to live to about 28 years and bear on average about 6 children. These figures are similar for one of the most primitive tribes in existence today, namely, the Yanomami Indians that inhabit a forest region of South America. In such societies, aged people are not very common, and death from old age is certainly the exception rather than the rule.

In Western societies with good health care the expectation of life at birth is now about four times higher than in primitive ones: more than 80 years for females, and just a few years shorter for males. There is not much infant mortality, and most subsequent mortality in the following decades is due to accidents, suicide, homicide, or occasional intrinsic disease, such as cancer. For a population, the shape of the survival curve is like that shown in Figure 1A. It is clear that the force of mortality starts to increase only quite late in life. With constant annual mortality, the survival curve is 'exponential' as shown in Figure 1B. Through history a major factor that increased longevity was the development of agriculture, which ensured a much more reliable supply of food. Even so, civilisations as advanced as the ancient Romans and Greeks had survival curves that were not very different from exponential, as shown in Figure 2. There was high infant mortality and expectation of life at birth was only about 25 years, and at one year was about 34 years. Infectious disease was the main cause of death, and it was not until most of this was eliminated in the twentieth century that the expectation of life rose very substantially. This was due to several major advances: the first was the discovery that disease was caused by infectious agents, usually bacteria or viruses. Then hygeine was greatly improved so that the chances of infection

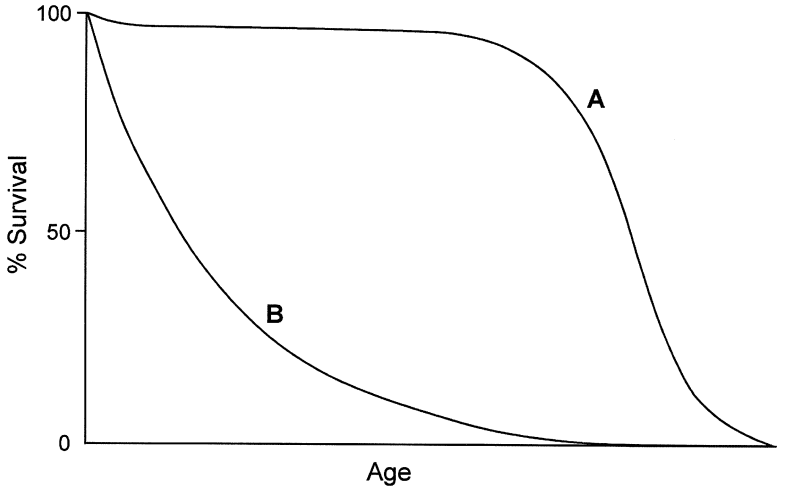

Figure 1. **A**, the survival of individuals in developed countries with good health care. In the early decades, death is mainly due to accidents (including homicide). Later the force of mortality increases, and few reach the age of 100 years or more. **B**, an exponential survival curve, which occurs when mortality is constant with time. Thus, after a given interval of time, 50% of individuals are alive, then after the same interval, 25% are alive, and after the same interval 12.5%, and so on

were greatly reduced. Finally, immunisation and antibiotics eliminated much of infection which still occurred. In addition to that, the quality of health care gradually improved through the twentieth century in developed countries, and even in many undeveloped ones. It is not uncommon for individuals a third world country to have an expectation of life at birth of 60 years.

Many inanimate objects have exponential survival survival curves. A simple example is a collection of wine glasses in a restaurant, as there is a constant probability that they will be broken at any time. Their survival is just the same as Figure 1B. One can extend the example to plastic glasses that do not break when they are dropped on the floor. Instead they accumulate scratches, and when enough of these occur, they are likely to be discarded. Their survival time is therefore more like Figure 1A.

There are in fact two measures of longevity. The one I have so far discussed is statistical and applies to populations of individuals, as shown in Figure 1. The other measure is the maximum lifespan. Amongst any population there will always be one that lives the longest. In Western societies with accurate records of births, the documenting

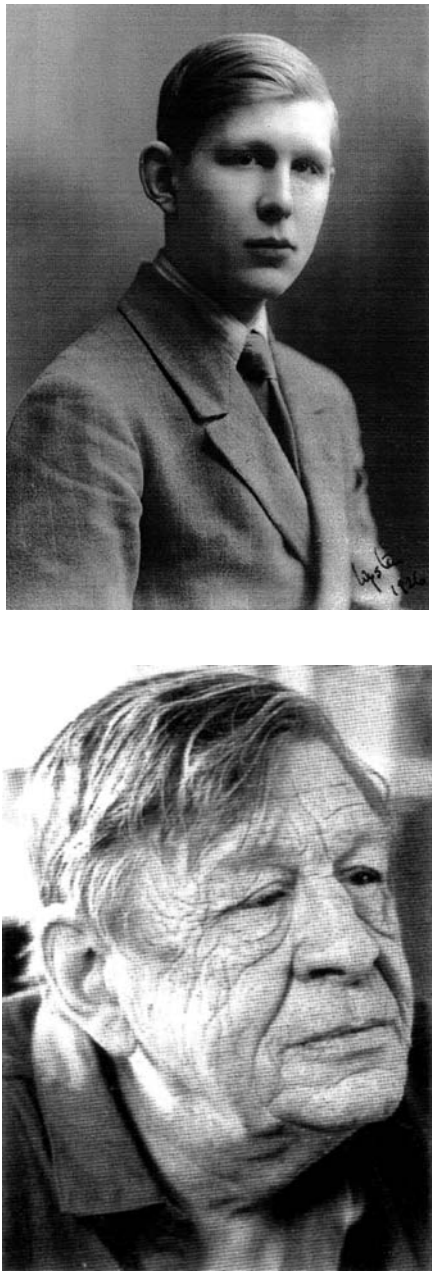

Figure 2. The poet W.H. Auden as a young man (top), and two years before his death in 1973 (below)

of longevities achieved is completely reliable. The oldest known individual was Jeanne Calment, who lived in France and died at the age of 122 years. Many reports of much longer lifespans will be discussed later in Chapter 9 and none have been authenticated. It is a fairly obvious generalisation that the larger the population that is properly documented, the greater is the maximum lifespan. This is an important point because many have made strong statements about the maximum lifespan of other animals based on rather small numbers of individuals in zoos. For example, it is often said that the maximum lifespan of gorillas or chimpanzees is very much lower than human lifespan, at around 50 or 60 years. Yet this is based on perhaps half a dozen animals kept in zoos, whereas human records include millions of individuals. If a few humans were kept in captivity, how long would their longevity be? Perhaps 70–80 years, which is 40 years less than Madame Calment. Although modern medical practice has greatly increased expectation of life at birth, it has not had much effect on maximum life span. Certainly there are far more centenarians than there used to be, but there is not much evidence that maximum lifespan is changing. There is no one alive today who is as old as was Madame Calment. One day there will be, but it will be one individual amongst many millions who will die younger.

Identical twins have the same genes as each other, but they do not have identical lifespans. They are more similar than that of non-identical twins or siblings of the same sex, so genes obviously have a role in determining lifespan. However, the differences between identical twins indicate that chance events are also important. Such events might be environmental or they may be intrinsic. The shape of the survival curve in Figure 1A is what would be expected if multiple events result in the increase in mortality at later ages. Thus, events can be occurring throughout life, but only when a sufficient number of them occur are the effects of ageing seen. (The lifespan of plastic glasses in a restaurant were mentioned earlier; they suffer multiple scratches and are then discarded). This very general interpretation is confirmed by experiments with animals which are genetically identical. When male and female mice are inbred for many generations, they end up with the same set of genes. These are known as inbred lines, and they can be exploited in all experiments in which the influence of different genes must be eliminated. When the longevity of a population of inbred mice is determined, it is found to be similar to Figure 1A, but with a maximum lifespan of about three years. As in the case of identical

twins, there must be chance or random events which are important in determining longevity. Old mice and old people have quite similar changes in their cells, tissues and organs, but humans have a lifespan which is about thirty times longer than mice. This used to be a major puzzle in the field of ageing research, but now scientists know why this difference exists, and why other animals have their own characteristic longevities.

Everyone understands the effects of ageing, and we have a rough idea how old people are from their appearance. It may therefore come as a surprise that scientists have some difficulty in actually determining the age of single individuals. There is no individual adult feature that can be examined which is an accurate indicator of age. There are many features which taken together give an overall estimate, perhaps within a few years. This is again what might be expected if multiple events occur, and at least some of them are subject to chance. Nevertheless the effects of ageing are extremely obvious. Figure 2 shows photographs the poet W.H. Auden as a young man, and two years before died. They show that time has enormous effects on our facial features.

Another feature of human longevity is very important. Why are babies born young? The answer is that the germ cells, the sperm and eggs, do not age in the way that cells in other parts of the body do. There is plenty of evidence that human parental age has very little, if any, effect on the longevity of their progeny. There is a significant increase in some kinds of mutations in sperm as males become older, and older women have an increased risk of chromosomal abnormality in their progeny, such as Down's syndrome, which is caused by an additional small chromosome Nevertheless, there is absolutely no evidence that the children of older parents are physically or mentally inferior to those from young parents, nor that they have a shorter lifespan.

It can be said that the germ cells enjoy immortality, but very few of them are actually transmitted from one generation to the next. It is therefore better to say that they are potentially immortal; if they were not, populations of animals would not survive, but instead become extinct. The differences between germ cells and body cells with finite survival time will be discussed in several different contexts in later Chapters.

It used to be thought that *Homo sapiens* was the longest lived mammalian species, but this might not be true. Recently it was discovered that an adult bowhead whale had a harpoon embedded in its body which was of a type not used for 200 years. So this whale

may have been at least that age. This is remarkable, and needs to be corroborated, but 200 years is still only a minute fraction of evolutionary time. The same can be said of giant tortoises, which live longer than humans. There are good biological reasons why a few species, including some plants, may have long life spans, and it should not be thought that it demands special explanation.

Although so much happens and so much can be achieved in one human lifetime, it can also be said that our lifespan is brief in terms of human history. Civilisations arose about 8000 years ago, and since then there have been about 350 generations of human life. Hominids walking upright appeared on our planet perhaps half a million years ago, a period comprising 20,000 generations or so. Anyone who lives to 60 years has experienced about one hundredth of one percent of human history. Why does this situation exist? Having developed to an adult, lived adult lives and produced children, why do we then disappear from the ongoing population? Why do different mammalian species have such different longevities? Why do most animals that breed quickly have short lifespans, and those that breed slowly have long lifepans? Why do human beings live as long as they do? Why is the average lifespan longer in women than men? Scientists now know the answers to these questions, and they will be found in this book.

Chapter 2. Body Architecture

The focus of this book is the ageing of human beings, but much of what is presented applies to other mammalian species with different lifespans. The ageing of birds is in many ways similar to mammals, but as the evolutionary relationships decrease, then more differences from humans are seen. In this Chapter, I review the evolved structure and physiological design of the human body, with particular reference to the many non-renewable structures.

Animal cells, unlike bacteria and plants, have no rigid wall, but only an outer membrane. This facilitates chemical communication between them and allows the whole organism to function as a unit. The cell enclosed by the membrane is a highly complicated functional unit. It has a nucleus within which are the chromosomes. Human cells have 46 chromosomes (except sperm and eggs which have 23). The chromosomes contain the genetical material, or DNA. This is an enormously long thin molecule and the genetic information is encoded in a linear array of just four functional units, abbreviated to A, T, G and C. The DNA in every cell has a total length of about 2 metres (adding together the 46 chromosomes) and the number of units is about 6 billion (6×10^9). This DNA is tightly packaged in the chromosomes, because the nucleus itself is only a few microns in diameter (one micron is one thousandth of a millimetre). The complete sequencing of the four units revealed that there are about 60,000 genes in the DNA of each cell (30,000 from each parent), and these code for the structure of proteins. Proteins are made up of a defined sequence of amino acids, of which there are just 20 kinds. The linear sequence of amino acids folds up in a particular way to form a specific functional unit, so that each protein has its own defined structure, and also a specific function. Most proteins are outside the nucleus, and held inside the cell by the membrane. Proteins are the very stuff of life. Many are catalysts or enzymes, which carry out all the metabolic reactions essential for living organisms. The membrane itself contains many proteins, tightly associated with lipids. (Lipids are the components of fat). The membrane determines what comes into and what goes out of cells, and it can transmit information to, or receive information from other cells. The total number of cells in a human being is enormous. In the brain alone there are about a billion cells (10^{12}), and in the body as a whole around 30 times as many. All these cells result from the division

of a single cell, the fertilized egg. As development proceeds, through embryo, foetus and child to adult, the cells follow various pathways which leads to their specific differentiation into the many cell types found in different tissues and organs. Many will never divide again through adult life, others keep dividing continuously, such as those which renew skin, or the lining of the gut. In many tissues, and muscle is a good example, the cells cease division, but can nevertheless be replaced by a pool of quiescent cells which can be stimulated to divide and differentiate into new muscle cells. Thus, the muscles essential for locomotion, and other movements, are capable of some regeneration and repair. But in the heart, very little if any repair is possible, because there is no similar pool of quiescent cells. Heart muscle cells also have a different structure from those in the other muscles. The heart is a pump and its ongoing function is entirely dependent on the activity of all, or a large proportion, of its cells, very few of which can be replaced.

This raises a crucial question. How long can an individual cell be expected to survive? There are cells and structures in the living world which can survive a very long time, such as bacterial spores or many plant seeds. These are in a highly dehydrated state, and they essentially have no respiration or metabolic activity. Cells in an active animal are very different as they contain a high proportion of water and they also depend on energy from respiration. Under these conditions, innumerable chemical reactions are occurring. Many of these comprise the wide range of activities characteristic of any living cell, but some provide background ‘noise’ which can interfere with those normal activities. In particular, respiration generates oxygen free radicals (often abbreviated to ROS - reactive oxygen species). These are short-lived but highly reactive. During their very short lifetime they can damage DNA, proteins, or lipids in the cell membrane. As we shall see later, the cell has defenses against these free radicals, but these are not perfect. It is known, for example, that the genetic material DNA is continually bombarded by free radicals and suffers damage to its individual components. Most of this damage can be repaired, but not all of it. The DNA is a double helix, consisting of two long strands coiled around each other. A break in one strand can be repaired, but breaks in both strands close together causes the molecule to fall apart, to fully break, and this damage is much harder to repair.

The adult brain consists largely of non-dividing cells called neurons which are entirely dependent on a good oxygen supply, and are very

active in chemical metabolism. These cells are therefore subject to damage from free radicals throughout their life. The DNA is never replaced, only repaired, and the repair is not perfect. This means that sooner or later a cell will suffer damage which may end its useful life. That is not to say that neurons commonly die from damage to DNA, because there can also be many abnormal changes in proteins. Although many such molecules are broken down and replaced, others resist such turnover and slowly accumulate. These abnormal molecules can then form insoluble aggregates, which strongly interferes with normal neuronal function, and may eventually kill the cell. In addition, proteins may be partially degraded to components called peptides, some of which are known to be harmful. Thus, for several reasons, neurons are not immortal. They have finite survival time, so as we get older and older the number of neurons gradually decline. In Alzheimer's disease and other dementias, the rate of cell death is accelerated, and much of this appears to be due to the accumulation of insoluble proteins or fragments of proteins. There are cells in the brain which are capable of cell division, but the cells responsible for brain function, the neurons, cannot divide. The inability of brain tissue to repair or regenerate itself is described in a well known text book of pathology as follows:

Localisation of function makes the brain inherently vulnerable to focal lesions that in other organs might go unnoticed or produce only trivial symptoms . . . . . This vulnerability of the brain to small lesions is compounded by its very limited capacity to reconstitute damaged tissue. There has clearly been a serious error by the celestial committee in its design of an organ that is vital for biological survival and yet is both the most vulnerable to, and least tolerant of, focal damage.

The biological fact is clear: an active living cell incapable of division can never be immortal, and if it cannot be replaced by other cells, then complex structures such as the brain will eventually age. The brain must last a lifetime, and therefore some of the longest living non-dividing cells we know are those in the brain and nerves of the longest-lived animals such as giant tortoises, or the whale mentioned in Chapter 1.

The heart is similar to the brain in that it consists of non-dividing cells, entirely dependent on a supply of oxygen, and very active in chemical metabolism. In this case, a large proportion of the available energy is devoted to rhythmic muscle contractions. For the animal to survive, muscle cells have to function without interruption throughout life. The heart is a highly efficient pump, but since it cannot effectively