Ageing: A Biomedical Perspective

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CHAPTER 1 The subject of gerontology

The gerontobiologist usually finds himself in possession of one of those enormous and heterogeneous card indexes characteristic of a subject in which no clear lead has yet appeared. In spite of its great interest, we need to recognize that the field of research into ageing presents quite exceptional practical difficulties, and these are responsible for the rather desultory character of much of the work undertaken in the past. (Comfort 1961)

TERMINOLOGY

It is difficult to conceive of any population, living or non-living, complex or simple, that does not alter with time. Change and life are synonymous, so that the definition of every biological system, be it cell, individual or population, should be qualified by its age, as well as the time of day it was observed. Ageing is one of the shorter time scales of life. Changes on the longer time scale of heredity require several generations; changes through evolution occupy countless lifespans. Although some processes of physiological function are repeated many times within a lifespan and others only once or twice, it is unlikely that repeat processes are ever identical. Activation of physiological systems takes place on a functional trajectory. Efficiency rises in early development and then falls. This rise and fall in vitality is evident when efficiency is measured as 'time of response', 'magnitude of response', and 'duration of response'.

Many terms have been used to describe different aspects of the life cycle, such as 'development', 'differentiation', 'maturation', 'aged' and 'senescence'. The common meaning of both 'ageing' and 'senescent' is 'to be old'. Ageing is to become old or senescent. Because of the lack of scientific clarity in the conventional use of these words various attempts have been made to restrict the term senescent to the scientific study of ageing, for example, by coining the word 'biosenescence'. However, most researchers persist with the use of 'aged' and 'ageing'. Senescence tends to have a restricted botanical usage, describing the terminal stages of maturation in fruit.

Definitions are not only important guide-lines for the articulation of research and the gathering of relevant facts, but are also important in the transmission of ideas. It is necessary, therefore, to restrict the definition of ageing in order to

have a useful category of changes for formulating problems. However, there is a disadvantage in the use of any term that has the notional effect of cutting the lifecycle into arbitrary segments. Some of the divisions may have no relevance when a broader temporal perspective is taken, and the terminology used may actually impede research and communication. In this respect, the restriction of ageing to define the terminal stage of life has been an impediment to delineating and understanding its subject matter. It has certainly limited discussion of the relationship of ageing to early development, and restricted the examination of the very young for signs of where, and how, ageing begins.

For example, mammals show a progressive deterioration in function with time, and four landmarks in the life history are: the time of fertilisation; the time of sexual maturation; the time when growth ceases; and the time of death. This raises the question, 'Is the timing of both growth and fertility related to the duration of life?'. An important subsidiary question is, 'Is ageing controlled through the interplay of factors known to be important in the regulation of development?'.

The commonly used definition of 'development' includes the processes of differentiation, growth and maturation which aid survival until the individual is reproductively competent. Ageing processes lead to a failure to adapt to environment and ultimately result in death. Points of difficulty arise in separating development from ageing, because many developmental events are the obvious precursors of ageing phenomena. Also, some retrogressive changes begin before, or shortly after birth and continue unabated, without affecting mortality, throughout life. This is the situation with respect to several well-defined degenerative changes at the histological level (Table 1.1).

Many workers would restrict the term 'ageing' to any changes which render the individual more likely to die as it grows older. This raises problems because some systems deteriorate early, but do not show up as an increase in mortality. Examples are the regression of the human female reproductive system, loss of scalp hair in men and the failure of human eyesight. This difficulty of definition has been recognized and the suggestion has been made that the term 'senescence' should be used to include only those events that contribute to the decreased resistance to death. However, it is really impossible to say that any particular degenerative change would not increase mortality. The force of mortality depends very much on the environment. It is no obvious disadvantage for people not to be able to run a 10 minute mile at the age of 50. Nevertheless, the well-documented decline in human athletic performance from the second or third decade of life could be included as an early ageing process in a less sophisticated society, where predators had the ability to run faster than human prey!

There is also the possibility that delayed secondary responses to earlier primary degeneration may increase the chances of death after a considerable lag. For example, there is a marked decrease in bone density in human females that is partly related to the decline in the female reproductive system. The mean

Table 1.1. Percentage distribution in grade groups* of human coronary artery sclerosis on autopsy

Age (yrs)	Cases	Percentage in grade groups Group		
0–9	410	89.5	10.2	0
10–19	194	56.7	43.3	0
20–29	325	27.4	69.9	0.9
30–39	618	17.8	79.4	0.8
40–49	927	12.8	66.0	2.7
50–59	1157	6.2	60.4	7.8
60–60	1009	0.3	49.4	14.9
70–70	358	0	34.1	21.8
80–89	58	0	20.7	41.4
90–99	4	0	0	75.0

*Severity of condition graded increasing on a scale 1-4

age for menopause is about 50 years, whereas the mean age at which half the bone loss has taken place occurs almost 20 years later. Comparable data for men show that the loss of bone is much less and starts at least a decade later. This faster and earlier loss of bone in women is associated with a dramatic rise in fractures of the long bones, which has no counterpart in men. In the later years, complications, such as pneumonia, arising from fractures, are an important cause of death in women.

Generally, differences between early and late events of development are that early events lead to perfection of function and later events result in deterioration. From this aspect gerontology may be defined as the scientific study of the irreversible deterioration in those structures and functions which have a definite peak or plateau of development in all members of the species. The expressions of ageing seem to be due to an imbalance between two kinds of process, those of early development which may be said to lead to 'perfection', and those that first become obvious after maturation, that lead to 'deterioration'. A central question of gerontology is currently concerned with determining whether there are really two kinds of process operating from the beginning of development; ageing is seen as the gradual dominance of uncorrected deterioration.

Although environment is important in revealing potential failures in biological organization, ageing is fundamentally a loss of precision in the systems specifying form and function. The causes are chemical deterioration, physiological errors and variability of gene expression. All are manifest as a loss of adaptability to environment due to a decline in tissues and functional reserves. The homeostatic systems of the body become less efficient in combating fluctuations in the external world. This appears at all levels of

analysis, from the reduced ability of the neuromuscular system to make a sustained and rapid response, to the failure of the body's physiological regulators to cope with an influx of nutrients (Figure 1.1).

DIVISIONS OF KNOWLEDGE ABOUT AGEING

Gerontology is the area of biology that seeks to explain human ageing, and has the practical goal of improving human well-being in old age. All countries now have increasing socioeconomic problems which stem from the need to support a rising population of elderly people. From this standpoint, the major biological aims are to discover how it comes about that different organisms have characteristic lifespans and to examine the possible connection between development and ageing. The important aims of applied research are to provide recommendations for diet, pharmacology, environmental regulation, and socioeconomic structure that will improve and maintain the quality of human life in old age. It is in this very broad sense that gerontology is a unified body of biological knowledge. It has clear guide-lines and subject divisions concerned with both principle and application, which have been hardly explored.

We know that ageing is both intrinsic and extrinsic to the organism. It has an origin in the evolutionary inheritance of genes which is expressed through the

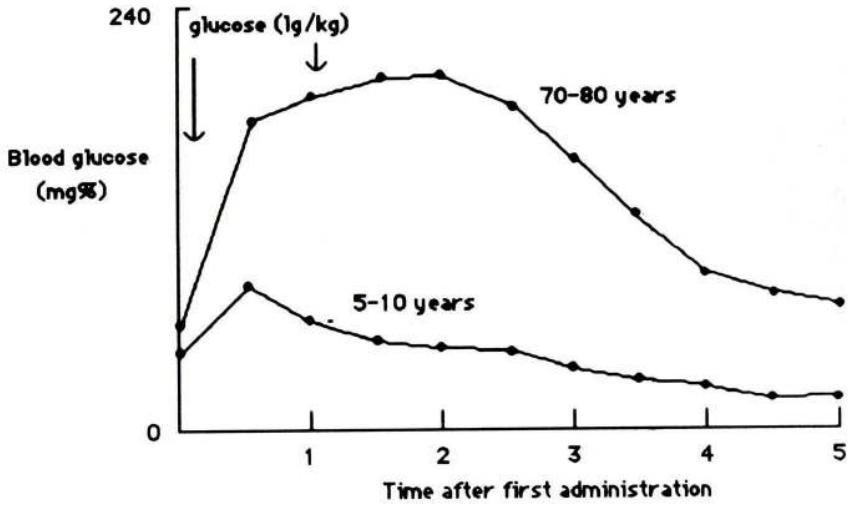


Figure 1.1. Changes in blood sugar after the administration of glucose to young and old human subjects

interaction of the genotype with particular environments. We also know that ageing is manifest at all levels of biological organization, in individual cells and integrated functions. But in no case has the balance between intrinsic and extrinsic factors been defined. As yet we cannot define any special mechanisms which control the rate of ageing. Whilst there is some evidence for 'pacemakers' that cause cells to degenerate and die, and which appear to operate through some kind of humoral mediator, we do not know whether there is a central 'head' clock, a group of organ clocks, or an intrinsic clock for each cell. We are very far from defining roles for cytoplasm or nucleus in such hypothetical clock-like mechanisms. Whilst it has long been known that ageing is expressed within cells on a very individual basis, we do not know whether these changes are intrinsic to the cells, or are the effects of a failure in integrative function between cell populations.

Natural selection operates to transmit genes from parent to offspring. Genes are the repository of advantageous characters which aided parental survival and they control the average lifespan for a species in the wild. They are also intracellular molecular processes whose products interact with environmental forces to control development. Thus, an explanation of ageing is bound up with research into the mechanisms of both transmission genetics and molecular genetics. With regard to transmission genetics questions remain unanswered with respect to the connection between parental selective advantage and the heritability of longevity. In particular, since ageing is a universal phenomenon present in all forms of life, what is its role in evolution? At a molecular level the way in which DNA is protected from molecular ageing is largely unknown.

Gerontology, in its search for answers, covers a wide span of knowledge, which bridges major divisions of biology such as ecology and molecular biology. As a consequence of this interdisciplinary perspective, the subject matter is often arranged or classified in terms of the levels of complexity at which ageing is expressed and can be studied. This is the 'molecules to societies' type of classification, which is commonly used to classify biological information in general. It allows the material to be treated in discrete blocks at a specialist level, and sticks close to the narrow compartmentation of the specialist that produced it. A shortcoming of this kind of arrangement is that it tends to fragment information relating to a broad subject like ageing that only makes sense when placed with interrelated views of different specialists.

Another, less common approach is to arrange information according to the broad questions which motivate people to study a wide subject, such as ageing. In its simplest form this method applied to ageing divides people into three groups.

1. Darwinian biologists, who are interested in the evolution of ageing and ask how it comes about that species have different lifespans.
2. Biochemists and physiologists, who ask questions about the physicochemical basis of cellular deterioration.

3. A group consisting of a range of scientists, clinicians, social and behavioural gerontologists and medical biologists, who are motivated by questions concerning the alleviation of the undesirable aspects of old age that lead to a loss of independence.

A more useful variant of this kind of motivating division creates subject matter categories defined according to the way people see the problem and try to solve it with a range of techniques and professional skills. This method of classification of information on ageing provides four research perspectives (Table 1.2). Each is self-sufficient, although each alone will only provide a partial answer to the overriding question 'What is ageing?'. The advantage of this method is that each section is multidisciplinary and provides a basis for unifying the specialist detail obtained from the 'levels' approach of individual researchers. A variant of this mode of division, by perspective, is the basis of the sections of this book.

The broad unifying philosophical perspective of gerontology is that ageing is the inevitable outcome of life being organised as an interlocking system of unstable chemicals, and sequential biochemical reactions, that tend to drift towards disorder. Therefore, life is a biochemical strategy to overcome chemical deterioration. This viewpoint should place the study of chemical deterioration as the central perspective of biochemistry and molecular biology. However, gerontology has made little impact on biochemical thought. This is due partly to the separate historical development of each subject area, but the neglect really stems from the fact that ageing is seated in the working of genes, which are only now beginning to reveal their secrets. Genetically controlled clock-like mechanisms, synchronized with lifespan, are involved in the manifestation of many biomedical phenomena, such as the failure of reproduction and the initiation of cancerous growth. This genetic perspective makes gerontology of general academic relevance to many fields of biology, as well as having important sociomedical implications. In this respect, gerontology relies totally on developments in biology which still tends to promote the study of 'youth' and 'maturity'.

Many would say that ageing will never be explained until we have gained an understanding of youthful processes, particularly their chemical durability. Ageing is not universal. Many protozoans, unicellular algae, lower invertebrates, the vegetative parts of higher plants and malignant cell lines derived from the tissues of higher vertebrates, appear to be capable of unlimited cultivation as clones without a loss of vigour. Germ cells of all organisms are also protected from ageing. Therefore, ageing appears to be the inevitable outcome of a bodily organisation where reproductive cells require the support of a relatively large mass of other cells not directly connected with reproduction. The ways in which cells are protected from ageing or have structures, both cellular and molecular, that are rejuvenated is one of the great mysteries of biochemistry.

Table 1.2. Scope for research on ageing. General definition: gerontology is the scientific study of deterioration in those structures and functions that have a definite peak or plateau of development in all members of the species.

Subjects of study	Processes of interest
Molecules	Chemical deterioration
Enzymes	Enzyme synthesis/denaturation
Chromatin/histones	Differential expression of genes
Organs	Physiological regulation
Organisms	Reproduction
	Behavioural adaptability
	Environmental influences on ageing
Populations	Natural selection of lifespans and life cycles

An understanding of protective mechanisms will inevitably reveal the molecular basis of ageing. Because of all of these complementary relationships, biochemistry and gerontology will merge in the future.

From all of these points of view, experimental gerontology is much more than the study of terminal processes. Increasingly, life is viewed as a continuum where past physicochemical inputs to the whole organism via the impact of previous environments influence future events at the cellular level. Gerontology provides a much needed forum where extreme specialist views may be moderated and, as such, the subject will play an important socioscientific role in the future development of biology and medicine.

To gain a better perspective of ageing, we must pay more attention to the way in which the conditions of early life, particularly nutrition, influence terminal processes. In the biological field, there is also a need to carry out more investigations into ageing in a wider range of organisms—plant, animal and microbe. Also, more work is needed on ageing in natural populations.

TOWARDS A GERONTOLOGICAL KNOWLEDGE SYSTEM

Finding Logical Places for Information

Every generation makes new demands on knowledge. New problems highlight the inadequacies of old academic divisions of information which are often irrelevant to the needs of contemporary society. A recurring theme of information handling is therefore to provide specialists with a common meeting ground, and also offer a broad educational framework for specialization. The tasks are to find logical ways of arranging information that transcend the viewpoint of the specialists who produced it.

Each individual's knowledge is a personal world of information set within,

and against, the worlds of others. There is a need to provide filing systems with logical structures so that personal or local information can be correctly sited within the architecture of the knowledge system to which it currently makes a major contribution. This requires the provision of containment cells for the information which also augment and extend the knowledge system, provide for its reappraisal, and offer bridges and links to other worlds of information.

It was Francis Bacon writing at the beginning of the 17th century who first highlighted this problem. He attributed the creation of specialists to the 'architecture of fortune' which made some minds good for handling one subject rather than another. Taking a metaphor that everyone is surrounded by 'worlds of knowledge' his aim was to create a navigation system for specialists to access known information of their 'homeland' which also listed each subject's deficiencies in 'the lands yet unvisited'. This Baconian system provided logical pegs on which to hang the subject-based knowledge of his time. His approach was interactive in that it signposted areas that would be fruitful for gathering new data and information. His writings also set out the elementary principles for constructing personal knowledge navigation systems.

Conceptualised Filing Systems

In making a complete inventory of all subjects known to him, Bacon was following a universal intuitive approach to learning. We all learn by organizing our experiences, categorizing them so that a whole fund of varied information can be subsumed under one concept that is named. In this way we can make sense of our bewildering and multifarious environment, classifying our experiences and slotting them into our growing conceptual filing system. It is the organization of pieces of information into concepts and the connection of one concept with another that turns data into knowledge.

It is important to look further into the question of the nature of concepts and their value as the 'structural steel of thinking'. In particular, we need to know how we can encourage effective conceptual learning. Concepts may be studied in their own right but usually a lasting personal body of knowledge is produced when a learner relates different concepts to each other which had previously been considered in isolation.

Drawing pictures and diagrams, mental or real, is an act of making a logical connection between one conceptual element and another. Data may be fixed in place firmly to illustrate a concept, but any particular array of concepts is an arbitrary arrangement defining the point of view of the compiler in selecting and connecting them. Constructing a conceptual array and moving within and between arrays constructed by someone else, are distinct learning experiences in their own right.

At a practical level we need research in two interdependent areas:

1. The standardization of methods to conceptualize clusters of information. The aim is to define an area of knowledge, and allow it to be extended and customized with data so that the knowledge system provides the logic for filing it.
2. The standardization of methods for holding multimedia data. The aim is to have the flexibility to blend the different media, and transfer the clusters of information between different kinds of conceptual arrays.

Both objectives may be addressed by assembling a conceptualized version of local knowledge which is then used as the filing system for information. A conceptualized information cluster is particularly relevant and useful for aiding individualized information handling for learning, management or instruction. As a natural development of the local knowledge paradigm, information clusters based on concepts provide a powerful environment for extending and transmitting the locally generated knowledge system. Such conceptual landscapes make sense of the various topographical elements encountered by a person in their day-to-day activities. They may be representations of the actual biophysical view through an office window, or a mental view of a set of social interactions seen through the mind's eye.

The joint handling of local information and the concepts it supports is vital to the development of the ability to launch propositional operations. The creation of conceptualized local information clusters provides the 'locally structured whole' necessary for dealing with problems and possibilities not encountered before.

Making Conceptual Learning Frames

Areas of knowledge where more than one special subject provide vital information are pictured as grouped concepts to ease the passage of learners across subject boundaries. Bacon grouped his concepts as lists of headings and subheadings, but a more powerful, holistic, open-ended procedure is to arrange them as a two-dimensional cellular map. Each cell represents a named concept and is placed adjacent to others most closely related to it. If the conceptual cell is defined graphically, it may be used as the logical containment structure to hold the data and information. An example of a map of the concepts that have a bearing on human population dynamics is presented in Figure 1.2 as an array of interlocking hexagonal cells. The viewpoint of the compiler is emphasized by arrows showing that each concept may be considered independently. A different compiler might have also connected the concepts of ageing and disease to indicate that in modern society most people die from age-dependent degenerative disease.

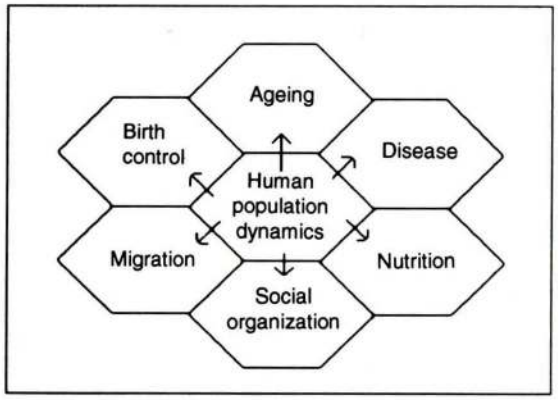


Figure 1.2. Conceptual map of human population dynamics based on a cluster of interlocking hexagons

The advantage of using hexes is that they impose a rigid standardized two-dimensional mapping space on the learner. They also offer six ways to and from a concept, which is about the maximum number of connections that an average person can easily memorize.

These two-dimensional, interactive clusters are called 'learning frames'. A learning frame is analogous to a climbing frame; moving within a climbing frame stimulates neuromuscular coordination; moving within a learning frame stimulates conceptual coordination. Each hex is a window into deeper concepts, other worlds, and illustrative information.

In the form of an overview, a conceptual learning frame allows users to concentrate on the understanding of key concepts based on the notion that each contributing discipline has certain key concepts or descriptors. These delineate the core of the subject to which all data and information are related. A learner can use these core concepts as windows through which to view the underlying worlds of information and decide which one to enter.

Gerontology is a conceptual subject and in order to provide the reader with a logical information structure six conceptual pillars which emerge from the consideration of ageing as a biomedical phenomenon have been used as the framework to encompass the total subject matter of gerontology. Each pillar defines a major perspective and since they are all interconnected it is important to devise a simple method of representing these interconnections through a network of subsidiary perspectives.

This network analogy has been developed as a method of viewing the whole subject of gerontology and its component parts, using a system of interlocking hexagons, with each hexagon standing for a distinct body of knowledge. For the purposes of learning, understanding, and classifying information about

ageing, pathways through this knowledge net may be highlighted by directional markers.

The knowledge net is outlined in Figure 1.3. The pathways are represented as arrows starting from the four numbered hexes which contain the descriptors of the conceptual pillars. They delineate the interfaces of gerontology with biology (natural selection), medicine (geratology), molecular biology (activation of genes), and chemistry (molecular deterioration). The arrangement of information in this book is based on the knowledge net, and each of the subsequent chapters deals with one of the conceptual pillars. Broadly speaking, scientists divide into two groups depending on whether they gravitate towards the left or right of this conceptual framework.

The largely separate concerns of research with 'lifespans' and with 'medicine' set points of entry to the study of ageing taken by the biologist and the biomedical scientist. Both approaches lead to the study of homeostasis at organ and organism levels.

The other two major points of entry are through a consideration of molecular deterioration of biological nucleic acids and protein polymers. These not only provide the blueprints and catalysts of life, but also the structural framework of

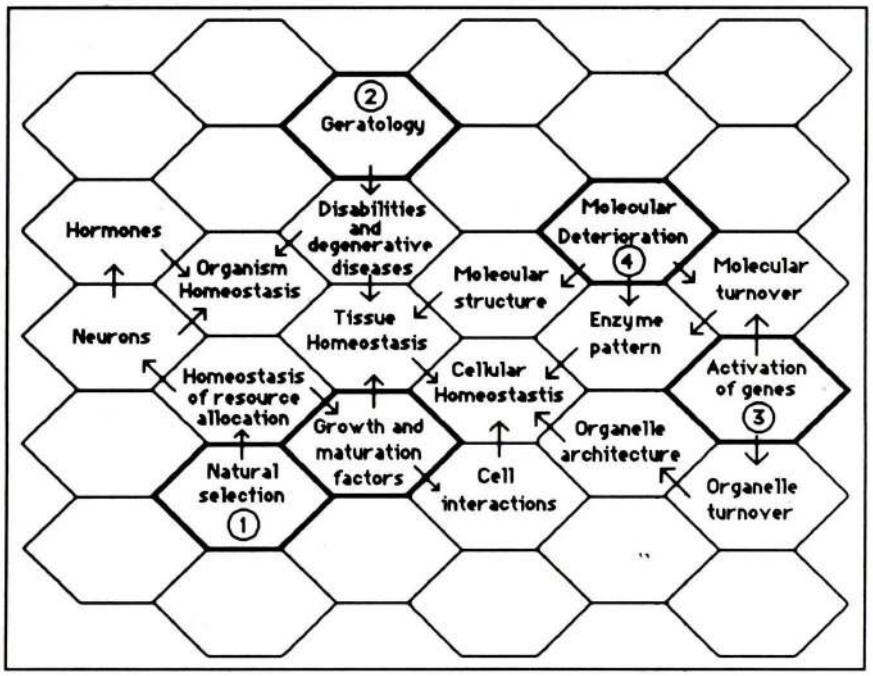


Figure 1.3. The overview knowledge net of gerontology

cells and tissues, and the mechanisms of activation of genes that specify the arrangements of proteins in cells and tissues. These approaches lead to the study of cellular homeostasis.

The common ground for all four approaches is homeostasis of tissues and cells which is bound up with cell-to-cell interactions and the relationships between cells and the structure of their extracellular matrix.

Entry point 1: natural selection (Figure 1.4)

Genetic factors predetermine the potential maximum span of life for every species, and to a certain extent the actual length of life for individuals within the species. Environmental factors, in their turn, may greatly affect the longevity; by unfavourable impacts they may considerably shorten the length of life. Natural selection affects lifespan primarily by setting genetically determined differential allocations of the limited resources available to species at the organism, cell and organelle levels. The levels of allocation are determined by the relative survival advantages in relation to the major mortality factors in the wild.

Different levels of resource allocation to organs change during development. One of the obvious examples of this is the selective allocation to support brain growth in vertebrates during early development. Later, the balance shifts towards growth of the skeleton and limb musculature.

Resource allocation is regulated by a range of different systems, such as the neuroendocrine system and other, less well-defined systems which produce substances loosely defined as 'growth and maturation' factors. There is thus a comprehensive area of knowledge dealing with the natural selection of various regulators connected with the needs of the homeostasis of resource allocation.

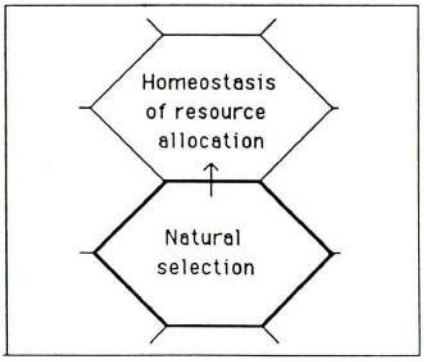


Figure 1.4. The biological perspective

Entry point 2: Geratology (Figure 1.5)

Geratology, the medical perspective, leads from the clinical speciality of geratology, commonly referred to as geriatric medicine, or 'geriatrics', to the study of disabilities and degenerative diseases which affect the elderly. This area is commonly perceived by clinicians as delineating the study of the loss of social, psychological and physiological reserve capacity. The ample reserves of youth eventually come close to the limiting level necessary to sustain life against the common day-to-day demands on the individual. Failures to respond to the sociophysical environment, pathogenic organisms, and biochemical errors eventually require medical care.

An important assumption behind geriatric medicine is that by studying the loss of organ function in relation to specific manifestations of tissue degeneration the slope of decline in physiological reserve can be reduced. This leads to a wide range of clinical approaches to the disabilities and diseases of ageing through environmental modifiers, physical aids, nutritional regimes and pharmacological support. In this respect, geriatricians deal in general medicine. However, they lack the firm biological analysis of functional failure which forms the basis of the rest of medicine that is orientated towards children and young adults and the middle aged. A particular feature of geratology is that is has to cope with the interactions of multiple systems, and with conditions which are not curable but have to be dealt with by establishing a multidisciplinary management programme for each individual.

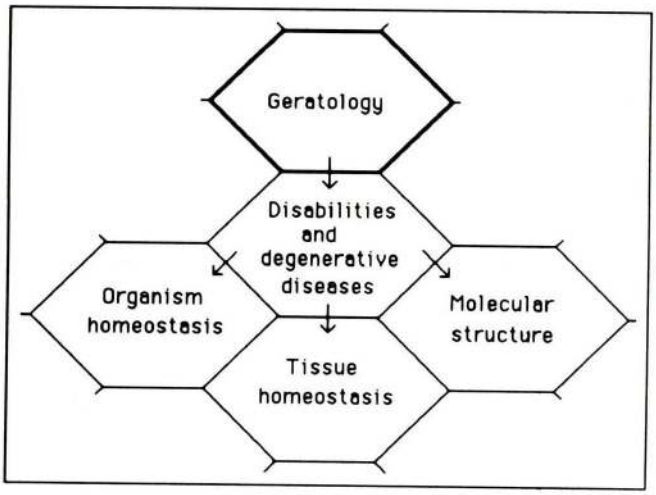


Figure 1.5. The medical perspective

Entry point 3: Activation of genes (Figure 1.6)

Molecular homeostasis lies in the realm of gene biochemistry, in particular, it is expressed in the regulation of genetic information in response to departures from chemical and structural norms. This area of biochemical and cytological knowledge surrounding the 'activation of genes' is a fifth pillar of gerontology. It can be argued that it is the most fundamental point of failure. This is so because genes set the level and direction of information flow to the various systems that maintain the molecular and organelle integrity of cells. As we grow older it appears that at this level the genes governing surveillance, repair and replacement, either become less able to function as they did in early development, or they tolerate a certain level of cumulative damage. It is beginning to seem as if both viewpoints are valid, and that ageing will be eventually explained mechanistically at this level.

Entry point 4: molecular deterioration (Figure 1.7)

From a chemist's point of view it is unexpected that the purine, pyrimidine and amino acid polymers of human cells remain largely intact over many decades of life. They are bathed in a warm saline solution containing potent oxidizing and reducing agents, and are subjected to cosmic radiation. Where a loss of biological properties of these polymers has been demonstrated the changes appear to be akin to the chemical 'ageing' characterized by oxidation and

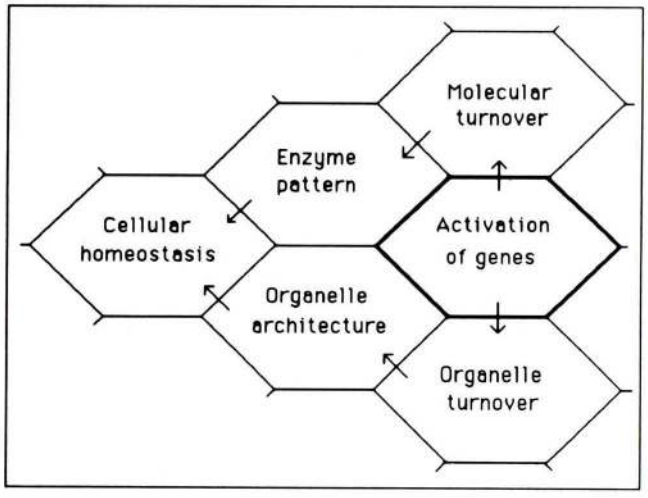


Figure 1.6. Activation of genes

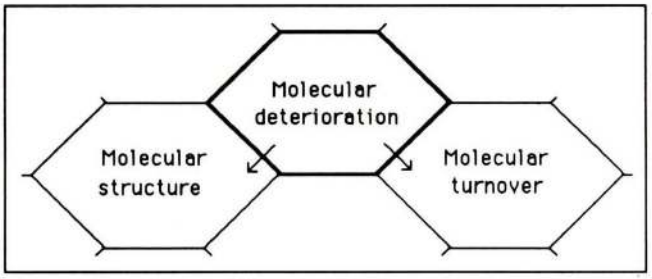


Figure 1.7. The molecular viewpoint

cross-linking that is responsible for the deterioration of leather and plastic. It is assumed that the apparent stability of living systems in the face of inevitable damage to macromolecules is a function of replacement synthesis, either of entire molecules or parts of molecules. This is part of the well-established phenomenon of molecular turnover.

As soon as atomic markers could be used to mark proteins and nucleic acids it was found that newly synthesized macromolecules have a well-defined average lifespan after which they are destroyed and replaced. Turnover must involve some kind of surveillance mechanism to check molecular integrity, but as yet we have no firm idea of how it works. Despite this chemical point of entry to gerontology being the obvious and fundamental one few chemists have taken turnover as a research viewpoint and most of what we know about molecular deterioration comes from studies of the molecular structure of extracellular polymers such as collagen and elastin which seem to be irreplaceable.

Entry point 5: organism homeostasis (Figure 1.8)

Deaths from ageing occur because the individual cannot cope with departures from youthful norms, and this emphasizes that the principle of homeostasis, or self-regulation, occupies an important reference point for gerontologists. Generally, ageing may be defined as a failure of homeostasis. This failure, although expressed proximally at the whole organism level, is generated distally at the cellular and molecular levels.

Organism homeostasis is studied in terms of the integrative controls exerted by the nervous system and the endocrine organs, separately and together. This, being a classical area of physiology, is well-defined in terms of detailed structure and function. Because of this it has been the first area of concern of those gerontologists seeking to explain age-related failures of homeostasis.

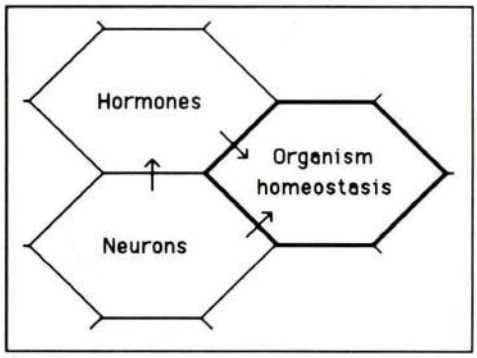


Figure 1.8. The perspective of organ function

Entry point 6: growth and maturation factors (Figure 1.9)

However, organs continue to function at a basal level and regulate their cellular integrity in the absence of their normal supply of neuroendocrine controls. From this viewpoint there are clearly other, may-be more basic, controls that might go wrong. We are beginning to glimpse these basic locally acting or intrinsic systems, which maintain cells in terms of their characteristic pattern of enzymes and organelle architecture. Relevant information comes from studies of the cell-to-cell interactions characteristic of early development. This work has delineated the importance of membrane glycoproteins as information transmitters responding to locally acting growth and maturation factors, and

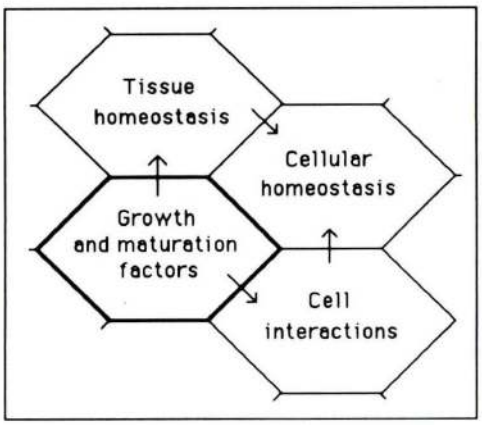


Figure 1.9. The tissue perspective

there can be little doubt that this area of research will be of future relevance to ageing of tissues and their component cells.

Cellular homeostasis (Figure 1.10)

A comprehensive knowledge base for gerontology has to take this current research into account, through a perspective dealing with the maintenance of cells in organs. One viewpoint deals with the ways in which cells maintain their internal integrity. The other deals with the maintenance of the tissue of which they are a part. On the one hand we ask questions such as, 'Can the universal failure of cells to maintain the youthful specifications of their organelles be explained by a failure in intracellular "hormones"'. On the other hand, the similar questions about the histological integrity of tissues are concerned with failures of intercellular feedback of growth and maturation factors which maintain the correct numbers and patterns of cells.

MACHINE ANALOGIES

Many advances in science have come by posing complicated problems in terms of the workings of machines, and a number of mechanical analogies have been proposed to define ageing.

It is not surprising that ageing has long been viewed as a clock-like process. This has led to the view that a biological 'clock' determines the rate at which the organism deteriorates. This raises the possibility that if the clock could be

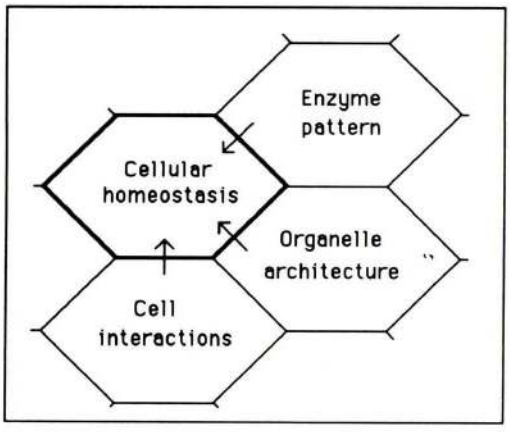


Figure 1.10. The cellular perspective

tampered with, particularly to make it run more slowly, longevity would be increased.

There are really three possibilities for the involvement of clock-like mechanisms in the ageing of the whole organism, particularly if ageing is due to the sum of the functions of its various parts, i.e. the different cellular populations. In one important machine analogy the relationship of the organism to its parts has been thought of as analogous to a well-maintained bicycle, kept going by interaction between its wheels, chain, pedals, and frame. If all its parts age at the same rate there would be a good chance of the entire machine collapsing at once. On the other hand if only the front wheel aged and became distorted so that the whole bicycle was put under stresses and strains the machine would end its life because of the age-related failure of only one of its key parts. A third possibility is that the bicycle dies not because any part becomes defective, but because through poor maintenance the parts no longer interact smoothly. An uncorrected bent wheel, a loose crank and un-oiled bearing could bring about accelerated damage in other parts.

These changes in bicycles have been taken to illustrate three different possible ways ageing could be timed on the cell tissue level:

1. All the parts could be intrinsically timed to age at similar rates.
2. One key part or 'pacemaker', ages faster than any other and its malfunction damages the other parts although they could have continued in their normal function.
3. No single part ages intrinsically, but the interaction between parts becomes defective. The poorly connected parts damage each other, causing them to fail.

As judged by the different times at which various organ systems reach their peak performance and the different rates at which they deteriorate, it is clear that there must be several clocks operating, even in the simplest organisms. Also, since the appearance of some features of ageing can be preferentially accelerated and some can be delayed by experimental treatment, the clocks must function independently of each other.

Translating this type of model into physiological reality is difficult. A common physiological cause of ageing in the various organs within an individual is unlikely on the grounds that they differ so much in both structure, function and survival value at different ages. In addition, the available experimental evidence supports the idea of organ ageing being largely chemically self-contained and not dominated by systemic factors. This multifactorial physiological model of ageing is more likely than the unitary model which states that there is only one system which is the limiting factor in longevity. On the other hand the 'bicycle analogy' usefully highlights the possibility of a central feature of ageing being a fundamental time-related deterioration in biochemical systems of surveillance and/or repair. This

possibility is emerging as a major theoretical basis of ageing and is being supported by experimental work.

Another mechanical analogy, based on the continuous playing of a gramophone record, highlights the most fundamental characteristic of cellular function, namely, the capacity repeatedly to transcribe coded information, apparently with decreased fidelity. If it is assumed that life is controlled by the repeated playing of the same record, from what is known about the biochemical transcription of DNA, the 'music', i.e. the integrated function of the cell, is not quite the same at each playing. This is partly because a 'blurring' of the record of life may occur by repetitive use, with 'cracks' appearing due to chemical deterioration. Also, the 'music' of life itself affects the score. In this form, the analogy represents development in its broadest sense. It takes account of feedback from the transcribed genetic information governing cellular function, which is able to alter the 'groove pattern' for the next playing.

All machine analogies suffer from the emphasis they must place upon the role of mechanical 'wear and tear'. This is a concept that seems to have little relevance to the functions of cells and organs, which have no moving parts, and have a capacity for self-repair. From the point of view of the way in which cellular processes are interrelated and driven, a more realistic analogy is the 'assembly line' model of error multiplication. In this model, a product is made by the sequential positioning of components on the assembly line. Replacement machine tools that control the relative positions of the components are assembled on another line. An error in assembly of a tool, not serious enough to prevent its use, would result in a mis-specification of the product. If the tools were also used to make replacement tools, the original error would be multiplied through the assembly system resulting in a total failure in production.

Such an error 'catastrophe', has been postulated to occur in the DNA/RNA transcription process, where errors in the nucleotide polymerase tools required for the assembly of amino acids in the correct sequence to make proteins could produce mis-specified templates and template enzymes (Figure 1.11).

The selection of placement mechanisms to ensure precision of assembly lines, and processes for error detection and elimination, would have been a very early and vital aspect of biochemical evolution. A general model of the role of evolution in the origins of ageing is illustrated by the 'space probe' analogy. This likens the individual life to the voyage of a space probe that has been designed and programmed to pass close to a distant planet and transmit its findings back to base. Once the mission has been completed the craft will continue to function, but, gradually, various parts will break down due to inevitable chemical deterioration and accidental damage. Eventually the probe will 'die' when its main transmitter fails.

The space ship model may be taken to represent an organism that is programmed by evolution to have a well-defined lifespan. This is determined statistically in direct relation to the effort put into the construction and/or replacement of its parts that are subject to random error and damage. It assumes

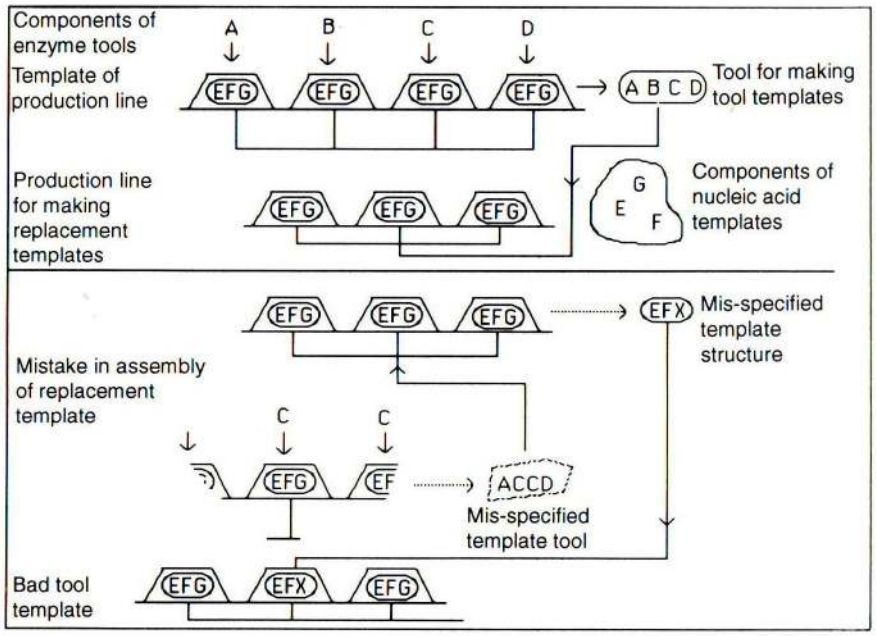


Figure 1.11. The 'assembly line model' of error multiplication

that lifespan is selected by the evolution of a programme of molecular replacement so that the organism has a good chance of staying alive for the period necessary to pass on its genes to the next generation. On this model, death in old age occurs because the DNA programme was not designed in the first place to eliminate completely all faults and errors in the cellular machinery, which eventually accumulate and dominate the organism's activities. This situation is illustrated in Figure 1.12.

In the model errors were set to occur at random over a range from 1–6 per unit time. If the accumulation of 30 errors results in death, doubling the rate of error repair would extend lifespan by about 40%. A three-fold increase in the rate of repair would extend lifespan indefinitely. However, if accidental deaths were a major cause of mortality so that no individual lived long enough to accumulate 30 errors, investment in repair could be set at the lowest rate if this resulted in an average reproductive replacement of one to one.

NATURAL SELECTION AND THE EVOLUTION OF AGEING

Although the impetus to study ageing arises largely to cope with the practical and clinical problems of human ageing, gerontology has a much wider scope

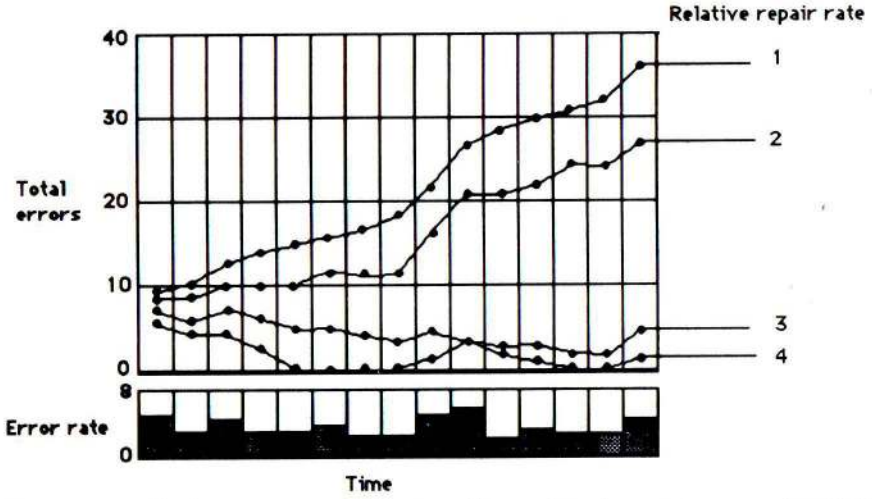


Figure 1.12. A schematic view of the accumulation of random errors over a four-fold range in the rate of error repair

than geriatric medicine. The area of investigation has lines of study extending deeply into biology. The past life history of the species as well as its present lifespan must be investigated. It is in this very broad sense that gerontology is a unified body of knowledge with clear guide-lines of principle which have been hardly explored (Figure 1.13). The subject is unified around the concept of the 'lifetime-environment', which provides resources for an individual yet is also a source of mortality, and the concept of 'species-genes' which allocate resources to combat mortality factors in the wild. Within this framework there is now a growing belief amongst biologists that we are close to a unitary evolutionary principle at the chemical level which explains ageing and offers testable predictions.

For most people ageing, is, on the initial approach, a difficult subject to appreciate in its evolutionary perspective. In the first place, it differs from other biological topics because it occupies a dominant position in our culture. The exponential rise in our chances of dying and 'old age' are the experiences of ourselves, our household pets and our favourite livestock. This age-dependence of death is not a feature of common wild animals which have surprisingly short lifespans, and most deaths in the wild are random and not age dependent (Table 1.3).

Each year a constant, large fraction of our garden birds die and it is very likely that the first songster of each spring is one of last year's replacements. This very short lifespan is greatly exceeded in aviaries where small birds captured in the wild can live for between five and 15 years.

This makes the point that senility is only found in animals that are under our

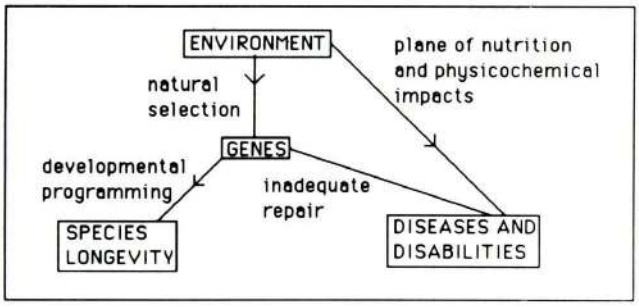


Figure 1.13. Environment, genes and ageing: the scientific area of gerontology

protection. In this sense, human ageing is a product of the last century of human socioeconomic development which has brought the distress and uncertainty that old age brings to the family circle.

In contrast to people under the umbrella of the welfare state, and domestic livestock, species in the wild die from environmental hazards, either when they are developing or when they reach maturity. With respect to small birds, high ecological mortality rates resulting from disease, food shortages, and predation, favour an increase in the rates of development and reproduction. When protected from these ecological hazards in cages, it is the continuous accumulation of internal biochemical damage that seems to be at the root of ageing. In this sense wild animals do not live long enough to accumulate an 'ageing dose' of errors. It is reasonable to assume that degenerative diseases of old age are high level expressions of this accumulation of damage. They vary according to the cellular system where the damage occurs, the possibilities for interaction of the cellular system with environmental factors, and the individual's genetic predisposition to the particular type of cellular malfunction.

Species longevity is inversely related to the pressures of natural selection which determines the period of development in the wild. Low chances of survival favour high rates of development to a relatively small mature body size and a high rate of reproduction. High chances of survival favour slow development to a relatively large mature body size, with reproduction spread over many years. Human diseases and disabilities occur after the period of maturation because the programme is not designed to eliminate errors that arise through inadequate repair.

In our modern protective social system the damaging effects of these errors leading to diseases of ageing may only be revealed in certain environments. For example, some forms of cellular ageing are accelerated by dietary changes. This aspect of comparative gerontology is a relatively new growth point. It is closely connected with population ecology and evolution thereby providing

Table 1.3. Mortality of small, British, wild birds

Species	Annual % mortality in wild	Maximum cage lifespan (years)
Blue tit	72	5
Wren	70	7
Robin	62	9
Blackbird	50	10
Song thrush	48	12

cross-disciplinary lines of communication with a number of other fields such as biochemistry, genetics, endocrinology and nutrition.

Investigations of the evolution of longevity can only take place within this broad area of biological knowledge. For example, comparative anatomy has produced a list of primate lifespan potentials calculated from brain/body weight relationships which form the basis for investigations into correlated biochemical variables. A shift in relative resource allocations from body to brain might explain lifespan extension in the pathway of human evolution (Table 1.4; Figure 1.14).

The field of animal behaviour has provided some evidence that the loss of behavioural adaptability may be important in natural selection within some wild populations. This highlights two other important growth points in the ageing field; behavioural gerontology and the associated areas of neurophysiology, endocrinology, and immunology, where we can expect a future demand for increasing amounts of temporal information.

The idea that errors in the immune system can prevent the body's defence system deciding between 'self' and 'non-self' has already brought immunology to the fore as a central feature of theories of ageing. In particular it is thought that some degenerative diseases arise through immunological 'self'-destruction.

Table 1.4. 'Cutler' lifespan potentials of modern and fossil primates

Primate	Potential lifespan (yrs)
Tupaionidea	6.5
Ceboidea	22.0
Cercopithecoidea	30.0
Hylobatidae	35.0
Pongo	50.0
Pan	55.0
Homo erectus javanicus	69.0
Homo sapiens (Australian aboriginal)	85.0
Homo sapiens	94.0

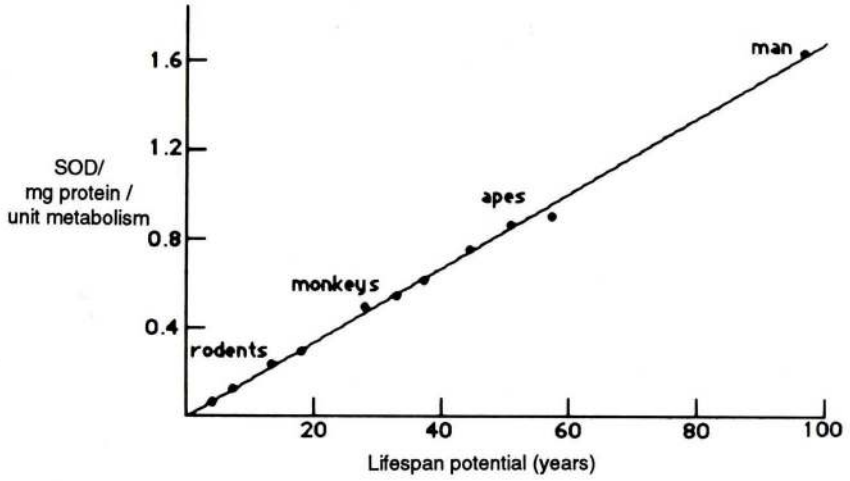


Figure 1.14. Relationship between the enzyme superoxide dismutase (S.O.D) and lifespan potential in rodents and primates

Any imperfection of function which results in a loss of adaptability could be classed as ageing and from this viewpoint it is likely that there are many causes and consequently many processes of ageing. The length of the life cycle is the end point of evolution and the way in which this is established by natural selection need not be identical or even similar in different species. This is not to rule out a small number of 'anti-ageing genes' that fix species' lifespans. For example, if lifespan is regarded as an ecological 'trade off' between the resources devoted to repair and that 'spent' on reproduction, then repair-genes would control the rate of ageing according to the extent that their activity approached a 100% correction of all defects.

From this evolutionary perspective, only in a prescientific sense is ageing one phenomenon. A better viewpoint would be to regard it as a symbolic term summarizing a diversity of different and unrelated processes. It is futile to search for one central process of ageing and a single definition with one all-embracing research approach.

GERATOLOGY

The Size of the Problem of Human Ageing

We need to study the principles of ageing in depth for sound social reasons. The beneficiary of any practical results arising from experimental gerontology is the

branch of medicine termed 'geratology', commonly described as 'geriatrics'. This deals with the clinical, remedial, preventative and social aspects of old age. It is essentially hospital-based and in the main deals with the practical problems of managing individual elderly people who are ill. However, there is also an important 'home-based' service which deals with disabilities which can be coped with outside the hospitals.

Research by specialists in geriatric medicine has increased with the development of specialized hospital services, but in most countries staffing and finance is still inadequate to provide a healthy and independent old age for each elderly individual. About 8 million people, about one in eight of the population of the United Kingdom are aged 65 and over (Figure 1.15; Table 1.5). Most of them are totally independent, mentally alert, but dependency increases sharply from the seventh decade onwards.

It is the cohorts over 75 (between 2 and 3 million, increasing to 3.5 million by 2001), who demand the greatest share of medical care. In particular, coping with the health problems of the over-85s will be the major health challenge for the next 30 years

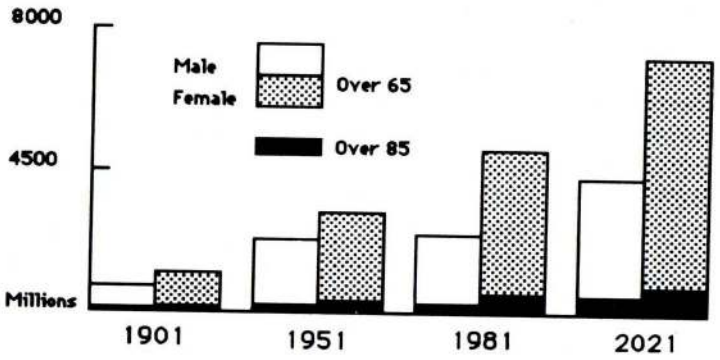


Figure 1.15. The over 65s in the United Kingdom; past and predicted future

Table 1.5. The proportions of over 65s in the population; past and predicted

Age group	Percentages of total population; Males, M; Females, F									
	1901		1951		1981		2001		2021	
	M	F	M	F	M	F	M	F	M	F
65+	4.2	5.2	9.3	12.3	12.2	17.8	12.6	17.8	14.6	19.6
75+	1.2	1.6	2.8	4.2	3.9	7.6	5.2	9.1	5.8	9.3
85+	0.1	0.2	0.3	0.6	0.5	1.6	1.0	2.6	1.3	2.9

The cause of this population explosion of elderly people is due to the decline in death rates in all age groups under 65 during the last 80 years. At the time of the census in 1851 the death rate of children between birth and one year of age was 153 per 1000 live births. The impact of public health measures starting with the sewage, sanitation and smallpox acts had reduced the death rate of children to 58 per 1000 births by 1941. With further improvements in combating microbial diseases the death rate is now 18 per 1000, and a male infant born now in the United Kingdom can expect to live to 69 and a female to 75.

Britain's death rates in the elderly have not decreased in proportion. In 1851 a man aged 65 had an expectation of life of 10 years and this has only increased by 2 years in the last 130 years despite all the advances in modern medical techniques. This tells us that the causes of death in the elderly are not now mainly environmental. They are internally generated as complex, interactive, metabolic failures, which are expressed at the cellular level as degenerative diseases (Table 1.6). The ageing process is defined as the sum of all of the chemical and physical interactions between body constituents that are damaging to the organism. These are intrinsic and inevitable outcomes of the way in which cells and organs are built. The diseases of old age result either directly from the process of ageing, or indirectly from the impact of extrinsic factors on faulty cells or organs. Longevity is therefore determined by the level of effectiveness of genetic mechanisms that either repair damage or give protection against it.

Most people in the developed countries die over the age of 65, and apart from accidental deaths, infectious diseases and some cancers that strike the very young, elimination of any of the diseases at birth would produce the same lifespan extension as eliminating it at the age of 65.

Elimination of a particular degenerative disease in the elderly would have little influence on life expectancy. This is because these diseases are an expression of an underlying biological process which pervades all tissues, and elimination of one disease would result in death from another within a short

Table 1.6. Gains in human life expectancy by elimination of particular causes of death

Cause of death	Gain in life expectancy (yr)	
	Elimination at birth	Elimination at 65
Accidents	0.6	0.1
Cancer	2.3	1.2
Cerebrovascular diseases	1.3	1.2
Diabetes	0.2	0.2
Heart disease	5.9	4.9
Influenza and pneumonia	0.2	0.5
Renal diseases	10.9	10.1

interval of time. Therefore, the basis of geriatric medicine should be to define the basis of molecular deterioration with a view to controlling it in such a way that its pathological expressions are delayed, without prolonging the period of dependence of the elderly population on medical or social care.

The medical and social problems of coping with an increased proportion of elderly in the population are not confined to the developed world. The health benefits arising from western medical technology are now producing the same problems in those developing countries that have adopted measures to combat premature deaths from environmental diseases. In Latin America for example the over 80s cohort will increase about twofold by the end of the century. Most of the world's population is beginning to encounter medical and social problems of caring for the elderly when they have not yet overcome the problems arising from the population explosion in the younger age groups (Figure 1.16).

Variability

Outward age changes are not an accurate index of chronological age. Neither are the less obvious changes that occur in the function of all parts of the body. This is because, intermingled with changes strictly related to time are other changes, of equal, or greater, importance, associated with individual genetic make-up and specific environmental factors and disease processes. These are due to the specific pattern of life of the individual. The quality and quantity of nutrition, amount of exercise, mental stimulation, exposure to infection, noise, and toxic chemicals all influence individual health. These individual impacts

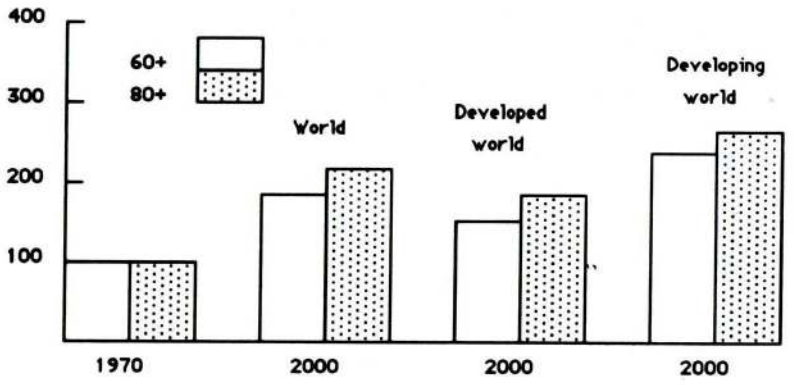


Figure 1.16. The increasing proportion of elderly on a world basis The numbers of elderly in 1970 have been set at 100 and the predicted increase to the year 2000 has been calculated, relative to these figures

interact with more fundamental chemical changes of ageing and it is not often possible to separate the random environmental forces in a person's unique physical and social environment from changes associated with chronological age alone. It is this kind of specific variability which is responsible for the wide spread of ages at death, obvious, even in the last decade of survival (Table 1.7).

There has been no definite case of anyone living beyond the age of 114 and centenarians are the extreme tail of populations that generally show an exponential increase in the probability of dying. Nevertheless, the existence of people who live well beyond the average lifespan has prompted speculation that the terminal cohort represents a kind of biological elite. After the age of about 80, all parameters of ageing in individuals in this group are better than the average. No doubt if everyone could achieve the status of the group then indeed we could all attain the objective of a healthy happy old age, but are they a distinct group or are they just the extreme of the normal distribution curve of health? If they are indeed 'normal' and the majority of the population are 'abnormal', is their normality a matter of inheritance or is it associated with life style? If the latter, then can something be learned by studying this group to help other people?

The latter question is central to a major study area of 'demographic geriatrics' which deals with the separate but interacting effects of ageing and lifestyle upon the general health of the individual. Another major problem area, which may be defined as 'organizational geriatrics', centres on the study of the systems of delivering care to the elderly, both in hospital and in the community. Delivery of services has nothing to do with the prevention ageing, nor with combating ill-health. Indeed, its development was necessary because of inadequate resource provision for preventive medicine. It is also concerned with the design of hospital facilities for the elderly and the making of social

Table 1.7. Variability of time of death in centenarians

Age	Survivors
110	1
109	16
108	140
107	830
106	3630
105	12300
104	44000
103	98600
102	233000
101	505000
100	1010000
99	18930000

diagnoses to avoid social mismanagement. For example, if only because of the slowing of perception and response in the elderly, special facilities have to be provided which take the loss of behavioural adaptability into account. The environment of the elderly has to be matched to their reduced capabilities.

Care of the Elderly

Medicine has tended to concentrate upon the problems of children and people of working age and the pattern of development of medical services has not encouraged the interdisciplinary pattern of research essential for investigating the elderly. Classically, medical research has been associated with the identification of groups of individuals with specific diseases followed by the identification of the factors common to that group. The assumption is that for each disease there is a specific exogenous, and/or intrinsic cause for each dysfunction. In this way for example, bacteria, viruses and toxins may be suspected and then confirmed by specific tests. In addition, groups of individuals presenting with the same symptoms can be treated in different ways and the results from each group compared as a test for a multiple origin of the symptoms. Unfortunately, if, as seems likely, the factors determining the rate of ageing are different from those causing age-related diseases and disabilities, this approach will not work with diseases of ageing.

Most of medicine is confined to the identification of the sick who are referred to a specialist. The development of these specialist medical services, for example, in respiratory medicine, cardiology, neurology, gastroenterology and genitourinary medicine, which concentrate on problems of the middle-aged ill, has led not only to a narrow physiological approach but also the exclusion of both the very old and children from the group of people studied. The medical profession is not built upon the biological, developmental perspective of the human life cycle. Its specialist services are sharply divided between the three divisions of the young, middle aged and the very old. Concentration solely upon the young or the middle-aged inevitably means that possibilities of the early genesis of terminal problems are either likely to go unnoticed or, if noticed, they may be considered irrelevant to the life a patient several decades into the future.

It would also be a great step forward if those people at risk with respect to particular degenerative diseases could be identified in advance. The concept of predictive ageing tests (Table 1.8) has been seriously promoted. However, their cost, serious disagreements as to the exact best test battery, great logistical difficulties in establishing a good baseline, and the poor predictive framework in morbity and mortality, have prevented their routine application to the under-60s.

Social care of the elderly is based on the academic area described as social and behavioural gerontology. It includes knowledge and methods distinct from

Table 1.8. Tests proposed for the battery assessment of biological age

Anthropomorphic measurements
Standing height
Sitting height
Trunk height
Biacromial diameter
Anterior-posterior diameter of the thorax
Weight
Fat-free weight
Hand or triceps skinfold thickness and elasticity
Grey hair score
Physiological tests
Vital capacity
Tidal volume
Maximum breathing capacity
One-second expiratory volume
Systolic blood pressure
Heart size
Hand grip-strength
Osteoporotic index of metacarpal
Nail calcium content
Visual acuity
Critical flicker frequency
Dark adaptation
Audiometry at different frequencies
Serum cholesterol, albumin, copper, elastase, RNAase
Blood autoantibodies
Psychometric tests
Wechsler's intelligence scale
Digit span
Digit symbol
Vocabulary
Visual retention test
Reaction time
Light extinction test

geriatrics, which are applied to those aspects of human ageing that affect social interactions. Unlike geriatrics its age range begins in the late teens. Although there are some connections between juvenile development and ageing, the complexities of ageing produce arguments in favour of studying ageing independently of development. Developmental psychology deals with the earlier juvenile phase of life. Social and behavioural gerontology has links with biology and medicine in that age changes affecting the peripheral and central mechanisms governing behaviour may produce adverse effects. Secondary social consequences affect other people through personal involvement with old people or through the general and economic aspects of ageing. In this

perspective, the constituent disciplines of social and behavioural gerontology range from architecture to social history and its practitioners deal in the main with applied problems yielding benefits through improved social welfare and psychological understanding. Any advances in physical health and longevity should be matched by advances in human achievement, personal adjustments and social relationships, which will require well-planned social and economic reorganization, together with changes in social attitudes, beliefs and values.

Many behavioural gerontologists believe that basic laboratory research on behaviour with non-human models is unlikely to yield much information that can be applied directly to solve human behavioural problems. Consequently research in social and behavioural gerontology deals mainly and directly with human populations through 'social experiments' and surveys. The motivating questions of research are concerned with the value of education in fostering physical and mental health in later life, the relationship between disuse and deterioration in physical and mental abilities, the formulation of the practical problems arising from human ageing, such as age-discrimination, and the resources that should be devoted to dealing with them. Research is also concerned with the production of valid methodologies by which the required social research may be carried out to establish a relevant social policy (Table 1.9).

OBSTACLES TO PROGRESS

Research into the reasons for the decline in our capacity for adaptation lags far behind studies on the basic mechanisms of growth, maturation and tissue maintenance. Also, research into the biology of ageing has not kept pace with the spectacular successes of clinical medicine. Now, world-wide, there is a great, and ever-growing discrepancy between the amount of money and knowledge available to keep young people alive and that devoted to maintaining the aged in good physical and mental condition.

The present relatively low level of support for research on ageing is partly connected with the way in which gerontology has developed from what was the low status medical field of geriatrics. It was only in the 1950s that attention began to be focused upon the problems of the old people who had been considered unfit for rehabilitation and who had been deposited in the workhouses and empty sanatoriums. Doctors in these units started the development of geriatrics as a separate medical speciality by demonstrating that rehabilitation could be successful in the elderly.

Within biology, ageing is still largely separated from mainstream thought. As an isolated body of knowledge it has been omitted from most biological and medical curricula, leaving a large gap in the training of those young people needed to develop gerontological research. To these historical impediments may be added a current bias against research proposals, arising from ignorance

Table 1.9. Some questions related to the formulation of social policy for dealing with special problems of the elderly

1. What steps are being taken to reduce or eliminate age discrimination in employment?
2. What is being done to re-examine the statutory age of retirement and other aspects of retirement in the light of modern attitudes towards work, leisure and equal opportunities for men and women?
3. To what extent are the needs and abilities of older people being drawn to the attention of the producers of goods and services?
4. What further effort is needed to reduce industrial, highway and domestic accidents to older people?
5. To what extent will housing and social services be adequate to meet the demands of the over-40s and over-60s in future years, bearing in mind changes in the age structure of the population?
6. Has enough been done to assess the special housing, health and social needs of older people and to meet them?
7. What more might be done in health and welfare education to improve the overall well-being of older people?
8. Is sufficient effort being put into the compilation of statistical information and the re-establishment of data banks relevant to middle age and old age; e.g. with regard to accidents, consumer behaviour, leisure, income and expenditure patterns?
9. What steps are being taken to learn from the experience of other countries and to influence 'state policy' with regard to older people?
10. Who is developing the social policy options that will be needed if there are substantial or even modest improvements in the further life-expectancy of older people in the future?
11. Why is not more being done to stimulate basic and applied research in gerontology?

on the part of those in competing fields; the high cost of maintaining stocks of vertebrate animals of known pedigree into old age; and the difficulties that many specialists encounter in moving into what is essentially a complex interdisciplinary area of research, where it is difficult to relate findings at a chemical level to problems at the level of organs and whole bodies.

There is also a largely unassessed factor arising from the fears of many scientists, who could potentially contribute to gerontology, that the study of ageing might provide a way of prolonging life and so add to our present unsolved social problems. In this regard there has been a swing away from the stated aims of the early gerontologists who were concerned with the possibilities of extending life indefinitely to the more circumspect aims of countering specific ills in the elderly.

The diagrammatic representation in Figure 1.17 indicates the difficulties of formulating a fundamental research programme to help the elderly. Although, theoretically, there is likely to be a limited range of initiating chemical events, which occur frequently enough to ensure that they will happen in all members

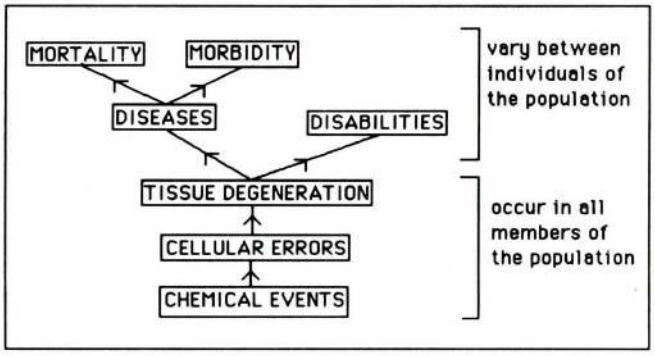


Figure 1.17. The hierarchy of phenotypic expressions of ageing

of the population, their higher level expressions are very variable, presumably because there are so many possible intervening limiting factors.

There are two approaches to restore the present unbalanced accumulation of knowledge, both of which require a deliberate increase in the proportion of our national educational and research budgets that are devoted to gerontology.

A preliminary objective must be to provide new educational resources and research training programmes backed by appropriate funds for research. More support must be given to research in fields other than biochemistry. While there can be no doubt that ageing will be ultimately explained in molecular terms, it is not easy to integrate the findings of molecular biology into the levels of organization familiar to the physiologist and experimental psychologist. An awareness of this methodological gap is important to the future balanced development of gerontology. Generally, research into any unknown area begins with 'the study of things before the study of the causes of things'. In this sense, biochemical observations only have significance to the extent that they fit the time course of loss of adaptability of the whole organism. There is a strong feeling among gerontologists that molecular biology often receives support on the mistaken assumption that studies at this fundamental level will automatically provide answers to problems, which, of necessity, arise, and can only be solved at a higher level of chemical organization.

Since ageing is characterized by the loss of organization and order at all levels the separation of data and researchers by level is the main problem in presenting gerontology as a unified subject. Like the concept of 'the universe' in a wider perspective of biology, the concepts of 'ageing' and 'gerontology' are each a series of levels of organization and complexity ranging from the sub-atomic level, through the atom, the molecule, the colloidal particle, the nucleus, the cell to the organ, the organism and its psychological and sociological entity. It follows that the laws or regularities which we find at one level cannot be expected to appear at a lower level. The conditions for their

appearance do not exist there. Just as in chemistry it is no use searching in homogeneous solutions for laws which hold good for the behaviour of liquid crystals, still less is it valid to search for laws that hold at all levels of the ageing individual.

There is much talk in gerontology, as in other branches of biology, about the reducibility or irreducibility of biological facts to physicochemical facts. These old controversies are unnecessary if we realise that we are dealing with a series of levels of organisation. We must seek to elucidate the regularities which occur at each of these levels without attempting either to force the higher or coarser processes into the framework of the lower or finer processes, or conversely to explain the lower by the higher. From this point of view, the appearance of irregularities in cellular integrity with age, as is for example revealed at the structural level with microscopes, will always have their validity, and will, in this sense, be unaffected by anything which either biochemistry, on the one hand, or psychology, on the other, may discover. The loss of muscular strength in old people will always remain the same and have the same significance, however much our knowledge of biochemistry and biophysics may advance. This is the reason why prediction is possible at a level of organization, such as the morphological one, which strictly speaking we do not yet understand at all. Nevertheless, the important point is that although the losses of order established at the level of experimental morphology are irrefutable, they will, in the absence of biochemical experimentation, remain forever meaningless. Meaning can only be introduced into gerontological knowledge by the simultaneous prosecution of research at all levels of complexity and organization, for only in this way can we hope to understand how one level is connected with the others.

The lack of this broad philosophical framework in the past has led to much fruitless methodological argument as to whether one should first develop a general testable hypothesis, describe the ageing organism in detail, or enumerate possibilities that have then to be eliminated, one by one. Whilst the decisive approach in science is inevitably an experimental one, all viewpoints are valid. The popular, yet biased, position of model-making, which has resulted in many theories of ageing, has also mitigated against the collection of information on the natural history of ageing, and has resulted in much time and effort being devoted to topics long after they have ceased to yield important results. This is a general situation for any subject in its prescientific era, and it can be argued that much of the confusion in the minds of journalists who examine laboratory reports for recipes to maintain fitness and prevent age-related diseases comes from their reading the unjustified, scientific dogmatism of some scientists.

Taking the standpoint that general gerontology is defined in terms of levels of organization at which ageing is expressed and the processes which connect the levels one with another, the subsequent chapters are arranged to express

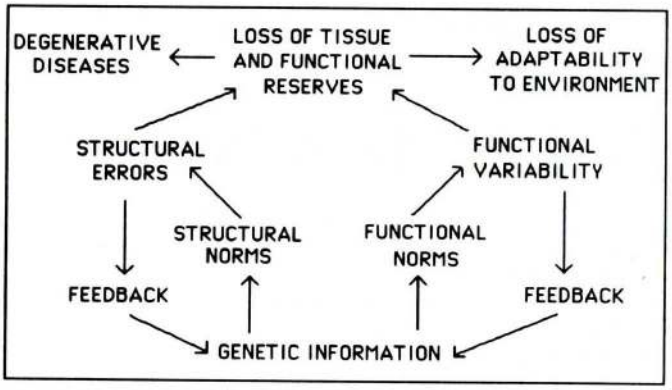
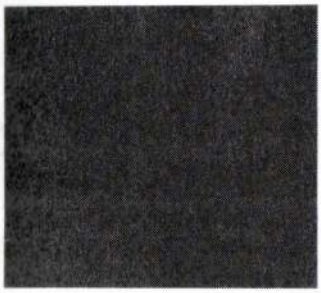


Figure 1.18. Gerontology as a unified system of knowledge

this standpoint. In sequence they deal with the four main features of ageing: age-dependent mortality, the involution of organs, the malformation of cells, and the failure of homeostasis to maintain structural and function norms. In an educational context this viewpoint presents a unified, cross-curricular system of knowledge (Figure 1.18) in which ageing is perceived as a failure of genetic information to detect all changes in structural and functional norms, and to respond perfectly to feedback from changing norms.



CHAPTER 2 Geratology

Ageing of the population has become a social and economic problem of the greatest magnitude. By 1989 the number of old people of pensionable age will equal the number of children, whilst by the same year the number of persons of working age will be approximately three millions less than in 1946. This situation would be very grave for every nation and every Government because a great number of helpless and useless old people would become a heavy burden on the younger working population. (Korenchevsky 1960)

MEDICAL CARE OF THE ELDERLY

In the past, great efforts were made in compiling scientific reviews and textbooks to separate changes of normal ageing from those of age-related diseases. Increasingly however, studies on selected old people, screened to exclude the presence of any disease, have failed to demonstrate many of the changes previously attributed to normal ageing. Because of the rarity of elderly persons who are devoid of any disease the concept of normal ageing is receiving less emphasis, and is being replaced by the pragmatic consideration of those age-dependent changes to be expected in a representative sample of the elderly which require care in a medical context. Geratology is a discipline which focuses the necessary research for applications to diseases and disabilities of the elderly. This chapter takes a broad interdisciplinary sweep through the area of knowledge from the problems of establishing a caring environment for the elderly, by way of a demographic statement of the human problems, to a theoretical basis of biological research which holds hope for clinical intervention. Many of these aspects will be expanded in more detail in later chapters.

Care of the elderly differs from that required by children and adults mainly because it has to be delivered in a very broad sociomedical context, and within the constraints of a general, ongoing failure of physiological and cognitive homeostasis. According to whether it deals with the organization of care, or the study of particular disabilities, the relevant knowledge may be divided broadly into the following areas:

1. The correction of cognitive disorders.
2. The cure or alleviation of diseases of major tissue and organ systems.
3. The study of the expression of ageing within a demographic setting.

Organization of Care

The aged and their doctors

The special medical features of the elderly population are connected with the interaction of multiple systems; biochemical, physiological, and socioeconomic. The doctor has to place these features in the context of, not only the patient's current health status, but also past experiences, and the salient features of the current environment. Also, as the age of the patient increases, emphasis has to be placed increasingly on the recognition of how dysfunction relates to the whole person rather than upon the definition of the dysfunction alone In relation to the deteriorating physiological baseline of the elderly, regular screening is an important aspect of care, a requirement that it has been suggested could be carried out by non-medical health personnel as part of what might be termed 'episodic health maintenance'.

It is particularly important to have an overview of the health problems of the elderly in relation to the population as a whole and their geographic and social situations. In the ideal caring situation the provision of medical services has to be backed up by a broad-based far-reaching community-based operation.

There are also special ethical dilemmas peculiar to the clinical care of the elderly, and geratology is particularly dense with ethical issues connected with how extensive and aggressive a treatment should be. Common areas of concern in general care are connected with the diagnosis and treatment of infections because of host resistance impairment, and the likely adverse responses to anaesthetics. Not least of these dilemmas is related to the cost of treatment, because it is likely that medical care in the last years of life will consume more medical resources than at any other time in the patient's life history.

From all of these points of view, physicians dealing with the elderly probably require special professional and personal qualities to become competent and efficient providers of health care to the aged.

The team approach to the elderly

Because of the scope and complexity of problems that elderly patients present, new conceptual models for diagnosis and management have emerged. This is epitomized in the multidisciplinary medical team with common goals, a diversity of skills, and a professional group orientation to achieve these corporate goals. The team approach has emerged because the problems of old people need a multidisciplinary cross-domain approach so that the medical assessment of needs may be translated into appropriate services and settings for care. A team is the best way to co-ordinate efforts to provide an integrated medical and social service for a patient or group of patients, which involves

systematic communication across a number of inter-personal barriers. This orientation is particularly effective in the comprehensive assessment of the frail elderly in relation to needs for long-term care

Problems in Delivery of Ancillary Services for the Elderly

Care of the elderly has special features connected with broad environmental context in which treatment has to be carried out. This includes hospital nursing, care in the nursing home and domiciliary care in the family context. The major clinical problems in gerontological nursing are related to the requirement for care management to have a rehabilitative approach to a loss of function. This perspective takes in the dual objectives of regaining and maintaining the maximum functional ability, and preventing or delaying an additional decline. It is expressed as the concept of 'interdependence-nursing', which is care involving the coexistence of dependence (expressed by the term 'needing another'), and independence (i.e. self-reliance). This is also the main philosophy required in nursing homes, where the inmates require access to a wide spectrum of services and facilities.

The home may be an important site of rehabilitation for the elderly to ameliorate particular disabilities. Often, a special caring environment has to be constructed through emphasis on the provision of assisting personnel, and devices such as aids for mobility, eating, dressing, hygiene, communication and recreation. It is in the home that problems relating to sexuality, sleep and nutrition first arise, with special home issues, many of which are related to failures in behavioural and physiological homeostasis, such as hypothermia, protein-calorie malnutrition, drug non-compliance and drug-nutrient interrelationships. From the point of view of age-related changes in drug kinetics and pharmacodynamics, the home is not the ideal place in which to administer drugs to the elderly because of the need for multiple prescriptions and special administration techniques to cope with possible adverse reactions, and non-compliance.

The home is also increasingly seen as the focus of a wide range of social services and family support problems which have to be provided to cope with the presence of elderly relatives. This gives prominence to the home/community interface in the support of geriatric patients. An increasing global issue at this level is the costs of paying for health care, which may be more than three times greater, and take up a large fraction of the family income, compared with the younger groups. This is seen particularly in the specialty of clinical pharmacology where in the United States drug expenditure in over-65s accounts for 25% of drug expenditure in the population. There is also a need for the elderly population and those caring for them at home for information about the availability of financial help and services.

DISABILITIES AND DISEASES

Two important concepts in addressing the issues surrounding the medical treatment of human problems of ageing are those of 'impairments' and 'disabilities'. Impairments are scientifically defined deficits at the anatomical, physiological, mental and psychological levels that result from the age-dependent organic dysfunctions which are classified as diseases. Disabilities are performance-defined deficits in vital functions resulting from these impairments. Performance deficits represent restrictions in the manner or range of activities considered normal within the normal physical and social environment. Some of the common functional limitations connected with ageing may be described under the following headings:

1. Vision.
2. Dental.
3. Hearing.
4. Falls.
5. Urinary incontinence.
6. Cognition—depression, dementing disorders, paranoia and confusion.
7. Gynaecological problems.
8. Gastrointestinal problems.

Major diseases of ageing are associated with the following organs:

1. Brain—Parkinson's disease and stroke.
2. Lungs—asthma, bronchitis and emphysema.
3. Endocrine organs—hyperthyroidism and diabetes.
4. Malignant diseases—breast, colon and rectum, and lung.
5. Heart—coronary heart disease and hypertensive heart disease.
6. Musculoskeletal system—osteoarthritis, rheumatoid arthritis, osteoporosis and Paget's disease.
7. Blood—anaemia.

DEMOGRAPHY

Mortality and Life-tables

There are two ways of demonstrating that ageing occurs in a given population. One is to measure particular physiological functions in groups of individuals selected as being representative of different age classes. This is termed cross-sectional analysis. The other way is to measure the function in the same individual as it grows older. This is termed longitudinal analysis.

Using either cross-sectional or longitudinal methods to measure the speed of people running a fixed distance would show that running speed declines from the fourth decade onwards, at first gradually, then more rapidly. This age-related failure in physical performance would be classed as a selective mortality factor in environments where people regularly had to escape from fast running predators. In our modern world, it is now expressed in international sport as the narrow age range for champion sprinters. Since it is indicative of a loss of a previously developed capacity for neuromuscular function, it may also be classed as a general ageing process.

The second method to measure ageing is to delineate the force of mortality in a given population. This may be expressed as the proportion of people in a given age class dying each year in relation to a standard population size. This is usually visualized as a 'mortality curve' which is a plot of the percentage of a given birth cohort alive at different times after birth. We say that ageing is occurring if the force, or chance, of mortality increases with time, and provided the old are exposed to the environmental conditions that are not more severe that those experienced by the young.

For populations that have a high level of social protection from environmental hazards, such as extreme climatic changes, restrictions in food supply, and infectious diseases, the mortality 'curve' is, to all intents and purposes, a plateau, with a steep decline in old age. After a slight drop due to post-birth mortality very few deaths occur until the cohort is close to its maximum lifespan then most individuals die within a relatively short time interval. This gives a 'rectangular curve' with a long 'plateau of fitness'. Such a curve is characteristic of populations where deaths occur from internally generated physiological failures classed as degenerative diseases. Comparisons of mortality curves in different countries during a given period provide an indication of the differences in environmental exposure. Plotting demographic data for the 1930s gives a range of curves representing a corresponding range of environmental situations (Figure 2.1). They show the situation in underdeveloped countries with low environmental protection and poor nutrition (India and Mexico) coexisting with countries with food in abundance, and high standards of medical care and public health (United States and New Zealand). Where comparisons have been made using the historical census information of developed countries it has been found that curves shift during development from the 'Indian type' towards the 'New Zealand type'.

Mortality curves are concerned with numbers in time, and the branch of science dealing with defining mortality curves in various populations is termed demography. Individuals of a population have potential mean lifespans, possible maximal ones (that very few members of any cohort reach), and each individual has a definite chance of dying at any time. Rates of death are the numbers of deaths in any time interval that actually take place among the number of individuals at risk.

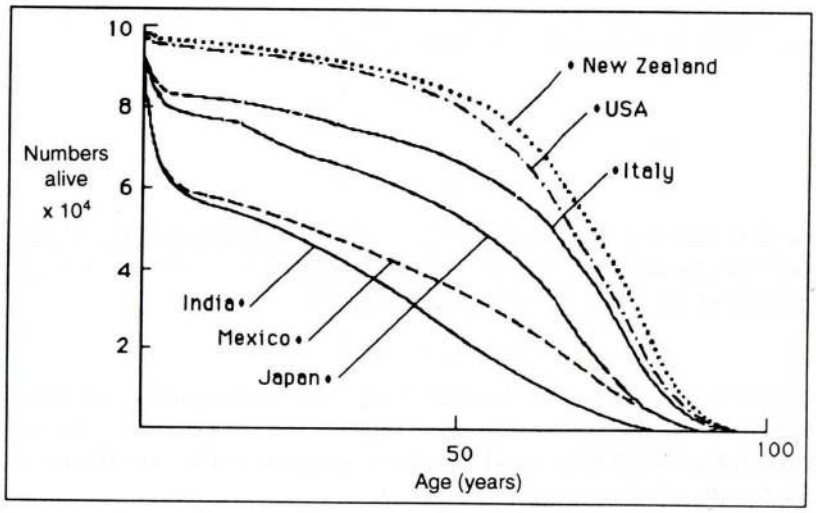


Figure 2.1. Morality curves of six countries in the 1930s

$$\text{Death rate per given time} = \frac{\text{Number of deaths in that time}}{\text{Population at risk}}$$

There is some latitude in choice of denominator for this equation, for instance the size of the population at risk will usually be changing because of mortality. It may be necessary to choose among at least three measures of its size; the 'initial size', and the 'arithmetic' and the 'geometric' mean sizes related to the time interval under consideration.

Also there are varying degrees of involvement in the risk, and the demographer may be general or very specific in his choice. At the beginning of the time interval the probability of an occurrence happening throughout the time interval in an individual at risk is the same as a rate, expressed as a decimal of 1.0. For instance, if a death rate is 10% per week, an individual at the beginning of the week has a probability of 0.1 of dying within the week.

Crude rates of death are obtained by division of the number of occurrences in unit time by the population during that time, or at the beginning of it.

$$\text{Crude death rate} = \frac{\text{Number of deaths in unit time}}{\text{Population during or at start of that time}}$$

In the first quarter of the 19th century Gompertz showed that for humans an increasing rate of death with age seemed to occur at a particular rate itself. This suggested that there might be some uniform factor involved in the process of

senescence. According to Gompertz's analysis the change in numbers of an ageing cohort of individuals with time occurs according to:

$$dN/dt = -R_mN$$

and

$$Rm = R_0e^{at}$$

where N = numbers of individuals, R_m = rate of mortality and R_0 = rate of mortality at age zero, e = base of natural logarithms, a = constant, t = time.

Converting the Gompertz equation produces:

$$\log_eR_m = \log_eR_0 + at.$$

Using this equation to plot the data of mortality curves produces a straight line which crosses the axis for zero age at logeR0, and has a slope of a. Between 20 and 80 years of age the mortality curve for humans conforms to this form of the Gompertz equation (Figure 2.2).

It is also possible to produce Gompertz plots of mortality due to specific causes (Figure 2.3).

To show the type of problems that have to be handled by demographers here is an historical situation for Sweden.

In 1750 the crude human birth rate per year was approximately 83 per thousand, and in 1934 it had fallen to 13.

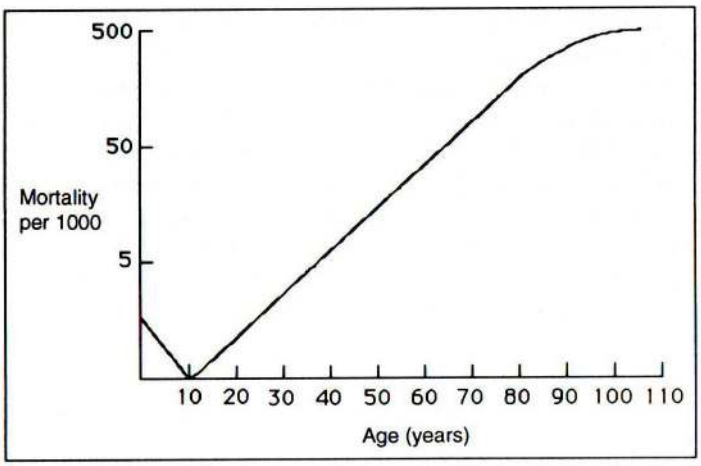


Figure 2.2. Gompertz conversion of morality curve for a population characteristic of the developed world.

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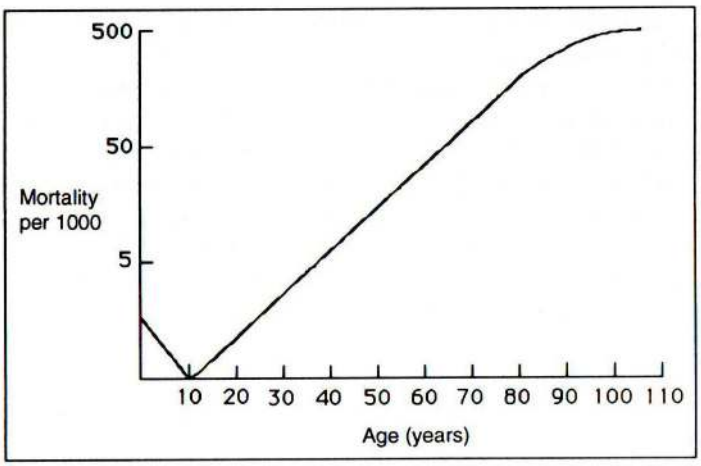

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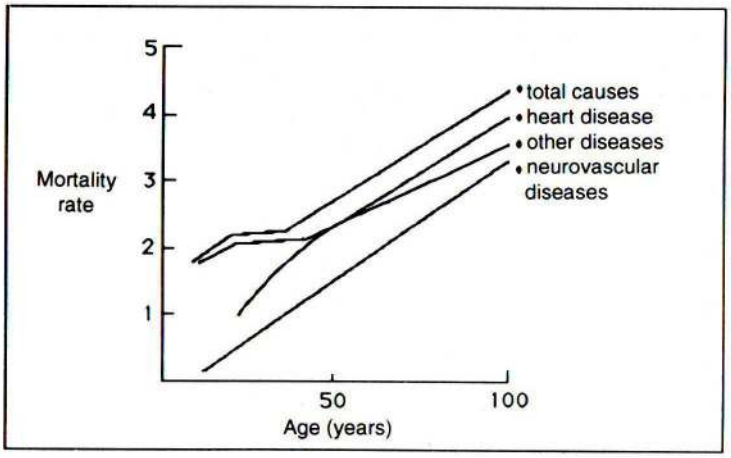


Figure 2.3. Gompertz plots typical of specific and general causes of mortality in developed countries (mortality rate expressed in powers of 10).

A demographer would be asking questions such as,'Did the birth rate per reproductive female decline? Was there a change in the number of reproductive females, or did the number of births each year remain the same, while total population size increased greatly because of a greater survival rate of older age groups?'. In fact it turns out that, with further data, the latter two features accounted for most of the change in crude birth rate.

The crude rate of increase in population is the crude birth rate minus the crude death rate. Thus, the crude rate of increase also reflects change in the age distribution, which is not defined in crude rates. These measures are valuable descriptions of events for the described time but without further data they cannot be used for predicting future population size and increase.

Crude rates are in units of calendar time, as opposed to generation time. Age-specific rates are more useful for predictive purposes, being defined as the number of occurrences in a specified class in unit time, divided by the number of individuals in that class. Age classes are such specified classes, and age-specific rates of births and deaths are widely used in predictive demography. When age-specific death rates are to be calculated (Table 2.1) from a population with a wide range of ages, or from a repeatedly censused population of the same age, data are conveniently arranged as a life-table. This is a formal arrangement with a set of symbols in general use, but the arrangement can be changed to suit particular purposes. To make a life-table the population is divided into age classes, and each age class occupies a new line down the left-hand side of the table; data and calculations for each specified age class are arranged across the line under column headings of symbols (Table 2.1).

Table 2.1. Life table for a laboratory population of voles (*Microtus agrestis*). From the age of 3 weeks, the mortality was recorded from 98 male and 46 female voles. Data collected over 3 years from several cohorts were grouped.

Stage	Weeks old (x)	Interval at age x	Survivors (x) to (x + 1)	Numbers dying	Age-specific mortality*
2 weeks from conception	−1	1	10000	2107	210.07
Birth	0	2	7893	1121	71.0
Weaning	2	1	6722	—	0
Sexual maturity					
Female	3	5	6772	235	6.94
Male	8	8	6537	470	8.96
	16	8	6067	1222	25.17
	24	8	4845	1223	31.55
	32	8	3622	1129	38.96
	40	8	2503	1180	58.92
	48	8	1323	709	66.98
	56	8	614	425	86.52
	64	8	189	94	-
	72	8	95	0	-
	80	8	95	47	-
	88	8	48	0	-
	96	8	48	48	-

*Age-specific mortality rate per 10^3/week

Age-independent Mortality

Populations showing age-dependent mortality may be contrasted with others where individuals are lost at random independent of age. A useful analogy to indicate random death independent of age is that of the accidental breakage of drinking glasses through continuous use. In this situation the probability of breakage would have nothing to do with the age of the glasses, and if new glasses were continually added to the 'population' in order to keep pace with breakages, the same percentage of those present at the beginning of every time interval would be expected to attain the end point of breakage during that interval. The age-specific mortality rate would be constant.

In other words the drinking glass model represents a situation where the percentage of a given number of individuals dying at each 'age' is in constant proportion to those of that age present. This model may represent the mortality in natural populations subject to infection with disease or suffering predation, both occurring randomly, and with no age-discrimination of individuals that are to die. The mortality curve is similar to that of the decay of a radioactive isotope.

In a harsh environment the ideal curve of a protected population, which is

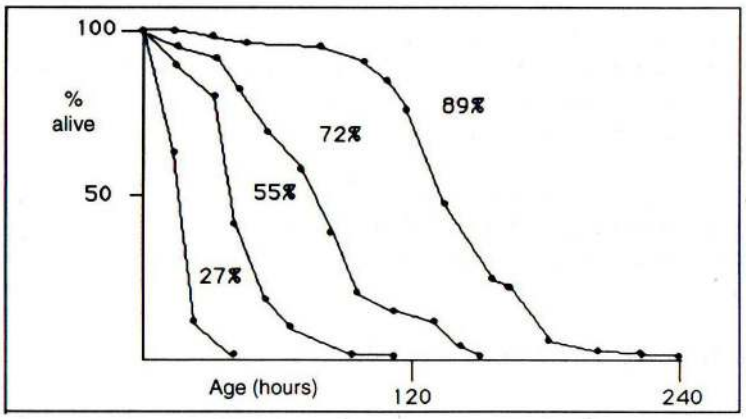


Figure 2.4. Mortality curves for populations of adult rat fleas in relation to percentage humidity of the laboratory environment.

characterized by a rectangular 'plateau of fitness', is modified by random age-independent impacts of environment. In this ecological situation, after a very high infant mortality, significant numbers of individuals continue to die 'accidentally' according to the 'drinking glass model'. This is represented by the responses of populations of fleas to the adverse effects of decreasing the humidity (Figure 2.4).

The changes in shape of the mortality curves for fleas from 'bad' environments, with less than 30% humidity, to 'good' environments of above 80% humidity, are similar to the historical changes in the mortality of human populations undergoing socioeconomic development. For example, the demographic data for India over the last 100 years is expressed as a series of curves which shift from the 'inflection type' to the 'rectangular type'.

No conclusions can be drawn from the shape of mortality curves about the rate of ageing or the processes involved in ageing. The curves merely give comparative information about the degree of age-dependence of deaths when one or more populations are compared in space or time. On the other hand, a 'function curve' obtained by plotting, for example, the rate of a physiological process against age of the individuals in which it has been measured, provides precise information about the rate of deterioration of a process, which may be important in contributing to mortality. For example, most physiological functions decline steadily at the rate of about 10% per decade after the age of 30.

Population Dynamics

Demography is the numerical aspect of population structure. A population has a size that depends on the operation of many factors, not all of which can be

described mathematically. These factors begin to affect the numbers of individuals under certain circumstances, may limit population growth or existence under other circumstances, or may have a steady influence at all times.

There are five basic types of numerical change in populations: input from births and immigrations; output from deaths and emigrations; and changes of total size.

As organisms age their biological attributes change as, for example, when they become sexually mature, and thus the distribution of ages in a population affects the attributes of the whole population.

HISTORICAL TRENDS IN LONGEVITY

The importance of environment for ageing is highlighted by the fact that during the last 100 years the proportion of old people in all countries undergoing social and economic development has steadily grown, whilst that of the young has considerably decreased.

Such a global trend does not occur because the majority of old people live longer; it is due to the historical declines in marital fecundity, deaths from perennial disease and warfare and infant mortality. The decline in early mortality is generally thought to be the most important. With improvements in medical care more and more babies have been able to survive the environmental hazards of early life which often proved fatal to a high proportion of infants in past generations. Although mortality has also decreased in the old-age groups it has not been on the same large scale as in the younger groups i.e. some old people live longer, though not the majority of them.

The data in Table 2.2 refer to the situation in Britain over a period of about 40 years from 1911 to 1953 as defined by official population statistics. The proportion of old persons 75–85 years and older was about 40% higher in 1951 as compared with 1911.

The experience of insurance companies points in the same direction. For example, over a similar period the death rate amongst policy holders of the Metropolitan Life Assurance Company decreased in men and women aged 55–64 years by 41 and 61% respectively, and in men and women aged 65–74 years, by 39 and 54% respectively.

This is a general phenomenon of prolongation of lifespan in the developing world, and is not associated with a reduction in degenerative abnormalities in the elderly. In this respect it has been said that some years are added to their life but not life to their years. Therefore ageing has become a world-wide social and economic problem of the greatest magnitude.

This situation was foreseen in the mid 1940s. Hopkin, in 1947, on the basis of changes of population in England and Wales, forecast that by 1989 the number of old people of pensionable age would equal the number of children, and that

there would be a drop in the number of persons of working age (defined by the age range 15–64 for men, and 15–59 for women) of approximately three millions compared with 1946. At this same time the British Registrar General forecast that a critical situation would occur even earlier, namely by about 1971. Benjamin's computation (from the data in Table 2.2) in the 1950s demonstrated a similar trend. He calculated that the people over 65 would increase from 1094 to 1406 per 10 000 of total population. It was pointed out by the British pioneer gerontologists Korenchevsky, also in the 1950s, that this situation would be very grave for every nation and every Government 'because a great number of helpless and useless old people would become a heavy burden on the younger working population'. Most of these predictions are now being realized throughout the world with very little in the way of advance economic planning to cope with the change in the age distribution resulting from development.

Environmental Factors

These historical changes in the structure of populations are linked with trends in the death rates from various diseases. One of the first computations revealing this phenomenon was made by Benjamin in 1954 using the data of the British Registrar General's Office. He found that in 1952, as compared with 1901–1910, the following changes occurred in the death rate (per million of population), which could not be explained by population changes.

There was an increased death rate from cancer (+306), heart disease (+1061) and other circulatory diseases (+206). Decreased deaths were found for tuberculosis (−1454), pneumonia (−1000), other respiratory diseases (−1848), premature births, birth injuries and congenital malformations (−286).

Table 2.2. Population of England and Wales (1901-1951)

Age intervals	1901	1911	1921	1931	1939	1951
0	1143	1069	877	749	690	850
5	2099	1995	1895	1635	1415	1375
15	1953	1805	1756	1734	1592	1283
25	1616	1651	1520	1605	1671	1446
35	1228	1344	1411	1368	1465	1538
45	892	978	1167	1235	1244	1369
55	597	637	769	932	1026	1045
65	331	377	434	536	646	742
75	121	126	151	182	225	309
85	15	18	20	24	29	43
All ages	10 000	10 000	10 000	10 000	10 000	10 000

Taking the North American life expectancy as a population mean, where there is likely to be a maximum expression of genetic potential and maximum environmental protection, it would be expected that other populations would be found with a lower proportion of individuals living beyond the eighth decade. This is indeed the case, and the risk of dying between the ages of 45 and 65 also varies substantially from country to country, and within large countries. For example, between 1962 and 1964, out of 20 countries compared with the United States, 17 had death rates in the age group 45–64 that were lower than in North America. Between the ages of 65 and 74, 10 countries had lower death rates, whilst between the ages of 75 and 84 only six countries had lower death rates. The reasons for these differences are not known with any degree of certainty but when a generous allowance is made for environmental differences there is still room for genetic explanations. With more detailed investigation of demographic data, geographical patterns emerge of differences of death rates. One of the first investigations in this direction demonstrated that in the United States, the highest rates from all causes occurred predominantly near the East Coast, but equally high figures came from states very far removed from this area, such as Nevada and Hawaii. In general, the areas with the lowest rates were concentrated in the West Central and mountain areas. These geographical differences in death rates increase with age of cohort.

Marked geographical differences are also found in death rates from various diseases. Early work in the United States showed that deaths from cardiovascular and renal diseases were lowest in the West Central areas. In general it appears that high death rates from these diseases are associated with city development. There are also geographical differences in the age-incidence of malignant growths, which are now thought to be largely environmental in origin. Deaths from these three causes tend to be correlated in the United States although this is not always found in other countries.

At a local level, rural farm areas have the lowest statistical death rates from all causes. Here, another causative factor may be low population density. In this respect, historical analysis of burials in relation to population size indicates that the proportion of people in isolated rural communities who survived into the late 60s and 70s is broadly related to the local population density during their lifetime.

Other correlations that meet standards of statistical significance are for deaths from cardiovascular and renal disease to be linked with regions of high rainfall and low elevation. High fluctuation in mean January temperatures has been linked with low death rates from cancers, apart from lung cancer. Such correlations are interesting but almost meaningless in defining the mortality factors at work, and are little use in devising practical methods to reduce the death rate.

Centenarians

At present, the usual span of life in North America and Europe ranges between 65 and 75 years, but a few old people reach ages of 100 years and over. This phenomenon is illustrated for changes in the number and age of centenarians from 1900 to 1953 in England and Wales from the Registrar General's data (Table 2.3).

Although the census data relating to centenarians are collected as carefully as those for other age groups, the returns from the very old are not very reliable from the point of view of drawing scientifically valid conclusions as to geographical differences in ageing. In many parts of the world, registration of births may not have been mandatory in the last century. If certificates do exist they are usually not examined. Bearing in mind the likelihood that long-lived survivors will tend to exaggerate their ages, and couple this with possible poor memory, and lack of paper records, the claims for very old age may be uncertain, particularly with regards ages exceeding 115 years.

In spite of this difficulty, results for a range of countries show that the usual age of centenarians is below 107 and people are very rarely found over the age of 110–115. The American demographers, Pearl and Pearl in the 1930s critically checked all the evidence as to the date of birth of nonagenarians and centenarians who were living at the time of record and whose records were used for the investigation. Moreover, records of their immediate ancestors were also examined to reveal the importance of having nonagenarians or centenarians in the previous generation. In this way the Pearls selected 52 people aged 100–104 years and 7 aged 105–109 years for their investigation. They also found three living centenarians 111–112 years old and one who was 113 years of age. The age of the oldest centenarian recorded, 113, is about the same as that given in the census records of most countries down to the present.

The longest lifespans recorded in Holland, Switzerland and Sweden during the period of observation were 106, 105 and 107 years respectively. In Finland,

Table 2.3. Average number of centenarians in England and Wales 1900–1953

Period	Number of centenarians (per 2 million of population)	
	Men	Women
1900–1909	1.8	4.4
1910–1919	1.9	5.0
1920–1929	2.2	6.1
1930–1937	1.8	7.1
1938–1945	1.8	8.3
1946–1953	2.5	11.1

Japan and Spain it was 110 years. In Portugal four centenarians claimed to be 123–124 years old, two 125–127 and one 130 years old.

Today, most human demographers would agree with Ernst, who, in 1938, after critically analysing all the literature on centenarian cases with the greatest longevity, recorded by different authors, and investigated by himself, concluded that very few trustworthy cases existed for people reaching a great old age. He found that of two centenarians who lived over 110 one was nearly 112 and one over 113 years at death. Backman in 1945 also concluded that 113 years appears to be the longest span of human life known.

Since these studies were undertaken a few claims have been made for much longer-lived individuals in specific parts of the world, such as isolated mountain communities in Ecuador and the southern republics of the USSR. These claims of lifespans over 115 years suffer from problems of reliability in checking statements of oldest inhabitants. Nevertheless careful statistical analysis of the population data of different countries indicates that maximum lifespan does vary geographically, but perhaps not so much as is sometimes reported by journalists. Birth records are particularly suspect in remote areas, but it does appear as if the exceptionally long-lived survivors invariably occur in small isolated communities where lifestyle and the social role of the elderly favour long life. Often the aged in these communities have a high social status. This together with the simple diets that characterize these communities opens up possibilities that unique environmental influences, such as diet and individual social well-being, produced the extended lifespans.

In summary, although it is often difficult, or even impossible, to check claims beyond the age of 120 years, there can be no doubt from reliable demographic records that some human populations have a much higher proportion of individuals over the age of 80 than is currently found in Britain and North America. However, the number of these extra 'long-livers' is still only a very small fraction of a given birth cohort.

It is also clear from many demographic studies of centenarians that the proportion of females considerably exceeds that of males. For example, in an early British study carried out by Korenchevsky, even during a period of observation as short as 50 years, the number of centenarians was detected as steadily growing in the case of women, although less definitely in the male population. The census data obtained in other countries agree in most cases with these British conclusions regarding the longer lifespan of female centenarians.

An important question from this work relates to the possibility of extending the maximum span of human life to the exceptional maxima claimed by a few individuals in census records. Most of the early investigators were very sceptical as to the value of these data as being indicative of possibilities for extending average human life well beyond the average of developed nations. Warthin, as early as 1929, went as far as concluding that it is neither possible nor probable that the average length of human life could be raised to the heights

prophesied by over-zealous advocates of life extension.

The opposite viewpoint was taken by Korenchevsky who felt that there was a potential biological process for expanding human lifespan that takes many possibly hundreds of years. According to this view, old age was due to abnormal pathologies and that it is 'impossible to find at present a human being who ages physiologically'.

The current attitude is that present day maximum lifespans in the developed world are physiological maxima. This stresses that they probably depend more on genetic endowment of the human species than on environment. Heredity should therefore be considered as a major physiological cause of longevity. The area of debate surrounds the question as to whether simultaneous pathological ageing overlaps a normal 'physiological ageing', obscuring its processes, and may be limiting our attainment of a much greater proportion of centenarian lifespans. In support of this view, despite the historical increase in mean lifespan, the centenarians of today suffer as did their ancestors from the same pathological senility, with all its degenerative abnormalities and diseases. In other words progress in medicine and hygiene has resulted in the prolongation of life by improving medical treatment and environment, but has not yet produced any favourable change in tissue degeneration of the elderly. From this perspective, if gerontological research aims to add life to years and not just years to life it should not only be directed towards the correction of degenerative abnormalities and diseases arising from present day environments, but also to those physiological systems responsible for the maintenance of youthful structures and accurate physiological systems.

Sources of Variation in Time of Death

Expressions of ageing that are not regarded as diseases, such as cosmetic changes, and many other less obvious alterations that occur in the function of all parts of the body, are not an accurate index of chronological age. Intermingled with non-fatal changes strictly related to time are other changes, of equal, or greater, importance, associated with individual genetic make-up and specific environmental factors and organ processes. We have seen that these relate to the specific pattern of life of the individual, such as the quality and quantity of nutrition, amount of exercise, mental stimulation, exposure to infection, noise, and toxic chemicals, which all influence individual health.

Most studies with the aim of analysing mortality through the examination of population statistics ultimately reveal the role of poor environmental conditions in shortening lifespan. For example in a study of mortality of Belgian coalminers in the 1940s it was found that about 60% died before they reached the age of 50. The causes of death were: tuberculosis 66.5%, acute pulmonary diseases, 15%, cardiovascular diseases 15%, and other causes 3.5%. Coal miners also died

earlier than other workers in metal factories, and those who worked in the open. There are several harmful environmental factors, in addition to hard work, which affected coalminers of that period, such as a high incidence of tuberculosis, atmospheric coal dust, and the unhygienic conditions of subterranean work associated with a lack of proper ventilation and light and water drainage.

The prospects for a long life have considerably increased for European miners during the last 50 years. This has taken place through improvements in the underground environment in which they have to work, and the socioeconomic improvements in the home environment.

Generally, economic factors are interwoven with the expression of population statistics. One of the pioneering works on the socioeconomic aspects of the demography of disease was carried out by Hersch in the 1920s. He used the official statistics for Paris to investigate the effect of poverty on the death rate, in particular on that of infants during the first year of life, and for all ages on the mortality from tuberculosis. He classified and divided 20 districts of Paris according to their economic position as indicated by the proportion of poor inhabitants exempted from personal property tax. Death rate from all causes was definitely proportional to the economic state of the districts. When the richest district of Paris was compared with the poorest, the infant mortality rates per 1000 livebirths were about 3 and 15 respectively, a proportion of 1:5 in favour of the rich. Death rates from tuberculosis per 10 000 of population were about 11 in the richest district and about 64 in the poorest, a proportion of 1:6 in favour of the rich.

In 1921 Stevenson studied the same problem of the relation of poverty to longevity in London, and compared his own results with those of Hersch in Paris. Stevenson's results were much less striking but indicated the same trend. Thus, in London, the death rate from all causes in the wealthy districts was about 13 and in the poorest about 17. The respective values for infant mortality were 100 and 128, and for deaths from tuberculosis, 1.4 and 2.2.

In the USA in the 1940s the working environment was clearly influencing death rates. In four groups of the male population, professional men, skilled, semi-skilled and unskilled workers, the respective death rates per 1000 increased in rank order as 7.0, 8.1, 9.9 and 13.1. The relationship between the per capita income and mortality rate in 92 cities in the USA which had 100 000 population or more indicated, as would be expected, (Table 2.4) that mortality also varied inversely with personal income.

Dividing the mortality data into 15 'broad diagnosis groups' each group containing different special diseases reveals the connection between poverty and mortality from respective diseases. The 'diseases of poverty' were infectious diseases, influenza and pneumonia, tuberculosis, appendicitis, syphilis, pellagra, chronic cardiovascular problems and nephritis, diseases of the throat, nose and ear, and hernia.

It is sad to see that, after a steady global decline in mortality from

Table 2.4. Per capita purchasing income and mortality in the USA (1939-40). Mean data for 30 cities

Income group	Dollar income	Deaths Total*	Infant**	Maternal**
Lowest	668	1211	47.9	4.3
Middle	789	1097	41.1	3.1
Highest	918	1092	37.5	3.2

*per 10 000 age-adjusted population
**per 1000 live births

tuberculosis, since the mid-1980s death rates have been increasing, particularly in the developed world, where the disease is once again correlated with poverty and poor living conditions. Currently, TB is the world's major fatal infectious disease.

The other aspect of the working environment that could influence lifespan is work-load. This aspect was explored statistically by Pearl using British population data for 1910-1912. He divided persons into five groups according to the magnitude of physical labour performed by them in ascending order from light work to the heaviest work (Table 2.5).

From the age of 40 there is no difference in the rate of mortality between persons in the two groups. Thereafter there is a direct and definite relation between the magnitude of the age-specific death rate. One problem in the interpretation of this trend is that no account was taken of differences in nutrition. That is to say malnutrition is an important factor in shortening longevity in all age groups and the result may be an expression of the fact that

Table 2.5. Differences in male mortality in relation to work load

Age groups	Differences* between groups 'light' work minus 'heavy' work	
	Indoor occupations	Outdoor occupations
15–19	0.15	0.56
20–24	−0.23	0.19
25–34	−0.32	−0.13
35–44	0.25	0.19
45–54	1.56	1.42
55–64	4.84	5.82
65–74	16.09	24.79
75+	47.39	48.07

*per 1000 living persons

older people are likely to be more sensitive to malnutrition as well as the stress of work-load.

It is important to bear in mind the strong environmental influence of nutrition on population statistics. For instance, whilst the connection between plane of nutrition and longevity is firmly established, we have little idea of the relationship between human diet and lifespan.

With respect to over-feeding in humans a very large number of the data of insurance companies shows that in every age period the mortality of overweight men and women is considerably greater than that of persons with what is termed a 'normal' bodyweight. Between the ages of 20 and 64 years this 'overweight' insurance factor increases the chances of mortality by 50%. In particular, mortality from degenerative diseases of the cardiovascular system and kidneys, and from diabetes has been found to be respectively, 140, 191 and 383% higher in overweight men. Cancer incidence is also higher. One set of figures showed that mortality from cancer in people of normal weight was 111 per 100 000 which increased to about 140 in people 15 to 25% overweight. Other work has suggested that this phenomenon may be related to the particular environment chosen for the investigation.

Dieting can improve the survival chances of the overweight. Typically, weight reduction under medical supervision reduces the mortality difference between groups from about 140% to about 115%.

The statistical effects of changes in diet on a given population was demonstrated by studies on the effect of food restriction on European lifespans during the Second World War. During the war, the caloric food restrictions were rigorous but efforts were made to maintain adequate levels of vitamins. Work on data from Sweden, Finland and Norway indicates that the national state of health was never so good in these countries as during the war; in particular the mortality from arteriosclerosis, including coronary artery disease decreased considerably from pre-war levels (Figure 2.5). After the war, with the removal of food restrictions, the mortality from these diseases increased rapidly rising to, and in Sweden, even exceeding, the pre-war level.

Following this line of investigation much has been made of the study of an isolated population on the Japanese island of Okinowa. Accurate birth records have been kept for over 100 years and these indicate that Okinowa has 2 to 40 times more centenarians than in any other part of Japan. The Japanese authorities also have accurate nutritional data from large-scale surveys carried out since the end of the Second World War. This information takes the form of random interviews and direct experimental measurement of the foods eaten during a period of 3 days. From this data it emerges that the nutrition on Okinowa differs considerably from the Japanese national average (Table 2.6). During the 70th decade the incidence of deaths from cerebrovascular disease, cancer and heart disease is about 60% lower than elsewhere. Thus, at the very least, this information backs up the earlier European research with regard to the

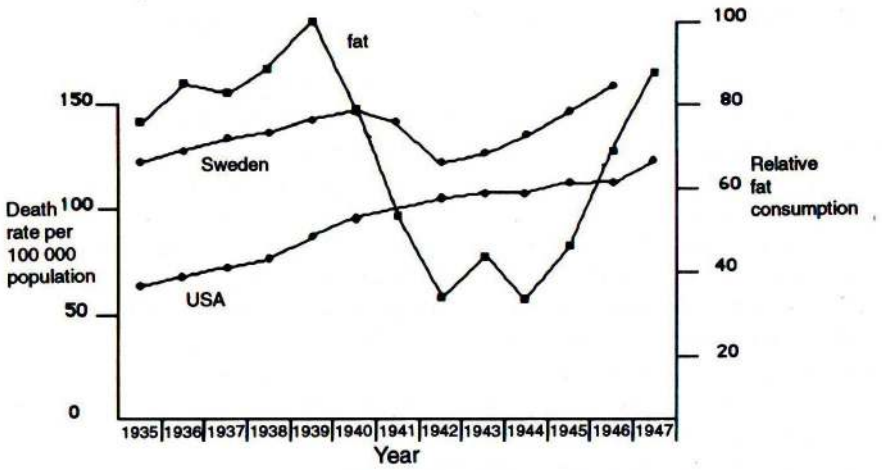


Figure 2.5. Mortality rates* and fat consumption in Sweden 1935–47.

Table 2.6. Differences in diet between the population of Okinowa and the Japanese national average

Nutrition	Percentage of mean
Total energy	
Children	62
Adults	80
Sugar	25
Cereals	75
Vegetables	300
Fish (meat)	200
Death rate 60–64 years	59

harmful effects of a high plane of nutrition, although it does not throw much light on the origins of ageing.

To summarize, many early studies on human populations pointed to a wide range of environmental features that affected lifespan. In particular, diet, population density, occupation and working conditions, and socioeconomic status were found to be correlated with geographical and local differences in life expectancy. All of these findings were borne out by later work and currently there is no doubt that differences in individual variations in mortality in all countries, particularly those of the developing world, are still strongly influenced by these environmental variables.

EXPERIMENTAL STUDIES ON DIET AND LIFESPAN

We have seen that human lifespan and patterns of degenerative diseases are both bound up with a complex set of genetic and environmental variables, which interact at the cellular level in biochemical regulators governing tissue form and function. These have yet to be clearly defined. From this point of view the outlook for a practical breakthrough of clinical relevance is bleak indeed. However, amongst the mass of mostly indirect data, one line of enquiry stands out as being of relevance to future advances in our understanding and control of the diseases and disabilities of human ageing. This is the statistical finding that human ageing appears to be connected with diet. It is an important connection because diet is a relatively easy thing for all of us to manipulate if we have a clear perspective of any advantageous outcomes. Unfortunately, the basis of the influence of diet in humans is not so clear cut as it is in experimental animals.

The laboratory evidence on mammalian species is very clear, that intermittent starvation, or a diet restricted in calories but adequate in all other respects extends longevity of laboratory rodents. This research viewpoint is related to one of the first general theories of ageing proposed by Rubner in 1908. Rubner's theory was based on the observation that longevity differences between species seems to be inversely related to the rate of metabolism. The idea was that lifespan is not related to the fundamental organisation of the body but is imposed by the rate of metabolism. In agreement with this, research in the 1920s stressed the relationship between longevity and energy expenditure. This line of investigation was put on a sound biological footing by Northrop in 1917 who showed that lowering the environmental temperature of *Drosophila*, which decreases their metabolic rate, prolonged their life, while raising the temperature had the opposite effects. Thus at temperatures of 10°, 15°, 20°, 25° and 30° the total duration of life of *Drosophila* was 178, 124, 54, 39 and 21 days respectively.

Thus, from research on poikilothermic animals the idea has developed that the length of life decreases if they are kept in warm environments and or if they are liberally fed. From these two aspects it appears that longevity is inversely related to the opportunity for growth. With respect to the human situation the relationship might be relevant to an above normal rate of ageing being returned to normal by a decrease in food intake from the adlib situation of the developed world. On the other hand, it might be that dietary restriction stimulates a general anti-ageing system to reduce the rate of normal ageing. In either case, any theory of ageing has to explain the well-established effects of diet on the longevity of laboratory animals. It is one of the few repeatable experimental manipulations of lifespan and deserves much wider attention in the biological sciences.

Research into the effects of dietary restriction began at the turn of the century. It was nourished by the physiological ideas of Benedict, Northrop and

Robinson, who were interested in the connections between diet, development, fertility, and longevity. This probing was orientated towards ageing by McCay's work in the 1930s, and consolidated by Tannenbaum in the 40s and Ross in the 60s, who showed that dietary restriction not only extends the lifespans of laboratory rodents, but also changes their patterns of degenerative diseases. It is probably true to say that exploration of possible mechanisms has been mainly at the level of the mammalian neuroendocrine system, exemplified by the work of Meites and Timiras in America, Everitt in Australia, and Merry in the United Kingdom. Walford and Weindruch have extended this mammalian perspective to include immune reactivity and oxidative metabolism.

McCay, Tannenbaum and Ross, are the trio who put the mammalian model on a firm experimental footing. However, alongside the work on rodents there was, from the beginning, a much broader biological research stream, initiated by the work of Ingle Kellogg and Bell in the 1900s on the relationship between diet and the 'clock' of insect development. It led to the experiments of Dunham in 1938, who found that abundant food followed by restricted feeding extended longevity in waterfleas. After the Second World War gerontology developed as a specialism related to medicine, and the broad biological base was lost. Only in the last decade has the unitary evolutionary approach to ageing been taken up once more, particularly by the British workers Callow and Kirkwood.

It can be argued that any breakthrough relevant to the control of human ageing will only come when dietary restriction regains contact with its biological base. Actually, we have to rely on a biological and evolutionary focus to make the case that dietary restriction will extend human longevity. The likelihood that it will work in humans is simply because it does so in at least five different orders of the animal kingdom. If we try to present the case for the applicability of dietary restriction to human development we have to cope with valid, although unhelpful statements of fence-sitting academics such as, 'I can tell you that dietary restriction will work in rodents. I can't tell you it will work in humans'. In the absence of clinical trials, this remains the ultimate critical position, and people are left to act individually upon inherent biological probabilities. If, as seems likely from the laboratory work, dietary restriction affects lifespan universally at the level of oxidative metabolism, we should be exploring the pharmacological potential of there being intracellular phenotypic switches that divert a larger proportion of resources towards organelle surveillance and repair. This is just as likely to be revealed in fruitflies, waterfleas and cell cultures as in mice and men.

GENETIC FACTORS

Much of the early work on the factors determining human lifespan was carried out by the Pearls who were pioneering demographic analysis in the 1920s.

They clarified the span of life in terms of two groups of factors, the local environmental factors and the genetic factors. The genetic factors determine the morphological and functional constitution of the organism, and in a wide range of animals these have been statistically related to the two species' characteristics of 'average rate of metabolism', and the 'average rate of energy expenditure during life'.

Genetic factors predetermine the potential maximum span of life for every species, and, to a greater or lesser extent, the actual length of life for individuals within the species. Environmental factors may considerably reduce the length of life in a local context compared with its potential genetic value. The balance between the two groups of factors, genetic and environmental, in fixing the lifespan of an individual, differs between species and between environments within a species. When the literature on heredity and human longevity is carefully reviewed and critically analysed there is no doubt that lifespan is strongly influenced by heredity in all living organisms. For example, short-lived and long-lived lines of flies can be produced by selective inbreeding, and many experiments have shown the inheritance of longevity in mice.

In humans the evidence for the genetic influence has to come from demographic data. Pearl and Pearl first approached the problem by collecting information on two parents and four grandparents to define the 'total immediate ancestral longevity' (TIAL) of two groups of people. A long-lived group (LG) contained 365 persons. In this group at least one of the offspring stemming from six 'immediate ancestors' reached the age of 90 or over. The 'comparison group' (CG) was selected more or less at random without special attention to age, and consisted of 143 persons. Not one of the offspring in this group reached an age of 90 or over, but their six immediate ancestors were also known and investigated. The main results are presented in Table 2.7.

Roughly one half of all individual matings (46–57%) of the LG nonagenarians

Table 2.7. Parents and offspring from a group of nonagenarians and centenarians compared with a group with no longevous individuals

	Percentage of matings in groups of different longevity					
	Long-lived group			Randomly selected group		
Numbers of long-lived parents	None	1	2	None	1	2
Matings producing:-						
Fathers	10.5	32.9	56.7	23.8	39.2	37.1
Mothers	8.2	38.4	53.4	26.6	43.4	30.1
Their offspring	13.4	40.8	45.8	57.4	30.8	11.9

and centenarians were composed of two long-lived persons for two generations back. The respective figures for CG subjects varied from only about 12–37%. Only one tenth (8.2–13.4%) of LG matings which produced nonagenarians and centenarians for two generations back, had not been composed of long-lived persons. For CG matings this proportion varied from 23.8–57.4%. The production of long-lived fathers, mothers and offspring clearly depended markedly on parental longevity. For LG subjects about 13% of nonagenarian and centenarian offspring were bred from matings in which neither parent was long-lived and the same fact was observed in about 10% of their fathers and 8% of their mothers.

These findings led the Pearls to conclude that extremely longevous persons may be bred in a small but not negligible proportion of all matings. They also suggested that longevity may reasonably be regarded as a single numerical expression of the integral effects of all the innate and environmental forces that operate upon the individual.

Korenchevsky, in his review of the data, felt that it is important to note that nonagenarians and centenarians were characterized by having long-lived parents in only about 50% of cases. He took this to mean that in about half of the cases a strong hereditary factor can be neutralized or overcome by some other factors, presumably specific environmental ones, which also participate in the determination of longevity.

Results similar to those of the Pearls can be obtained through investigating the data of insurance companies. Insurance data are especially valuable because the birth dates are carefully investigated. For example the mortality of policy holders, both of whose parents lived to be 70 or over, is about 30% less than that of policy holders both of whose parents died under the age of 60.

Other supporting data comes from pedigree analysis. Nollenburg's observations on inbred members of a German religious sect showed an hereditary longevity relationship not only between parents and children but also between uncles or aunts and their nephews or nieces (Table 2.8) although the latter effects are comparatively weak. The hereditary effect of mother or aunt appears to be more pronounced than that of father or uncle.

The latter relationship emerged from a study of the family records of Finnish and Swedish middle class and nobility by Jalavistos. This investigation also showed a stronger hereditary influence of the mother as compared with that of the father (Table 2.9). The expectation of life was calculated simply as a mean of the age at death.

A more direct approach to the genetics of ageing is to analyse data on twins; monozygotic twins conceived from the same ovum of the mother, and dizygotic twins produced from two different ova of the mother. Twin studies have revealed hereditary effects in that various physical and functional characteristics are closer in monozygotes than in dizygotes (Table 2.10).

In some pairs the similarity in longevity was striking: monozygotic twins in

Table 2.8. Longevity of parents in relation to age of their children

Age of children at their death	Ages of parents at death in relation to age of children			
	Father		Mother	
	Sons	Daughters	Sons	Daughters
20–30	—	—	52.6	55.5
30–40	63.0	63.3	58.4	59.0
40–50	50.6	56.6	57.1	58.5
50–60	55.5	56.3	62.0	56.8
60–70	62.4	56.7	60.4	55.4
70–80	63.6	59.0	63.3	62.5
80–90	63.3	62.2	63.4	61.9
over 90	63.5	59.7	73.7	69.4

Table 2.9. Life expectancy of children in relation to age of death of parents

Age at death of parent	Life expectancy of children					
	less than 50		50–69		over 70	
	Sons	Daughters	Sons	Daughters	Sons	Daughters
less than 50	36.5	41.0	41.5	44.0	44.5	45.1
50–69	39.0	40.2	42.0	43.9	45.0	46.8
over 70	42.0	40.5	43.5	44.1	48.0	46.2

Table 2.10. Intrapair differences in longevity in 58 twin pairs

Age groups	Average intrapair differences in longevity (months)					
	Monozygotes			Dizygotes		
	Males	Females	Total	Males	Females	Total
60–75	47.6	24.0	42.3	107.9	88.7	103.1
All over 60	47.6	29.4	36.9	89.1	61.3	78.3

one pair died on the same day from natural causes at the age of 86. In two other pairs, which differed considerably in their social and marital histories, deaths occurred 5 and 25 days apart respectively at the age of 85.

Many measurements such as physical resemblance, results of psychometric investigation, the predisposition or resistance to involutional, or senile psychoses, and marital reproductivity, showed that monozygotes were more similar than dizygotes.

Vogt's studies on pairs of monozygotic twins, 55–81 years old, revealed a striking similarity in each pair in greying of hair, baldness, tooth defects, detailed structure and pathological changes of eyes, decrease of hearing, and in mental disturbance. In some pairs some of the above mentioned abnormalities were absent or slight in both twins.

PARENTAL AGE

For hereditary studies the age of mother and father has to be recorded at their death; on the other hand, for the study of parental non-hereditary influence, the parental age must be considered at the birth of their children.

Jalavisto, using Finnish population data, observed a definite decrease in the longevity of the progeny with increasing maternal age. Others have studied the effect of the mother's age on the longevity of her progeny at birth. The results of all these investigations show that the older the age of the mother the greater the incidence of stillbirths, premature births, and the neonatal mortality rate. In particular, abnormal births occurred in a greater proportion of mothers under 17 than in those in their late 20s. (Table 2.11). The paternal influence on the longevity of progeny is negligible in comparison with these maternal age effects.

The effect of maternal age is indirectly shown in the frequency of mongolism and malformations involving various parts of the body. An investigation of 545 cases of mongolism by Penrose revealed that the incidence rose rapidly with maternal age (0.23–0.25 for children born from mothers aged 15–29; but 0.71, 2.2, 8.29 and 20.8 for children of mothers aged 30–34, 35–39, 40–44 and 45, respectively. This increase is also seen for all malformations, major and minor (Figure 2.6).

BIRTH ORDER

A pioneer investigation of the influence of birth order on ageing in 1606 pairs of sisters and 2246 pairs of brothers was carried out by Beeton and Pearson at the turn of the century. This showed that, compared with their younger siblings, the elder brothers lived, on average, 4.6 years longer and elder sisters 3.2 years longer Another approach to birth order effects concentrated on records of premature and neonatal mortalities and stillbirth ratios. This work showed that abnormal births and neonatal mortality analysed by order of birth showed the same trend as that ranked by maternal age. Since elder children are born from younger mothers it is difficult to determine the effects of birth order from maternal age but the workers in this field feel that both factors are active and important in producing the result.

Table 2.11. Stillbirths* in relation to mother's age in England and Wales (1949)

Number of children	Median age of mothers							
	17.5	22	27	32	37	41	45 +	all ages
1	17.2	17.7	23.5	35.3	47.2		58.2	23.3
2	11.6	12.3	13.3	17.4	23.7	39.5		15.5
3		14.6	14.9	18.0	26.0	34.6	58.0	18.7
4	17.3	17.1	25.4	28.3	49.1	75.6	25.3	
5			20.2	23.9	34.9	49.1	87.2	29.8
6			25.0	28.1	39.6	55.3	59.2	37.2
7			18.8	25.5	34.5	51.9	81.5	35.3
8				29.6	33.8	51.4	70.4	39.0
9				35.3	37.6	53.4		44.3
10 +				49.2	45.1	57.4	90.0	54.7
All parities	16.7	16.1	17.9	23.4	31.5	47.6	68.9	21.5

*per 1000 live births

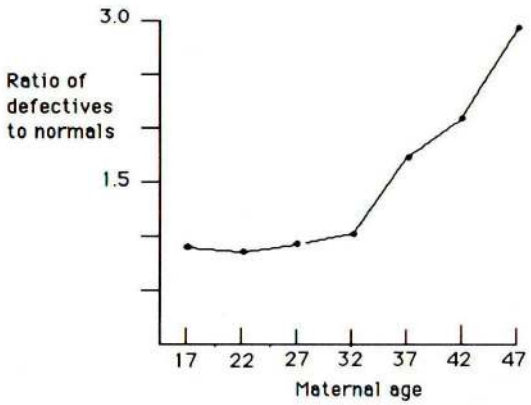


Figure 2.6. Proportion of malformations in children in relation to mothers age.

MARITAL STATUS

The socioenvironmental influence on ageing emerges when death rates are analysed according to marital status (Table 2.12). In a study of American figures, the mortality of single persons, and especially of the divorced and widowed, was definitely higher than those of married people.

Taking the ratio of mortality in the married group as a control, they obtained the following respective comparative mortality ratios for specific causes of death in single, widowed and divorced males represented sequentially as follows: tuberculosis, 2.63, 4.3, 3.30; diseases of the heart 1.34, 1.67, 1.93; pneumonia and influenza 1.82, 2.49, 2.40; cirrhosis of the liver, l.81, 2.26, 3.39; ulcer of the stomach or duodenum, 1.48, 1.63 2.56; nephritis 1.25, 1.89,1.67.

It has been shown in previous sections that hereditary and non-hereditary factors may be instrumental in an increase or decrease of longevity, depending on various quantitative and qualitative changes in these factors or in their operation in the organism. There may be good or bad heredity; young paternal

Table 2.12. Comparative death rates in the United States in the 1940s

	Relative death rate (set at 100 for married people)			
	Whites		Coloureds	
	Male	Female	Male	Female
Married	100	100	100	100
Single	140	117	155	143
Widowed	173	135	214	149
Divorced	218	174	203	167

age tends to prolong the span of life of the offspring, while older parental age usually shortens it; environmental agents may be favourable or unfavourable to the prolongation of life.

It is obvious that all those hereditary and non-hereditary factors which shorten longevity at the same time result in age-dependent phenomena which are characteristics of growing old. From the point of view of alleviating the undesirable aspects of ageing there is a basic difference between the nature of hereditary and non-hereditary factors. The former are in the main, unavoidable, indispensable, and, as far as we know at present unchangeable causes of ageing. For example, it is impossible to change the intensity of metabolism inherent in each species, although to a certain very limited extent this intensity can be regulated by exposure to cold or warm temperatures, by a limited or liberal feeding, and by the amount of physical activity. From this point of view we may say that hereditary factors of ageing belong to a group of basic primary causes of old age; they are physiological, i.e. normal for the organism, although they are able to finally bring about death of the organism. Presumably they are present in all individuals to a greater or lesser extent. For example, many older mothers give birth to normal children indicating that the basic rates of ageing of human ova can vary considerable between individuals.

Non-hereditary factors of longevity are the secondary causes of ageing, i.e. causes which may or may not be present in the organism. They are not necessary features of the morphological chemical and functional structure of the body; their main characteristic is that they are accidental and therefore unavoidable. For instance, not every subject is born from older parents, or is one of the last born children in the family. Hence, it could be that in any population which chooses to reduce the number of births to older mothers there would be a reduction in the number of stillbirths, neonatal mortality malformations, and mental and physical deficiencies.

GENETICS AND ENVIRONMENT: THE ATHEROGENESIS MODEL

Ageing and atherosclerosis are two closely interwoven complex processes. There is a close correlation between age and atherosclerosis which has been explained by the insidious prolonged course of the disease and hence the time required for its clinical expression. However, atherosclerosis is not simply the result of an intrinsic biological ageing process because most mammalian species and many human populations age without spontaneously developing heart disease.

Human atherosclerosis is a multifactorial, time-related process, in which the outcome is influenced primarily by genetics, and secondarily by pedispositions to many secondary risk factors expressed in individual characteristics such as serum lipids, blood pressure, secretion of sex hormones, behaviour and lifestyle.

Table 2.13. Decline in death rates from coronary artery disease (1968-78)

Age group (yrs)	Whites		Blacks	
	Male (%)	Female (%)	Male (%)	Female (%)
35–44	36.2	36.2	35.4	53.1
45–54	27.2	24.9	27.3	37.6
55–64	26.2	25.5	22.6	35.8
65–74	24.6	30.6	27.1	34.2

In particular, leisure-time exercise patterns are strongly related to mortality from all cardiovascular disease which accounts in some groups in the United States for 45% of all deaths.

It has been found that changing diet, reducing cigarette smoking, and treating hypertension all affect the relationship between ageing and atherosclerosis in American society. The outcome of medical and social campaigns on a mass scale have been to reduce the incidence of cardiovascular disease that peaked in the early 1960s. However, these behavioural changes appear to defer the onset of atherosclerosis which remains a major cause of death in middle and old age (Table 2.13).

The model of the causes and effects and progression of the pathogenesis of coronary heart disease is set out in Figure 2.7.

Atherosclerosis usually develops in our environment because of relatively slight but continuous elevation of low density lipoproteins in the plasma. This leads to progressive growth of the plaques, due to stimulation of smooth muscle cell proliferation and the increased deposit of intracellular and intercellular low density lipoproteins. Plaques develop slowly and insidiously over many years. and they generally progress from a fatty streak to a fibrous plaque, and then to a complicated plaque that is likely to lead to clinical effects as it blocks the lumen of the artery. It is evident that this process starts very early in life and is augmented by damage to the endothelium, high blood pressure and increased levels of cholesterol, and cell wall interactions which are not yet clearly understood. The pathogenesis of atherosclerosis is defined fundamentally as the interaction between elevated levels of low density lipoprotein and arterial endothelial injury to produce a progressive atherosclerotic plaque. However, as the pathogenesis proceeds from the genetic predisposition, inputs from the home and working environment, through physiological changes, to the terminal clinical problems, there are many points for potential deviation from a standard pattern. The various elements are not to be found in any one individual, but they will be common to a susceptible population, with some more prevalent and hazardous than others. At any stage, a change in individual habits can divert the path away from, or towards, a particular end point category.

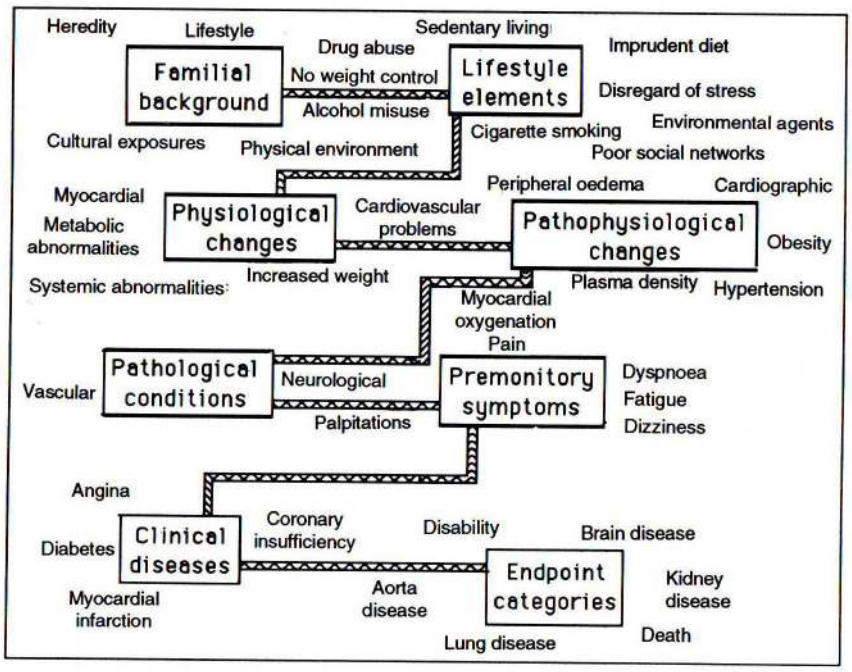


Figure 2.7. A model of the progressive development of coronary heart disease.

We know much about the interaction between smooth muscle cells and plasma lipids, about the environmental factors which increase the chances of a heart attack, and the various actions necessary to reduce this probability. The model is therefore very detailed, but in principle it will stand for the multifactorial development of any degenerative disease. In particular, the dramatic reduction in deaths from heart disease opens up questions as to the future impact of finding cures for degenerative diseases.

As outlined above, most people in the developed countries die after the age of 65, and apart from accidental deaths, infectious diseases, and some cancers that strike the very young, elimination of any of these diseases at birth would produce the same lifespan extension as eliminating it at the age of 65. Also, elimination of a particular degenerative disease in the elderly would have little influence on life expectancy. For example, calculations have been made for the white population of the United States of probable lifespan extension from the treatment and prevention of heart disease. This health programme has been the most successful application of geratology in combating a major degenerative disease. The calculations show that compared with 1970, when the first impact of the new programme was apparent, by 2050 the expected lifetime at birth will

have risen in men from about 68 years to 71.8 years, and in women, from about 74 to 81 years.

Many feel that the relatively low statistical returns expected from combating degenerative diseases must be because these diseases are expressions of underlying biological processes which pervade all tissues, and elimination of one disease would result in death from another within a short interval of time. If this is valid it points to a general strategy of research. On the one hand, gerontology should aim to define the general basis of molecular deterioration, then control it to delay all of its pathological expressions, without prolonging the period of dependence of the elderly population on medical or social care. On the other hand, it is important to determine the environmental inputs which promote particular diseases.

'NORMAL' AND 'PATHOLOGICAL' AGEING

Korenchevsky, generally regarded at the founder of gerontology, first defined physiological ageing as a universal norm; a disease-free baseline, which was obscured by the pathological conditions of the day. His starting point was the great variability, evident from cross-sectional analysis of human ageing. This variability is seen in the weights of organs, their structure, chemical composition and function.

He felt that values in old people that are close or equal to the highest values in the young show that ageing of the respective organ or function is not a necessary and unavoidable feature of ageing, and that such an adult or young value in old individuals most probably represents a feature that was not fundamentally sensitive to ageing, i.e. it has the potential of remaining physiologically normal in the elderly.

Values which in old people are equal to, or not very far from, the average values in adults, he classified as defining the condition typical for physiological old age. On the other hand, he felt that values in old individuals considerably below the average in young adults, are far removed from the adult values and therefore represent the features of definitely pathological old age. He concluded that, 'in the future, man will not only live longer, but his youth and adult age will also be extended: this is indicated by the occurrence of 'adult' or even 'young' organs or function in the minority of old people of today'. According to Korenchevsky gerontological research would only be able to 'add life to years' when degenerative abnormalities and diseases of present day pathological ageing would be eliminated and ageing would become a physiological process. This remains a fundamental largely untested theoretical standpoint that places the origins of diseases of the elderly in their past environment.

The conceptual separation of the normal from the pathological in the elderly is still causing difficulties today. Most pathology manuals do not refer to ageing

at all, and gerontologists are still debating the relationship between medical problems and the 'normal ageing process'. There is still active discussion about the meaning of 'biological age' in relation to 'chronological age'.

In a recent conference on the relations between normal ageing and disease one conclusion was that, 'the distinction between ageing and disease is more artificial than real', another was that it is not clear 'whether the myriad of changes which occur with time represent the developing basic substratum necessary for the appearance of disease associated with the aged. . . . No one really knows.'

LIFE AS AN ERROR-PRONE SYSTEM

Despite this uncertainty regarding what is normal and what is diseased, a consensus is gradually emerging in which disease is viewed as the outcome of environment impinging upon homeostatic systems that have been selected by evolution to accept an increase in unrepaired errors. The accumulated errors eventually reduce homeostatic reserves, either generally, or specifically, in organ systems which we term 'diseased', to the point where a slight physiological disturbance is fatal.

Species longevity is inversely related to the pressures of natural selection which determines the period of development to reproductive maturity in the wild. In modern protective human ecosystems, human diseases and disabilities become increasingly common after this period because development is not designed to eliminate functional errors that arise through inadequate repair, or a 'bad' environment. Such errors are likely to have a serious impact on the assembly systems for macromolecules. The importance of random external impacts means that the damaging effects of these errors may only be revealed in certain environments. For example, some forms of cellular ageing which produce cancer are accelerated by dietary constituents.

It is imperative that gerontology should gain an understanding of the chemical durability of youthful processes, particularly as ageing is not universal. Cellular immortality is a possible evolutionary strategy and ageing appears to be the inevitable outcome of a bodily organization where self-repairing reproductive cells require the support of a relatively large mass of other cells, the soma, which cannot be replaced if they suffer intracellular damage. The soma is not directly connected with reproduction, and is therefore 'disposable'.

An understanding of intracellular protective and replacement mechanisms will inevitably lead to the future preventive and pharmacological strategies of geratology. Geratology in its search for effective treatment of diseases of ageing must therefore communicate with and draw to itself, a number of other specialist fields, such as biochemistry, genetics, endocrinology, and nutrition. In particular, an evolutionary perspective is much needed in geriatric medicine to

promote the idea that lifespan is a 'trade off' between the resources devoted to repair and those 'spent' on growth and reproduction. This brings up the idea that repair genes might be accessible to pharmacological control according to the extent that their normal activity approached 100% correction of all defects. On this view of the therapeutic margin that might become available to medicine it is assumed that there is likely to be a small number of mechanisms of ageing, which regulate investment of resources in repair.

The biological propositions underlying this approach to geriatric medicine are:

1. Ageing is the result of somatic damage arising either internally from errors in the operation of biochemical and physiological systems, or externally, from the random impact of physical and chemical factors in the environment.
2. Special mechanisms exist to keep the germ line fully repaired, or selectively to destroy damaged cells in the germ line.
3. Species with different longevities should exhibit corresponding differences in their levels of somatic maintenance and repair.
4. Different development programmes should all exhibit an inverse relationship between longevity and fecundity.

These propositions have to be related the loss of adaptability at the cellular level, in terms of a gradual loss of cells, an increasing number of bad cells, and a failure of communication between surviving cells.

There is no doubt that old people contain less cells than at the peak of maturation. This cell loss is shown directly by the decreased actual and relative weights of organs, described as atrophy or involution, and by indirect measurements of the volume of the intracellular compartment of the body.

Atrophy of organs has long been recognized as the most typical histological feature of human old age, leading to Korenchevsky's dictum that 'ageing is a major involution of the living organism'. To Korenchevsky the ubiquity of involution placed it in his category of normal ageing, in contrast to cancer, which, not being ubiquitous, should be classed as pathological ageing. Organ involution certainly occurs in a great majority of tissues, being least or absent in the heart and liver, and more or less pronounced in skeletal muscles, gonads, spleen, kidneys and bone. From their magnitude and obvious disruptive effects on organ structure these changes would, by themselves, be expected to account for the loss of adaptability to environment characteristic of old age. However, there have been no direct tests to prove it. Indeed, the apparent large cellular reserve in most organs argues against cell mass being a major determinant of longevity.

After maturity there is a large alteration in the body's water compartments connected with a decrease in the proportion of body water, a trend which seems to start at birth. It is important to note that the rate of loss of body potassium,

and therefore the rate of death of cells hardly changes from the third to the ninth decade of life. This indicates that the process of cell deletion originates long before the exponential rise in mortality rate, and mortality is not related to either cell death, or increased whole body mortality, in a simple or direct way.

CONCLUSIONS

There has been no definite case of anyone living beyond the age of 115 and centenarians are the extreme 'tail' of populations that without exception show an exponential increase in the probability of dying. Nevertheless, the existence of people who live well beyond the average lifespan has prompted speculation that the terminal cohort represents a kind of 'biologically elite'. No doubt if everyone could achieve the status of this group then indeed we could all attain the objective of a healthy happy old age.

But are they a distinct group or are they just the extreme of the normal distribution curve of health? If they are 'normal' and the majority of the population are 'abnormal', is their normality a matter of inheritance or is it associated with lifestyle? If the latter, then can something be learned by studying this group to help other people? The latter question is central to a major study area of 'demographic geratology' which deals with the separate but interacting effects of ageing and lifestyle upon the general health of the individual. As yet we have no concrete answers to these important questions. The practical problem is to integrate the obviously important, theoretical and unitary, genetic viewpoint, with the wide variety of different old human phenotypes which are categorized clinically in terms of particular degenerative diseases.

In our present state of ignorance of the details, ageing has to be defined as the sum of all of the chemical and physical interactions involving body constituents that are damaging to the organism. This damage is the intrinsic and inevitable outcome of the way in which cells and organs are built. The diseases of old age result either directly from this process of ageing, or indirectly from the chance impacts of extrinsic factors on faulty cells or organs, the probability of which increase with age. On this view lifespan is determined by the level of effectiveness of genes that either repair cellular damage or give protection against such damage. A mechanism of ageing is therefore anything that reduces the effectiveness of the macromolecular production lines and assembly systems for cellular organelles.

Although environment is important in revealing potential failures in homeostasis, the pathological expressions of ageing are due, fundamentally, to a loss of precision in the systems specifying form and function. We can define these mechanisms as chemical deterioration, physiological errors, and variability of gene expression. As far as these are also expressions of the mechanisms of

ageing, they have yet to be connected functionally to a loss of adaptability to environment due to a decline in tissues and functional reserves. There is no doubt that a whole range, maybe all, of the homeostatic systems of the body become less efficient in combating fluctuations in the external world. Deaths from ageing presumably occur when there are insufficient homeostatic reserves left to return a vital system that has been disturbed to its norm. The so-called individual expressions of 'pathological ageing' are the result of random events impinging on this failing homeostatic reserve. Many years ago Simms showed experimentally with rats that this combination is sufficient to explain a Gompertzian rise in mortality at the population level.

Since age is associated with a gradual loss of precision in the systems that make and maintain those cells which have a high rate of turnover, it is likely that ageing is an expression of faults in the working genes. The emerging consensus of the way in which genes are expressed at the biochemical level enables several potential sites to be identified where ageing could affect the pattern of tissue enzymes. Some of these potential sites are associated with the complex structure of the gene, which is a functional combination of DNA with several well-defined histone proteins, and other nonhistone proteins that are not so well characterized. Other possibilities may be defined in terms of what we know of the way in which the DNA code is expressed and regulated.

Unfortunately, all current models of working genes are tentative in that, although the main properties of DNA and its associated structural and enzymic components are not in doubt, the details of the controls and feedback systems are far from clear. In this context, work on the ageing genome awaits basic information as to its mode of operation from cells in the early stages of development. Also, it must be said that we know very little in principle about the means used by cells to detect damage to the components of the genome and the ways in which repair or replacement are undertaken. Nevertheless, despite these uncertainties, it can be said that, so far, research on ageing has not brought to light any outstanding evidence for a major disturbance to the current gene model that might be of relevance to clinical strategies.

This exposition highlights the greatest problem in defining geratolgy as an applied body of knowledge, and of formulating a fundamental research programme to help the elderly. Although, theoretically, there are likely to be a limited range of initiating chemical events, which occur frequently enough to ensure that they will happen in all members of the population, the higher level expression is very variable, presumably because there are so many possible intervening limiting internal and environmental factors.

From every point of view, environment interacts with general biochemical changes. This interaction makes it virtually impossible to separate the fundamental changes from the random environmental forces in a person's unique physical and social environment that are associated with chronological age alone. It is this kind of specific variability which is responsible for the wide

spread of ages at death, obvious even in the last decade of survival. This variability also emphasizes the point that to produce general improvement in care of the elderly pharmacology should concentrate on the more limited proximal cellular causes.

Thus, the control systems that might be relevant to the manipulation of human ageing are but distantly glimpsed. In fact, the relevant research is spread widely through many branches of biology dealing with such apparently diverse topics as 'ecotoxicology', 'cytoprotection', 'leaf senescence' and 'protein turnover'. Such work is beginning to focus on intracellular 'messengers' such as prostaglandins, metallothionines, and 'ageing hormones'. This mixed bag of compounds appears to be concerned with the control of structural integrity of cell organelles. As important agents governing development and tissue integrity they have been defined widely in the plant and animal kingdoms, where they appear to be involved in responses to a variety of cellular stresses such as metallic and organic toxins (xenobiotics), They also play a part in developmentally timed self-destruction and remodelling systems, which are a part of resource partition physiology. From this aspect there is hope that such systems may be targets for drugs to control individual rates of error repair in people. However, on current knowledge, we cannot say whether it will be possible to stimulate repair of the extreme errors currently expressed in the form of heart disease, cancer and brain disorders, leaving most people to die, disease free, in their second or third century of life from the widespread errors responsible for Korenchevsky's normal physiological ageing.

CHAPTER 3 Ageing and evolution

Sooner or later, in the study of ageing and death, it becomes necessary to consider whether an ageing process is built explicitly into the design of living things or whether ageing is simply due to the random accumulation of damage within the system

The predictability of ageing phenomena both within and between species gives no doubt that they are part of a programmed process. Furthermore, the good correlation between the life-span of parents and offspring, and the similarity in the life-span of twins reared apart, points directly to a genetic basis to ageing. However, this need not mean that suicide instructions are written explicitly into the genetic programme. An alternative could be that organisms age as a result of the way that they are designed to do other things. (Calow 1978)

THE MODEL OF EVOLUTION

Evolutionary change is brought about by a complex set of processes and mechanisms which may be described as the evolutionary system. The system is composed of the following four main subsystems.

1. The natural selection system, which includes the many kinds of environmental pressures and stresses which determine the success of the various individuals in passing their genes on to the next generation.
2. The epigenetic system which includes the mechanism by which the information specifying form and function contained in the fertilized egg produces a fertile adult.
3. The exploitive system by which an organism selects and modifies a particular habitat from a range of possibilities, abstracts resources of materials and energy, and disposes of them physiologically to maximize their returns in offspring that survive to reproduce.
4. The genetic system comprizing the physiological and molecular mechanisms by which hereditary variation is brought into being and transmitted from one generation to the next.

These four sub-systems impinge together on the evolving individual, making the organism in its environment a complex self-regulating, cybernetic system with many feedback relations. Each sub-system is relevant to questions about

the origins and mechanisms of ageing.

The position of ageing within this broad perspective of developmental biology is exemplified by an outline syllabus which would be acceptable for a comprehensive course in developmental biology as in Figure 3.1. This, however, remains an ideal possibility, rather than a practical reality in most educational establishments throughout the world.

It was only in the mid-1980s that an ageing perspective began to penetrate research in genetics (Figure 3.2). Again, this viewpoint has been slow to enter the education system. Even today there is confusion about the genetic basis of ageing. A common attitude is that ageing is beneficial to the organism. This has a long history and may be traced to Weismann's view at the end of the last century that ageing was advantageous to the species, and an adaptive feature, because it provided space and released resources. As taken up by modern biologists it became part of what has been called 'group selection'. Ageing was good because it allowed constant renewal of the population and, by accelerating the species (group) turnover, increased the chances for adaptation to environment.

Weismann's 'prevention of overcrowding' theory may be contrasted with the more generally accepted non-adaptive stance that ageing is disadvantageous to the organism because it reduces the fitness for reproduction. Medawar developing the views of Haldane convincingly argued that selection on genes acting early in life will affect a greater proportion of individuals than late-acting genes. Genes expressed late in life will be expressed biologically in only a small fraction of a birth cohort with very low chances of survival. A major practical argument in favour of non-adaptive theories is that an aged individual is rarely found in the wild, so minimizing the opportunities for gaining any advantage

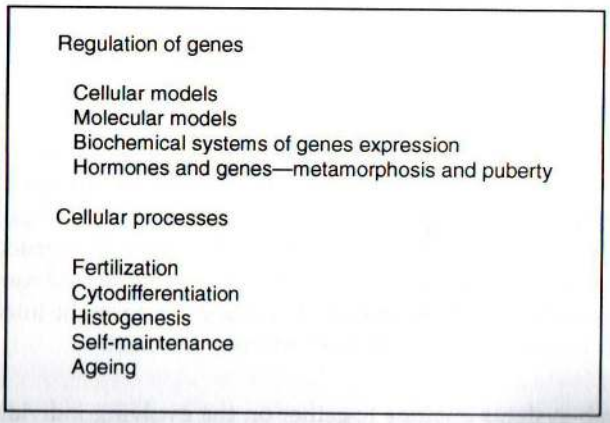


Figure 3.1. An outline syllabus for developmental biology which includes ageing as a subsidiary, yet integrated topic.

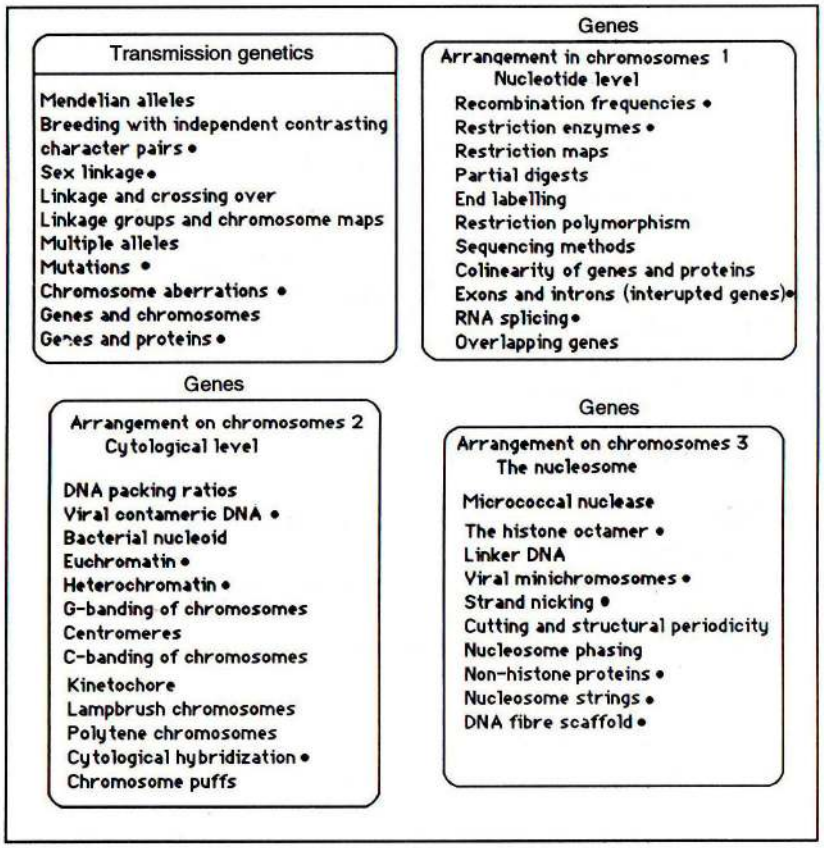


Figure 3.2. Pinpointed topics in the field of genetics that were the subjects of significant research into ageing published during 1988

from it. Therefore, the force of natural selection in the wild becomes progressively less and less as an individual grows older. Also, group selection, if it occurs, would be much weaker and, as a system, more unstable than selection for individual survival, which is thought to be the main route for evolution. For example, in a group composed mainly of ageing animals any individual which did not do so would be at a great advantage in gene transfer. Medawar's ultimate conclusion was that aged individuals in a protected environment would become a 'genetic garbage dump' for late-acting genes with deleterious effects. It would be advantageous to individuals for these harmful effects to be delayed by natural selection as long as possible. In a welfare state for humans, or a zoo, these late-acting deleterious genes would become a multifactorial handicap to aged individuals. According to Medawar, ageing was a latent

evolutionary feature in all organisms, and is revealed in environments which allow a high proportion of individuals to survive to an age well beyond the average in the wild.

After the search for late-acting genes and the predicted increase in genetic variance, had failed to reveal the phenomena predicted by Medawar's theory, theoretical discussions shifted towards genes that had good effects early in life and late effects in old age. This idea of dual acting, or pleiotropic, genes was formulated by Williams in 1957. The same gene could have beneficial effects early in life, thus promoting evolution of the trait, and harmful effects late in life when the small probability of survival would offer little chance of selection. The end-point of Williams' theory was the same as Medawar's, namely the expression of morbidity and mortality through the actions of late-acting genes. The difference between them is that ageing is a byproduct of selection for benefits earlier in life and is therefore non-adaptive.

The salient points are:

1. Selection of a pleiotropic gene expressed early in life may modulate the age which deleterious effects appear.
2. The system need not be associated with an increased genetic variance with age.
3. The theory does not specify the type of advantageous character involved.

The next section will examine possible processes and systems that might be candidates for early-acting pleiotropic genes.

THE NATURAL SELECTION SYSTEM

Geological events in the physical development of the Earth have their beginnings in the formation of cratons, the first relatively stable continental masses, on the margins of which mountains tended to form. The earliest known cellular organisms are bacterial prokaryotes fossilized in African rock strata about 3.3 billion years old.

All forms of life, except bacteria and blue-green algae are eukaryotes—that is they are based on cellular structures containing chromosomes, nuclei and a range of other highly organized structures called organelles. With regard to the development of an oxygen atmosphere the organelles called mitochondria are the most significant because they contain the enzyme systems for harnessing metabolic energy produced by aerobic oxidation of organic matter.

The first appearance of unicellular eukaryotes occurred around 1.5 billion years ago. Consequently for about 50% of the total time that life has existed on earth the only living organisms were prokaryotes. The oldest fossils that could

be regarded as multicellular or multinuclear organisms are not more than 700 million years old. Therefore, the period when evolution was exclusively at the prokaryote level was twice as long as that encompassing the entire evolution of multicellular eukaryotes. The earliest prokaryotes were probably heterotrophic and surrounded by firm cell walls. They secreted enzymes into the surrounding medium and absorbed soluble food that had been externally digested. The source of their food is assumed to have been organic materials produced by the physicochemical abiotic processes of the early planetary environments. This heterotrophic form of nutrition is still predominant in prokaryotes; only the relatively advanced structurally complex blue-green algae, together with a few groups of photosynthetic and sulphur bacteria, have evolved autotrophy. On the other hand, all three forms of nutrition exist in protozoan eukaryotes. Not infrequently, autotrophy, or ingestion, as well as digestion, ingestion, or absorption, can be carried out by the same cell. At the unicellular level, differentiation with respect to these three basic methods of nutrition does not appear to have required extensive evolutionary changes with respect to structure, physiology, and gene content.

Whether life evolved on earth, or came from an extra-terrestrial source, there is general agreement that a major boost to evolution on Earth was the appearance of atmospheric oxygen. This may have first arisen from the photolysis of water, and later by the action of photosynthetic organisms. The origin of a truly oxidizing atmosphere took place probably not much more than a billion years ago.

The most significant event of this evolution was the origin of photosynthesis, which took place relatively soon after cellular life first appeared (Figure 3.3). It is widely agreed that photosynthesis by blue-green algae caused atmospheric oxygen to increase during Precambrian time. Before such a buildup could occur, natural reservoirs known as oxygen sinks had to be filled. These are chemical compounds present in the Earth's crust or atmosphere immediately after the planet was formed that combine readily with oxygen. Sulphur and iron are two of the most important oxygen sinks. Stromatolites, mineralized structures formed by blue-green algae, first became abundant in the fossil record about 2.3 or 2.2 billion years ago.

The presence of red beds of iron-banded sediments in rocks younger than 2.3 billion years old has been taken as evidence that atmospheric oxygen had reached a moderate level by Proterzoic time, e.g. the iron compounds precipitated from soluble iron compounds rendered insoluble by aerobic oxidation. It therefore appears that nearly 2 billion years elapsed before oxygen had built up sufficiently for the evolution of animal-like protista to produce the first eukaryotes from which the multicellular plants and animals eventually developed. Acritarchs are fossils of single-celled planktonic algae that almost certainly were eukaryotes. They underwent adaptive radiation between 800 and 700 million years ago, and then became extinct at the time of the last major

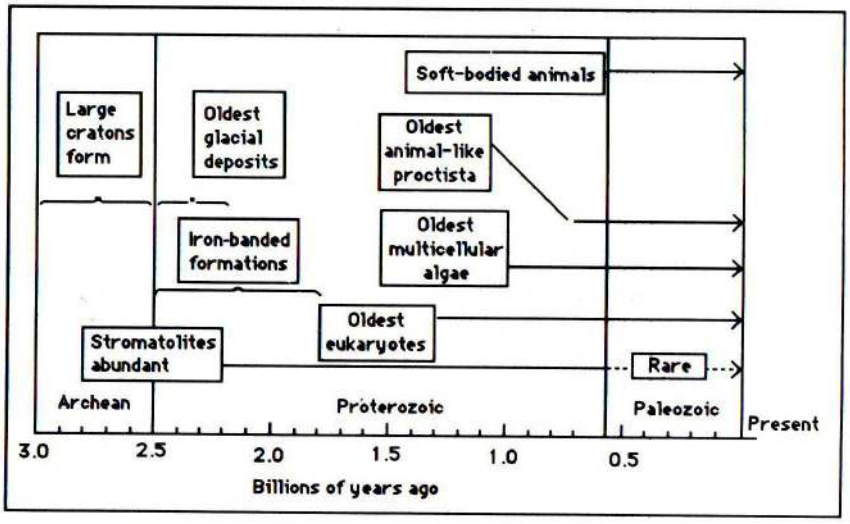


Figure 3.3. Major physical and biological developments during Proterozoic time

Precambrian glaciation, about 600 million years ago.

The fossil record of the blue-green algae is instructive in this connection. Their modern forms have molecules of chlorophyll that are identical with those of higher plants. Even the carotenoid pigment associated with their chlorophyll resembles that found in eukaryotes. Although some can carry out anaerobic metabolism most of them are aerobic and perform photosynthesis with hydrogen peroxide as the hydrogen donor. Hence by the time blue-green algae appeared photosynthesis similar to that of higher plants either existed, or was represented by a similar process. The oldest fossil blue-green algae are found in rocks about 2.7 billion years old, and they are therefore 500 million years younger than the oldest known bacteria, but 1.4 billion years older than the earliest eukaryotes.

The extent to which aerobic metabolism enabled bacteria to undergo additional adaptive radiation, even before the evolution of eukaryotes, may be estimated from the wide range of ecological niches in our contemporary world inhabited by aerobic prokaryotes.

The advent of an oxygen-rich atmosphere required many readjustments of cellular metabolism. One of them must have consisted of the selection of biochemical pathways by which cells could overcome the toxic effect of oxygen on obligate anaerobes. Of the many evolutionary strategies for acquiring this adaptation, one was particularly striking, the origin of bioluminescence in bacteria and presumably in their protozoan descendants. A theoretical aspect of bioluminescence important to gerontology is that it can be envisaged as evolving from mechanisms in the earliest aerobic organisms. In these earlier

organisms it was probably a part of the system that reduced molecular oxygen directly, and quickly, thus preventing it from exerting the toxic effects which oxygen has when it enters modern anaerobic cells. The cells that produce luminescence produce a compound luciferin aldehyde which reacts with the potentially toxic peroxide produced by free oxygen to form the corresponding organic acid and water.

The important influence of the oxidative metabolism on the evolution of ageing in modern organisms comes from a consideration of Rubner's Rule. This states that when metabolism is expressed in relation to lifespan, mammalian species metabolize about the same amount of calories per gram of tissue per year. This relationship has been taken to mean that oxidative metabolism sets a limit to lifespan and in some way the process of aerobic oxidation damages cells which carry it out.

If, as seems likely, the evolution of prokaryotes has been associated both positively and negatively with the availability of oxygen for aerobic metabolism, several hypothetical events may have encouraged the selection of organisms based on their capacity to utilize organic materials competitively. One such event may have been the gradually diminishing non-biological production of basic raw materials necessary for life due to an increased density of atmospheric ozone to form an outer layer cutting off the ultraviolet energy needed for their synthesis.

It is assumed that the primitive prokaryotes evolved a complex system of genetic information coding for a variety of proteins and that this system is virtually the same as that governing the cellular activities of modern prokaryotes. From this point of view the battery of enzymes governing the cellular integrity of the coding system would have been a vital acquisition in the evolution of earlier forms. In the dominant chemical environment of the early stages of planetary development ultraviolet damage and chemical destruction would have been an almost overwhelming hazard of life. In this respect, the modern prokaryote cell is primarily a biochemical strategy to overcome the inevitable physicochemical deterioration of purine, pyrimidine and amino acid based polymers. The maintenance of the simplest living system would have required processes for protection and repair directed against a range of physicochemical impacts. In view of the complexity of even the simplest growing and reproducing cell the first 'housekeeping genes' required to combat the environment would have been continuously active and operating in integrated feedback loops, at least as complicated as those found in prokaryotes today. These were the first anti-ageing systems at the cellular level.

To summarize, the appearance of atmospheric oxygen clearly provided the opportunity for the evolution of cells which gained energy from aerobic oxidation. However it also posed another hazard related to its direct reaction with organic substances of all kinds, and also because of the production of highly reactive, partially oxidized, intracellular intermediates during the

processes of metabolism. Thus the selection of genetic coding to increase the efficiency of ATP formation by aerobic oxidation carried the disadvantage of harmful side effects of highly reactive intermediates of this metabolism destroying the phosphorylation enzymes and the mitochondrial membranes in which they were embedded (Figure 3.4).

An important stage in the evolution of cytoprotective strategies may well have been the segregation of the nucleus from other enzyme systems by a membranous cytoskeleton, which is the feature of all eukaryote cells. One theory is that that eukaryotes evolved by the permanent incorporation of a symbiotic prokaryote within a prokaryote 'body'. Eukaryote chromatin, which is a complex of proteins surrounding a precisely structured DNA core, may have been selected, not only to improved the efficiency of intracellular regulation, but also to minimize the damaging impact of the mitochondrial oxidizing environment. Indeed the whole system of cellular compartmentation may have been part of this evolutionary strategy to protect against chemical deterioration of the genome.

The needs of cells vary as do the functions they perform, according to their place in the body, and the nature of the whole animal. As a rule the cells which make up any particular kind of animal exist as tissues, each kind of tissue consisting of one or several kinds of cells united in particular ways. The organism as a whole can be said to be made up of its component tissues, although it is important to realize that any organism has a quality of wholeness in form and function to which both cells and tissues are subordinate.

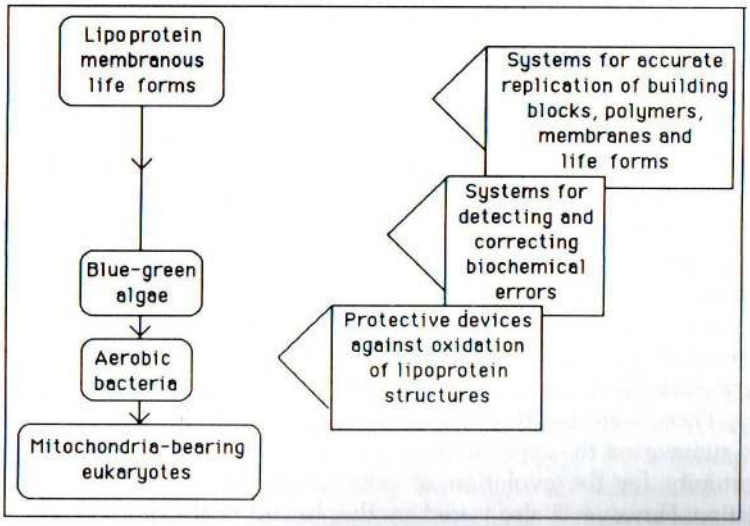


Figure 3.4. Theoretical sequence of the evolution of life forms and basic anti-ageing biochemical systems.

One model for the evolution of multicellular organisms from aggregations of single-celled eukaryote organisms draws upon the life-cycle of modern slime moulds. As part of reproductive development isolated free living amoeba come together to form a multicellular aggregate which shows division of labour; some cells control forward movement, some form the reproductive system. According to this model all the basic self-maintenance and cytoprotective systems present in single cells were taken into the evolving metazoa. Immortality in the primitive multicellular organisms is a corporate property of some of their cells which are able to repair all their defects and retain the capacity to divide and differentiate to give rise to all specialized tissues of the body. These genetic features have been handed down from their prokaryote ancestors.

Almost all single-celled organisms, whether animal or plant are of a minute size. The increase in size of any organism beyond microscopic dimensions is largely dependent upon the coexistence of many cells as a single functioning organism. In any multicellular animal or plant, the constituent cells are not all alike. This multicellular state creates its own potentialities and limitations with respect to the evolution of lifespan. Not only must the multicellular individual function as a whole in an effective way, but the life of each of its constituent specialist cells must be maintained. Whatever the whole animal may be, each of its cells requires steady supplies of oxygen, organic substances, mineral salts and water, and at the same time requires that its waste substance be removed from its neighbourhood as fast as possible. Growth and elaboration of the animal organism progresses only so long as these basic conditions may be maintained.

The animal body may be regarded as an assemblage of cells that has to cope with two basic sets of circumstances. There are those relating to the health and activity of the constituent cells in relation to the needs of the cells in the varying circumstances within the body, and to the need to coordinate their activities as a whole organism. Then there are circumstances relating the whole organism to a particular way of life among the various possibilities afforded by the environment. The first set of circumstances concerns body maintenance, somewhat as in large human communities where urban organization and city government are necessary to maintain the activity of the whole and the health of the individuals. The second set of circumstances concerns the adaptation of the whole organism to such external requirements as maintaining location, defence, obtaining food and oxygen, and reproductive repopulation of the community. Each kind of multicellular organism is a specialist with regard to its way of life but shares with all other organisms the same general problems of internal maintenance and organization relating to its tissue-based method of construction.

With regard to ageing, these needs of a multicellular, tissue-based organization led to the separation of the gametes from the rest of the body, which may be

termed the soma. This was not only a spatial separation. There was also a functional separation of the genes responsible for cellular maintenance and repair. The soma was allowed to age, but only at a rate that was commensurate with the maintenance of a body that could pass on its genes within its likely ecological lifespan. A full set of housekeeping and repair genes were kept active in the germ cell line to ensure the production of gametes with a 'perfect DNA code' and the correct intracellular environment for it to direct. This required the evolution of special resource partition mechanisms to govern the flow of materials and energy to the different cellular populations, which are required to support and disseminate the genome. The aim would be to save on limited resources by allowing part of the organism, the soma, to age, whilst the genetic information in the germ cells was protected.

Several suggestions have been made with respect to the special anti-ageing features of the metazoan germ cell line. These are, for the ovary:

1. A highly condensed genetic apparatus (chromatin) involving special histone proteins giving maximum protection to the DNA when it is not being read.
2. A minimum readout of information from the genetic apparatus, further protecting the DNA.
3. Low specific metabolic rate of the cell, which would reduce the rate of oxidative damage.
4. The presence of synapses between homologous chromosomes during meiosis to permit additional post-replication repair capacity, and for the gonads, processes similar to those in the ovaries.
5. The presence of an active state of division, spermatogenesis, which reduces the chances of slowly dividing cells, that are likely to have genetic defects, making contact with the ova.
6. Selection of the most viable cells by fitness (motility, membrane integrity, etc) to reach and fertilize an ovum.

Additional processes that could select only undamaged germ cells are the abortion of abnormal fetuses, and the decreased ability of defective organisms to reproduce. The effectiveness of these protective mechanisms may be judged by the relatively low level of congenital abnormalities even in the offspring of older mothers, where ova have had more time to accumulate random genetic damage.

THE EPIGENETIC SYSTEM

There are two important features of the developmental processes of animals whereby the epigenetic system operates in evolution. The first is the mutual

influences between different cell populations which bring organs and sub-systems into being. These interactions are often reciprocal and highly complex, and take place between neighbouring parts, initially of the embryo, and later, after organogenesis, between tissues and/or organs. In the embryo, mesodermal structures may induce specific types of differentiation in the ectoderm with which they are in contact; the tensions of the developing muscles have an influence on the form of the bones to which they are attached. Each part is to some extent moulded by another, so that together they tend to form relatively integrated systems. Later in development the balance of resource utilization between tissues is maintained by local hormone-like substances. For example, the mass of skeletal muscles in birds and mammals is maintained by a substance called sciatin, released by motor nerve endings. The formation of these integrated systems is an expression of the differential allocation of materials and energy in time to produce what we describe as organs or tissues. The interplay and balance of resource utilization between organs and tissues changes during development under the influence of 'developmental clocks'. In this context, ageing may be viewed as an expression of the operation of these clocks. It is also an expression of the lack of commitment to provide programmes and resources for complete repair of all defects. This eventually reduces the efficiency of integration that was built up during early development between organs, between cells, and between organelles within cells. The steady decline in homeostatic efficiency after maturation at all levels has to be visualized as a consequence of the epigenetic principle of resource partition.

The second feature of epigenetic processes important to evolution is the subtle balance between developmental flexibility on the one hand, and the lack of it on the other. Flexibility is shown when an adverse environmental influence during development causes an organism to exhibit an acquired character; development diverges to some extent from its normal course to produce an 'environmental modification'. On the other hand, organisms will very often succeed in reaching their normal adult state in spite of injuries or abnormal circumstances encountered on the way. From this point of view, development has a strong tendency to proceed to some definite end-point independent of environment. This relative inflexibility is demonstrated in the fact that adult tissues, such as muscle, nerve, lung, and kidney, are quite distinct from one another, and intermediates between them cannot be made naturally or experimentally.

These features indicate that the system of gene-controlled processes has a certain number of relatively distinct courses of change open to it in relation to variations in environment. We may say that the fertilized egg comprizes a set of genes and associated cytoplasmic material which gives it the potentialities for proceeding along one or other of a definite set of trajectories of change from the point of fertilization. Such predetermined time-trajectories of change Waddington has called 'creodes'. Each creode has a quality similar to stability, but involving

time, which he has defined as homeorhesis. Homeorhesis encompasses all of the feedback controls of the developing system which keep it moving along a creode and will resist it being defected from this path by external influences. If an environmental influence succeeds in diverting it for a time, development will tend to come back on to the creode when the disturbing influence is removed. The extent to which homeorhesis is effective determines the extent to which the organism, when it becomes adult, will exhibit some physiological or anatomical modification.

The relevance of this to gerontology arises in the first place because there is likely to be some genetic variation in the stability characteristics of the epigenetic systems of the different individuals in a population. Therefore, if a normal heterogeneous population is submitted to some environmental stress, certain individuals are more likely than others to be affected and acquire a character. If this character is manifest in an individual during early development, it may give that individual an advantage in furthering reproduction over those acquired by others. Natural selection will operate to increase the frequency of genotypes which enable their possessor to become adaptively modified phenotypically to this stress. Moreover, the organizing capacity of epigenetic processes makes it likely that the acquired character will be a relatively harmonious one. It will not be a matter of, for instance, getting large muscles, but lacking large bones to go with them. On the other hand, if the character is manifest after reproductive maturation, it may either shorten or extend longevity. A situation may be envisaged when a character advantageous early in life may become disadvantageous later. This is termed a pleiotropic effect, and was central to early theories of ageing, where it was presented in terms of a genetic 'burden' which terminated lifespan. However, following this line of argument pleiotropic genes in an individual could produce a relatively healthy old age, compared with the average. Also, theoretically, the flexibility of phenotypic regulators of creodes offer opportunities for the pharmacological direction of the body's resources to maintain the highest possible level of post-maturation homeostasis (Figure 3.5).

In summary, lifespan in an evolving animal is moulded by selection for its capacity to react in a satisfactory manner with environmental stresses. In this epigenetic context, longevity will be determined by certain creodes with particular homeorhetic characteristics. The effects of new gene combinations will be to some extent, at least, characteristic of the epigenetic system in which the new gene pattern is operating. This feedback loop ensures that the phenotypic alterations produced by new gene combinations are not completely independent of the demands which natural selection is making on the evolving organism. This type of situation may be responsible for the relatively precise relationship between lifespan and body and brain size of mammals which have increased steadily during the evolution of this group.

It is appropriate at this point to examine the question of rejuvenation in

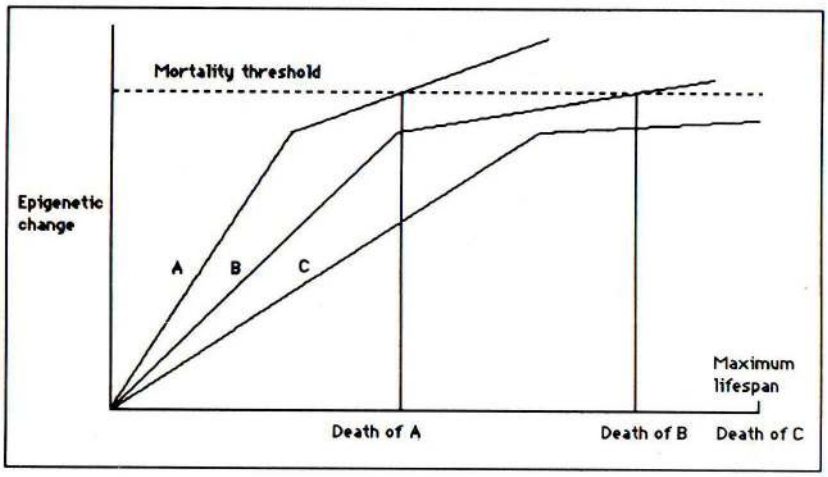


Figure 3.5. Mortality in the context of epigenesis. A, B and C represent three phenotypic creodes for development of a trait which is influenced by environment. In A and B it is a major cause of mortality. C dies from some other trait.

animals that appear to be able to withstand adverse environmental conditions by drawing upon bodily reserves and in so doing appear to return to a more juvenile state. The classical models are found in several groups of invertebrates; the coelenterate medusae, nermertines, ascidians and triclads. As early as 1915 Child drew attention to the fact that following long periods of starvation adult triclads come to resemble juvenile forms. This process of 'degrowth' has been equated with a reversal of the life-cycle. It has led to the idea that rejuvenation is a feature of triclads that allows them to live for several decades in the laboratory and points to a special mechanism of ageing in this group.

Starved animals after refeeding behave like juveniles with respect to metabolic rate, and sensitivity to environmental stresses such as cyanide and irradiation. For example, Child found that the resistance to cyanide in well-fed triclads increases as they grow, but decreases as they degrow. This has been taken to mean that degrowth increases the animal's dependence on oxidative metabolism. In other words it is behaving more like a metabolically active juvenile. The mechanism behind rejuvenation is obscure. Growth is associated with a decline in neoblasts, totipotent cells which are thought to play an important part in normal cell replacement, and it may be that it is the increased dominance of these cells which are stimulated to divide and repopulate the attenuated tissues after refeeding that is the rejuvenating force.

These experiments on triclads and the like indicate that these animal groups are the exceptions to the rule of somatic epigenetic ageing, but this is probably more apparent than real. The selective advantages of possessing neoblasts are

clear enough as a form of insurance in an unstable environment. Quiescent cells with the potential to divide and differentiate following the use of fixed somatic tissues to withstand prolonged periods of food shortage is a general specification for life in this type of niche. From this point of view the triclad phenomenon illustrates what might be termed a 'fresh start' life strategy which is common to other invertebrates which are able to regenerate from cut pieces, and fission processes which are part of normal development.

THE EXPLOITIVE SYSTEM

The exploitive system is currently a central issue of biology. At the ecological level, efforts are mainly directed towards the analysis of local races or recognizable varieties within a species showing a tendency to choose a particular habitat, or exhibit a specific type of behaviour, which brings them in contact with some particular aspect of the environment. For instance, in butterflies one particular coloured form of a species may be active in the early morning and late evening whereas the more common form, found in the same environment, may show a preference for different times of day. The two forms have different lifespans Laboratory studies have also shown that the different strains may be characterized by behaviour traits which cause them to choose different habitats.

Different strains of the fruit fly *Drosophila*, each identifiable by the presence of some visible gene mutation, select light against darkness, heat against cold, and wetness against dryness, when released into an experimental arena where these choices can be made. These are repeatable characteristics of particular strains, which indicates that they must have an important genetic component, and that selection for or against a particular behavioural preference would strengthen or weaken the trait. Again, the different forms have different lifespans.

Another type of behaviour preference shown in wild and laboratory stock is in the selection of sexual mates. Local races of animals and laboratory strains, with different mean lifespans, may exhibit marked mating preferences usually favouring mates of their own race. These preferences may be strengthened by experimental selection.

Such genetically-determined preferences for particular types of habitat or mate will strongly influence the kind of natural selection pressures to which the individual is subjected, and thus the operations of the genetic system by which inheritable qualities are passed on to the next generation. This relationship by which the genes specify the environment for an individual, which, by its natural selective action, helps specify the character of the genes appearing in the next generation, constitutes the exploitive feedback loop. It is within this model that mortality during development, which determines the chances of any individual reaching sexual maturation in a given time, would select physiological and behavioural traits governing the speed of attainment of sexual maturation. Wild

type mortality, and rate of maturation, are both important correlates with longevity in protective environments.

At the physiological level this exploitive system is directly relevant to gerontology from the point of view of the dietary selection of materials to yield building blocks and energy. This issue focuses on the importance of the evolution of mechanisms of resource partition to make the best use of what the environment has to offer, behaviorally and physiologically, for aiding survival and reproduction.

The evolution of cellular differentiation and the resultant separation of cellular populations into soma and germ cell components was a biochemical condition for the adaptive radiation of metazoan organisms. This was associated with selection for different lifespans in direct relation to the level of mortality factors. From this point of view lifespan is one of the most significant variables affected by evolution of the metazoa. Yet very little attention has been paid by gerontologists to the natural selection of strategies to invest food resources in relation to the time available for the use of these resources for growth maintenance and reproduction. The fact that there are great species differences in the length of life and the timing and frequency of reproduction implies that many different resource investment strategies have evolved. These various strategies of food utilization are related to the relative costs of different physiological processes which compete for the materials and energy provided in the diet.

Ecological research is concerned with apportioning these costs to define how organisms adapt to a potentially changing environment by accumulating and dispersing materials and energy between their various tissues optimally. This was first given expression in the models of life cycle strategies constructed by MacArthur and Wilson. At two extremes were the r- and K-strategies; the first staking all on early and explosive reproduction; the second taking a moderate reproductive rate but extending reproduction over a longer time. The assumption is, that in an unpredictable, fluctuating environment, a population will evolve a set of r-traits, of shorter lifespan, earlier maturity, larger number of offspring and lower reproductive effort per individual, than populations living in constant and highly predictable environments, where the opposite K-strategy would predominate. Lifespan is therefore a trait of all evolutionary strategies which involves the selection of advantageous combinations of genes, and is not simply the outcome of happening to find an environment where it is possible to survive accidental encounters with diseases and predators.

An important evolved characteristic at the species level is the way in which the costs of maintenance, with respect to the use of scarce materials and energy, are balanced against the time needed to reach reproductive size, and the timing and magnitude of reproductive effort. At some point the costs of increasing the parental investment with respect to each of these demands on environmental resources will exceed the benefits of the investment in both parents and offspring. Beyond this point the chances of survival of the particular genotype

will decrease. In the context of gerontology, important information is lacking about the following relationships:

1. The fecundity of parents in relation to their longevity.
2. Age-specific survival in relation to all aspects of reproduction.
3. The relationship between the investment in reproduction and the subsequent well-being of the parent.

This kind of information is needed to decide how important these relationships have been in determining the best-evolved lifespan strategy for a particular environment. Information is also needed on the optimum trade-offs in resource partition in relation to the survival of parents that during reproduction have to cope with different environmental variables.

Growth, Size and Metabolism

A starting point is to consider food and its utilization as being a major area of concern to gerontology, on the assumption that organisms should maximize their net return on food resources to maximize their age-specific growth rate, minimize the time to reach maturity, and maximize their reproductive output. These parameters would be set within an overall requirement to minimize the use of resources for maintenance.

In the Western human context, sexual maturity is reached in the early teens and actuarial data reveals that the age of minimum mortality for all causes is about 12 years (Figure 3.6). Thereafter there is a steady rise in the chances of dying. The well-established decline in most physiological functions from the end of the third decade, at a rate of about 1% per year, is a measurable quantitative expression of the relatively early onset of ageing in relation to the period of maximum fitness. Calculations made from historical data and archaeological remains of human burials indicates that peoples of the past could expect to live on average to between 30 and 40 years. This tells us that only in the past 200 years does ageing seem to have been encountered on a significant scale, presumably because of the steady improvements in protection from environmental hazards of all kinds. Although we have no firm evidence for the maximum ecological lifespan of early hominids, it was probably little longer than the time they took to become sexually mature.

One problem for palaeontological gerontology is that the fossil records of many lines of descent are incomplete. For other animal traits the convention is to establish rules which govern the correlations between size and structure of certain organs and body size by comparing a great number of specimens from related recent subspecies, species and genera, or families differing in body size. In this way rules emerge concerning the changes of size and structure of organs

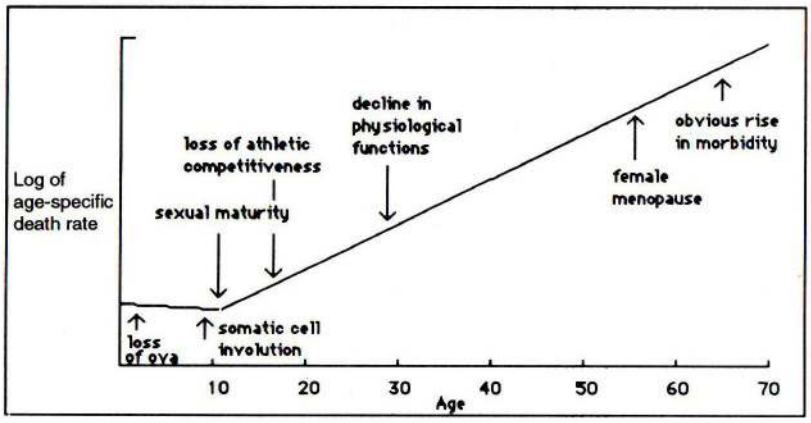


Figure 3.6. Trends in loss of fitness in relation to the probability of dying as a function of age.

resulting from changes of body size. Hence one replaces the phylogenetic series by an anatomical series of recent related animals. This is a sound method because the rules governing the correlations between body size and organs of recent species, genera etc., must have been the same in their common phylogenetic ancestors, otherwise the establishment of a general rule would have been impossible. In an effort to delineate evolutionary trends in mammalian lifespan Cutler used the relationship between lifespan and body and brain weights, including data for extant primitive mammals, such as pygmy shrew, opossum, and tree shrew, to calculate the longevity of those extinct species where information was available on body size and brain weight. His data for about 150 species of extinct mammalian species indicated that the mean lifespan potential increased steadily to the present time for all species whose evolutionary lines did not become extinct. There appeared to be a slowing down of the increase for some time prior to extinction. The lifespan of primates increased at a remarkably high rate and the closer the relationship with man the faster the rate of increase. Using data for 100 different hominid fossils it appeared that mean lifespan stopped increasing about 100 000 years ago.

Current thinking of how species in general evolved at the gene level is based on the idea that substantial biological changes occurred during evolution without corresponding changes in the number of structural genes. It appears that the essential change was in the rearrangement of regulatory genes. In particular, mammalian evolution can be accounted for on that basis, rather than by point-mutational changes occurring in genes that code for structural or nonregulatory proteins. Cutler believes that increased mean lifespan potential and extra brain capacity also resulted from such regulatory changes. He calculates that the hominids reached a maximum rate of lifespan increase of

about 14 years per 100 000 years. In this period of time there would only have been a change of about one amino acid in about 250 genes out of a total estimate of 40 000 genes per haploid genome. This supports the concept that only a few primary anti-ageing processes exist for hominids, and maybe for all mammalian species.

When environmental resources are in short supply, it is advantageous for a species to have a small body size and rapid rates of development and reproduction. This situation, coupled with a high mortality due to diseases and predation, is not favourable for the evolution of large-bodied animals. Nevertheless, large body size has many advantages; strength in capturing prey, fleetness in evading predators and success in mating and competition are examples.

Placed within the context of mammalian evolution Man has a pedigree related to increasing body size which extends back in time to the first small, primitive mammals which emerged about 150×10^6 years ago. The fossil ancestry of all types of modern mammals indicates that their evolution has been associated with increases in body size. Lifespan of modern forms is broadly related to body size ranging from between 1 to 3 years for small rodents and insectivores, weighing tens of grams, to 70 years for an elephant, and 100 years for man, weighing tens of kilograms. As would be anticipated from the ideas already outlined, connecting oxygen toxicity with the evolution of cellular ageing, lifespan of mammals is also related to metabolic rate. Early mammals evolved homeothermy and their relatively high rates of metabolism may well have been a burden in this respect. In fact it may have been an important factor in the selection of large body size in environments where food was in good supply and where mortality factors were low enough to allow time to grow.

However, the conventional view connects the major metabolic advantage of growing large with a decrease in the proportion of energy used for maintenance. From the mid-nineteenth century it has been recognized that in homiotherms the rate of oxygen consumption depends on the surface area of the animal. With increasing body size the volume of the body grows by the cube, but the surface radiating heat by the square, and hence the loss of body heat is relatively smaller in large animals. On a unit weight basis the rate of metabolism in small homiotherms is more intense than that in larger types. In consequence of the relatively low oxygen consumption in larger animals the rate of respiration and pulse rate are lower. In the mouse for example, the heart will beat 500–700 times per minute, in the pig 60–80 times, in the horse 34–36 times and in the elephant 25–28 times.

Differences in the Development of Parts

In discussions on the major steps of evolution it has often been pointed out that organisms remain well-balanced and harmonious systems; i.e. if there is an

alteration of a certain organ, other organs will be altered correspondingly. So for example in the parallel evolution of running animals from the various vertebrate orders Perissodactyla, Artiodactyla, Rodentia, Carnivora and Marsupialia, not only did the legs become longer and the moving muscles stronger, but there was always a corresponding reduction of the number of toes; a reinforcement of hip and shoulder girdles; the distal parts of the feet were rendered more solid and developed soft pads; the heart and lungs became adapted for better performance; the eyes became relatively larger, and certain patterns of behaviour were altered; e.g. strengthening of flight, instincts, and social behaviour. Confronted with these facts it is difficult to avoid the conclusion that animals cannot be changed by simply adding new characters, but that each transformation is governed by specific rules affecting the organism as a whole. These rules which govern systemic qualities like ageing are embodied in the way that genes work in evolution. There is not simply a change in the print-out of a DNA sequence. Through chromosomal linkage, position effects, pleiotropism, mutual reactions occur between the processes brought about by different genes and the modifications selected during ontogeny. Many, if not most, genes cause pleiotropic effects and in the course of development the processes caused or controlled by genes can interact in many ways. Effects on the organism as a whole will be produced, especially by genes affecting the production of hormones. A condition termed 'pituitary nanism' in mice caused by a recessive gene which blocks the formation of eosinophil cells in the anterior lobe of the pituitary, produces simultaneous defects in the thymus, thyroid, pancreas and gonads. The animals remain small and have relatively short lifespans.

It seems probable that numerous constructive genes which are effective in the formation of all organs, also affect longevity. The shape and size of the organs, tissues and structures are interrelated, and especially complex correlations link those factors which affect, or are affected by body size. It has already been pointed out that body size is often considerably enlarged in most lines of descent, and this is associated with quite complex alteration in the body's proportions, which may often seem to be directed. Of course such alterations of organ and body proportions are accompanied by corresponding physiological changes. Analysis of all differences caused by a change of body size must include longevity. Thus, ageing is bound up with any analysis of the complicated process of evolutionary transformation as a whole.

The outcomes of these phylogenetic transformations in relative proportions of organs and tissues are expressed during development in relation to selection pressures during ontogeny (Figure 3.7). As wild animals develop from birth, they encounter a sequence of different, age-dependent, mortality factors and it is essential that some organ systems develop preferentially, and sequentially, to cope with those changing selection pressures. It unlikely that maximum biochemical support would be demanded, simultaneously, by all organs in any one particular phase of development. For example it is important to all

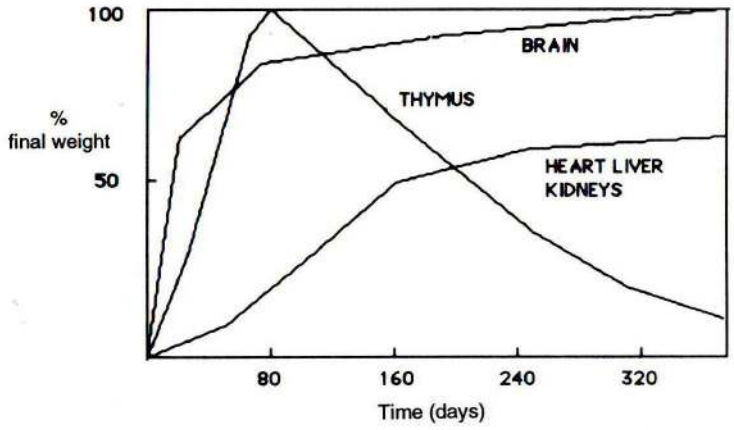


Figure 3.7. Growth of organs in the rat during the first year of life.

vertebrates that the brain should command a large share of embryonic resources, so that there is no deficiency in central co-ordination of responses to environment at birth.

In a newborn child the length of the head is approximately one quarter of the total length of the body; in the adult, it is only one eighth. After the first year of life, the human brain stops growing and thereafter takes a smaller proportion of materials and energy through the operation of special control systems which reduce its metabolic demands on incoming materials. We know very little about these organ systems regulating metabolic demand, which appear to be governed by some kind of 'biological clock', either intrinsic or extrinsic to the organ.

Preferential support for the brain ends long before maturity. The drop in brain respiration measured *in vitro*, and cell loss from specific areas both begin before maturation, but these processes are only specific examples of similar general changes throughout the body. It has already been stated that from an actuarial viewpoint, the human body is least vulnerable at the age of 12, and in this respect, there is no doubt that the allocation of resources after puberty is inadequate to maintain the average level of cellular support and co-ordination that had a survival value only a few years earlier.

Also, since species brain weight at birth correlates with gestation time, it has been suggested that long lifespans, in large-brained animals, evolved in response to the need to extend the gestation period to allow this preferential allocation of resources to the brain. This necessitated reducing the number of bouts of reproduction, giving an advantage to those animals that evolved the capacity to increase their lifespans, giving opportunities for more reproductive cycles.

Brain is one of the exceptional organs that undergo a large change in proportion to body weight during development. Studies of the ontogeny of

numerous animals and plants have revealed that the growth ratios of many single organs and structures in relation to the whole body remain constant for certain periods. In both situations, the genes define growth partition coefficients governing the diversion of resources in relation to the size of the body. An organ or a structure that grows more quickly than the body as a whole is said to exhibit positive allometry. If it grows more slowly it shows negative allometry, and if it grows at the same speed it is an example of isometry (Figure 3.8.) Similarly, allometric growth may occur of a certain part of an organ in relation to the organ as a whole. In some cases such allometric tendencies remain constant through the long periods of ontogeny. In other cases several different growth gradients follow one another, and then some growth ratios may change from positive to negative allometry, or to isometry, or vice versa. Such changes often coincide with birth, the end of larval development, or the onset of sexual maturity, but they may well occur during any other time. In many mammals and birds the head grows with positive allometry until birth and then negative allometry begins. Some organs (e.g. kidney and thymus) show positive allometry early in postnatal life and negative allometry later, after they have reached a peak or plateau of development. This preferential build-up of structural elements followed by a decline is also seen at the chemical level. These patterns indicate the dominance of processes that reduce metabolic support once the critical phase of development requiring the peak degree of complexity has passed. Where there is a sharp point of change, it has been assumed that there must be some kind of physiological switch to divert more or less resources to or away from the organ concerned. Whilst some of these may

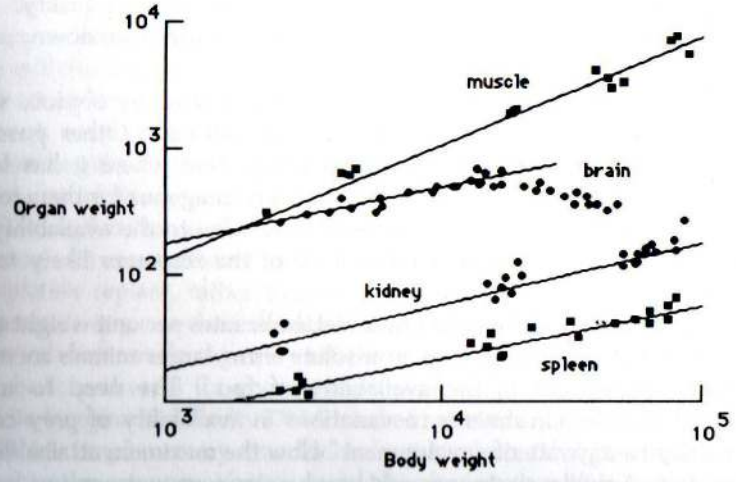


Figure 3.8. Differential postnatal growth of organs in the rat.

be endocrine switches it is likely that some other, as yet unknown resource partition system is involved in most cases. For example, the change in the relative size of the brain is associated with a fall in its absolute growth rate, and so far we have no evidence for the existence of a special systemic brain growth hormone.

As cells, tissues and organs, and the whole body grow in three dimensions, the process of allometric growth may be mathematically described by a function formula, $y = b \times a$, in which y is the size of the organ under consideration, x means body size and 'a' is a growth coefficient from which, for example, one can calculate the size of a certain organ at a given body size. The formula can be written $\log y = \log b + a.\log x$. In a system of logarithmic coordinates this function is a straight line. The slope of the line indicates the degree of resource partition according to which it departs from an isometry angle of 45°.

A wide variety of animals, vertebrates and invertebrates, are capable of initiating a growth rate considerably above the normal after being subjected to a short period of growth restriction. It does not appear to matter how the perturbation in normal growth has been brought about; e.g. by toxins, experimental food restriction, or in man after illness and starvation. This compensatory, or 'catch-up', growth is associated with increased feeding and metabolism and indicates that physiological mechanisms exist to keep normal growth below the maximum rate which can be called upon in an emergency.

Although we know very little about it, the mechanisms involved in 'catch up' growth are likely to involve, primarily:

1. The activation of growth control hormones.
2. Behavioural regulation of food intake and choice of food quality.
3. Biochemical systems that govern the rate of tissue breakdown.

The advantages of submaximum growth and feeding rates are obvious with respect to the needs to divert resources to parental care. Other possible advantages are considered in an ecological perspective where it has been suggested that for animal predators, it would be advantageous for them to set their controls relating food intake to growth in relation to the availability of mean size of prey, and hence to a mean level of the resources likely to be available at each feed.

Since larger animals usually have lower metabolic rates per unit weight than smaller animals, but lose more energy in absolute terms, larger animals are more susceptible to reductions in the availability of food. The need to make physiological fail-safe adjustments to variations in availability of prey could select for a day-to-day rate of development below the maximum attainable on very good days. A similar strategy would be advantageous to organisms facing short-term or long-term variations in the physical nature of the environment which subjected them to factors limiting their growth.

Apart from many demonstrations of compensatory growth, direct experimental evidence for any of these ideas in an ecological context is lacking. It is virtually impossible to make suitable measurements of food intake and growth in the field. In the laboratory it is the convention to express growth in the terms of average weight gain over weeks or months. For rodents in constant environments of laboratory animal houses, given standard food ad lib, such long-running smooth averages mask quite wide day-to-day variations. These variations have not been investigated in depth but it is likely that they are due to individual and group variations in physiological regulation brought about by subtle variations in the managerial environment and the microsociophysical environment of their cages.

If the preferential direction of resources towards growth is possible, is there a similar flexible phenotypic mechanism governing the use of resources for maintenance? One aspect of maintenance relevant to this question is replacement synthesis. Replacement synthesis associated with the universal biochemical phenomenon of turnover is important because it makes significant demands on materials and energy. The cell degrades many of its constituents only to replace them immediately. Turnover is likely to have been fixed genetically in the first prokaryotes if only because it is found in all modern bacteria. Another constant demand for energy is to pump sodium and potassium across cell membranes to maintain a differential gradient of these ions between micro-organisms and their external environment, and, in multicellular organisms, between the cytoplasm and the extracellular fluid. From protozoans to man there is also a continuous requirement to separate water from solutes in osmoregulation and glandular secretion. The advantages of this constant need to expend energy to support chemical turnover are difficult to reconcile with the economy of evolution. It has been proposed that turnover of macromolecules, particular proteins, removes components rendered defective by oxidation, etc. This is certainly the case with human red cell renewal where several weeks circulating in the blood, with no repair systems operating, results in a loss of glycolytic enzyme activity through enzyme denaturation. It is more difficult to see the advantages in dismantling the complex sliding-filaments system of skeletal muscle on average every few months.

One explanation is that it is simply a damage repair system that evolved because is is more efficient to dismantle a cellular unit with likely defects, and completely replace, rather than have systems for constantly checking every polymer. In any case the amount of energy devoted to turnover may imply that chemical errors or damage are a relatively commonplace feature of the universal metabolic pathways for energy production and utilization. For example it is easy to imagine how faulty DNA can be corrected by a turnover process using the DNA complementary strand. It has been suggested that genetic recombination falls into the category of error correcting devices according to the notion that the selective advantage of recombination actually allows the accumulation of errors in DNA to be corrected when recombination takes place.

The Concept of 'Metabolic Potential'

Possibly related to the less intense metabolic processes in large animals the periods of ontogenetic development are longer; i.e. their gestation or incubation periods are longer, and their postnatal growth is slower than in their smaller relatives. In the mouse for example, sexual maturity is reached in five to six weeks, and in a beaver this maturity is attained one year after birth. Numerous pairs of related animals provide similar data. The comparative data also indicate that the tempo of development and average life expectancy are affected by, and depend upon body size, and generally that populations of large homiotherms reach a higher individual average age than related small types. In the early 1940s Backman was the first to develop a mathematical formula for the correlation between the tempo of ontogenetic development and average age by which he could predict the major events of the life cycle such as birth, onset of maturity and senility, and death, using the individual time of each organism (its eigenzeit) as the basis of his calculation. This point was re-examined by Sacher in the 1950s He discovered that a better correlation was obtained between lifespan and brain size in mammals. His conclusion was that the extra brain size is related to a qualitatively improved homeostasis.

The importance of species size and metabolic rate in the evolution of mammalian longevity is apparent from statistical correlations showing that about 80% of the variation of lifespans between species can be accounted for by body weight, brain weight, specific metabolic rate and deep body temperature, the two major components being body weight and brain weight (Figure 3.9).

The areas on the graph enclose the points for brain weights superimposed on those for the body weights, with the two lines of best fit to the points.

For 85 species, the formula which accounted for most of the variation in lifespan was:

$$\log L = 0.62 \log E - 0.41 \log S - 0.52 \log M + 0.026T + 0.9,$$

where E = adult brain weight (g)
S = adult body weight (g)
M = specific metabolic rate (watts/g body weight)
T = deep body temperature (°C)

A connection was made at the phylogenetic level between the rate of metabolism and brain size by Friedenthal at the turn of the century, demonstrating that species with an unusually high metabolic rate in relation to their species size also have an unusually large ratio of brain to body weight, where body weight is inversely related to specific metabolic rate. Extra-long-lived mammals seem to have an extra amount of brain for a given body size, compared to their shorter-lived relatives. The additional brain weight can be expressed as an encephalization index, which can, in turn, be related to learned

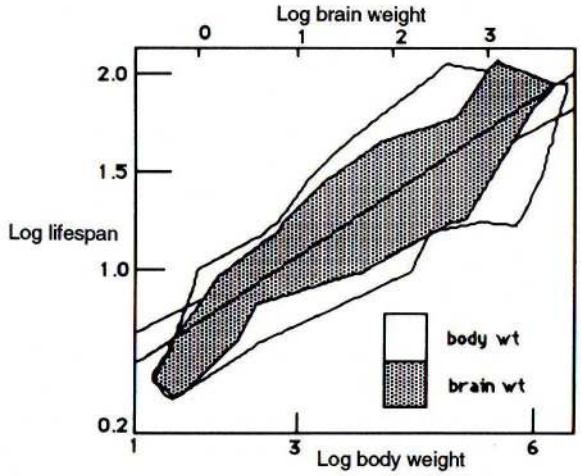


Figure 3.9. Regressions of lifespan against species body weights and brain weights for 63 mammalian species.

behaviour and cognitive abilities. By comparing body and brain weight, the potential lifespan for most mammalian species can be predicted within 10% of known value.

In 1908, Rubner, comparing data on the metabolic rates of five mammalian species, which differed considerably in body weight and lifespan, noted that the metabolic rate per unit body weight, multiplied by the lifespan, gave figures for the total metabolic energy per lifespan which were very similar. He proposed that differences in the length of life depended on the rate at which this fixed amount of energy (the metabolic potential) was used.

The concept was first explored by Pearl in 1928 who studied the transparent water flea Daphnia in which it is easy to measure the rate at which its heart beats. In males there were approximately 4.3 beats per minute, and in females 3.7. The male had a mean lifespan of 37.8 days and the female 43.8. Heart rate multiplied by length of life came to the same figure for each sex, 162. This figure may represent a store of vitality which when it is eventually exhausted leads to death. On the other hand, it may represent the point at which a deleterious process becomes the dominant force of mortality.

The central question is, does the rate of living apply to more advanced animals such as mice and men. A mouse is well past middle age at the end of one year, a man at 50, and this is more so if we consider the biological or physiological time rather than calendar time. Under laboratory care a mouse normally lives for about two years, an advanced age for so small a creature. It spends less than three weeks developing in the womb and attains sexual maturity a month after birth. Its heart beats 200 times a minute or four times

faster than a man's. In biological time it is travelling very fast.

Cutler followed up this work using data on 26 mammals to determine the ratio of 'maximum lifespan potential calorie consumption' (MCC) to 'mean lifespan potential' (MLP) as about 200 kcal/g/MLP. From this kind of study it appears that mammalian evolution is limited by oxygen toxicity in a remarkable way. It is however important to realize that the figure of 200 kcal/g/MLP is an average that hides a fivefold range, and that no satisfactory explanation has been put forward to account for the existence of species with extremes values. There are at least three different levels of metabolic potential in mammals; about 200 kcal/g body weight per lifespan for most non-primates; about 400 kcal/g in non-human primates and about 800 kcal/g for humans.

As part of his work with poikilotherms, in the 1920s Raymond Pearl investigated the relationship between metabolic rates at different temperatures and the influence of temperature on longevity. For example, raising the temperature of the water flea, *Daphnia*, from 8° to 28° decreased the lifespan by about 80%, increased heart beat fourfold, but the total number of heartbeats in the different lifespans was constant. One of the first insect studies was carried out on *Drosophila* where, between 10° and 30°, rates of larval and pupal development were faster and lifespans of adults were shorter in the high temperature range. Pearl analysed the experimental data on mortality and formalized the phenomenon as the 'rate of living' theory, which stated that the duration of life of an organism is dependent upon the exhaustion of a fixed quantity of a vital substance at a rate proportional to the metabolic rate. Since then there have been many studies of the two testable predictions of the theory:

1. Depending on species and general environmental conditions, there should be a predetermined metabolic potential which if realized quickly will result in a short lifespan and if realized slowly will result in a longer lifespan.
2. There should be an inverse relationship between metabolic rate and the rate of ageing.

Unfortunately, although there have been many studies on the relationship between temperature and longevity, these have often been the only parameters measured. Oxygen uptake, which was the important feature of Rubner's standpoint has not always been measured. Temperature alone is not a good predictor of respiration. Insects have compensatory mechanisms for adapting to temperature changes, and do not always take up a standard metabolic rate when transferred from different temperatures to the same standard temperature. Also, there has been confusion in the interpretation of data where winged insects have been used. The different physical activities of insects are not equally affected by temperature changes. In fact lifespan of insects is related to a number of different aspects of their environment at each temperature (Table 3.1).

Table 3.1. Lifespans of houseflies in relation to sex and activity at the same temperature

Population	Walking (counts/h)	Flying (counts/h)	Lifespan (days)
Males (50)	2400	15 360	25.4
Females (50)	2100	13 760	30.5
Males (40) and females (10)	2630	21 364	19.8 m 19.3 f

Flight is a major consumer of aerobic energy and has a marked effect on longevity, indicating the foraging insects, such as worker honeybees, would be suitable models for exploring this relationship. Bees also have an advantage in that they can be studied under natural conditions as they move to and from the hive.

Similar studies on honeybee workers dealt with the dependence of lifespan, and energy consumption, upon flight performance. The maximum flight performance during the life of a foraging worker is about 800 km. After reaching this level of activity the bees quickly die. Within individuals, there is an inverse correlation between a high mean flight performance and longevity, and a direct correlation between the time of onset of foraging and size of the corpora allata. Artificially applied juvenile hormone results in an earlier start to foraging and results in a correspondingly shorter lifespan, indicating that foraging is initiated by some kind of developmental endocrine 'clock'. In this instance flight metabolism draws upon a fixed lifespan potential.

Lifespan in insects is not only reduced by the level of general physical activity but appears to be reduced specifically by sexual activity in both sexes. This might occur because of an increased risk of death at the time of mating through the failure of some vital process. On the other hand, sexual activity could accelerate the process of ageing by commanding resources that would otherwise be devoted to somatic repair. The first idea implies that current reproductive activity, not past reproduction should govern the probability of death. If the second aspect is important, then lifespan should be governed by the total past reproductive activity of the insect. Specific tests of these two possibilities with *Drosophila* indicate that the effect of sexual activity on males is to increase the risk of death when the insects are sexually active. In experiments where males were kept with and without females, the presence of females shortened lifespan (Figure 3.10), and the additional costs in metabolic potential could have been incurred during either courtship or mating.

This raises the question of the relationship between lifespan and the partition of food resources and reserves between the various activities. Flight in blowflies affects food intake and its pathways of utilization. Longevity depends on the level of reproductive sufficiency and physical performance. Both factors act to

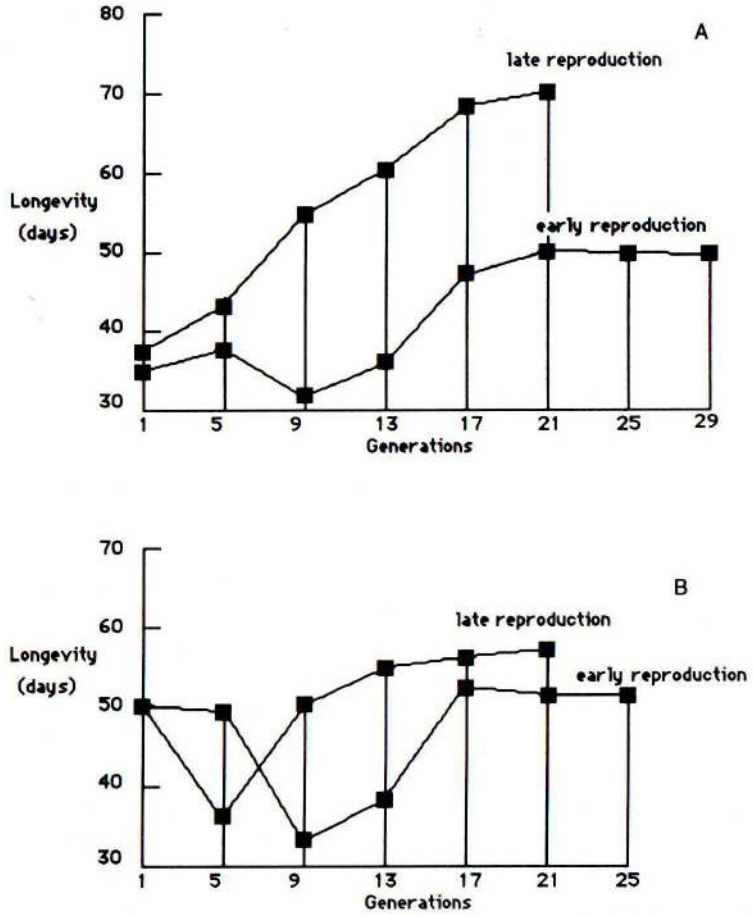


Figure 3.10. Mortality of male *Drosophila* caged with and without females. A Larval density uncontrolled. B Larval density controlled.

shorten lifespan. For example, in comparison with mated females, virgin blowflies ingest more sugar, with a later intake maximum, and live 40% longer. Their egg production is delayed from day 5 to day 10, and is only half that in mated females where 80% of eggs are laid between 6 and 12 days. Flight performance is generally higher in virgin females, reaching a delayed maximum (2 days later; day 14), and declines more gradually instead of being terminated suddenly as in mated flies; there is about a 40% drop in flight activity in mated females in 24 hours between days 11 and 12 whereas the same drop in virgins takes 6–7 days. There is an ecological explanation of the correlation of virginity

with long life in insects, like the blowfly that has a large egg supply, is that it pays to invest some of the resources potentially available for egg production in the search for a mate.

Therefore, there are apparent physiological control systems to inhibit the flow of food resources to reproduction when flight is more advantageous in ensuring survival of genes.

Female blowflies receiving their food by flight only (forced fliers) in comparison with corresponding groups that had access to sugar on the cage floor show a shorter mean lifespan (which is more pronounced in virgin flies). Forced flying reduces the sugar intake by about 30% over the entire lifespan, and shifts the maximum intake to an earlier point after eclosion. In line with this lower energy consumption, egg production is lower and tends to have an earlier maximum. This kind of experiment raises questions about the nature of the 'metabolic potential' limit to lifespan. It clearly is not the total amount of food metabolized or even how it is apportioned between activity, reproduction and maintenance. Although it was not measured in this particular experiment, it is likely that lifespan is more closely related to oxygen consumption, which indicates that the lifespan limit and the concept of 'metabolic potential' is somehow connected with mitochondrial processes.

The same kind of relationship between lifespan, onset of reproduction, and reproduction has been obtained by varying the breeding temperature in rotifers (Tables 3.2 and 3.3). In rotifers lifespan is inversely related to the level of nutrition, as is the reproductive period and the reproductive effort. A high food intake shortens lifespan, decreases the proportion of lifespan over which individuals remain fertile and reduces the number of offspring. In this model the rate of reproduction, expressed as the number of young produced per day of the reproductive period, increases with a rise in temperature from 15 to 23 °C, then declines up to the highest temperature tested of 25 °C (Figure 3.11). Above 23 °C the drop in reproduction does not have a marked effect on the decline in lifespan. From 17.5 °C lifespan decreased with temperature, at first rapidly then more slowly. Between 15 and 23 °C there was a good inverse correlation

Table 3.2. Effect of food intake (feeding interval) on lifespan and reproductive output of the rotifer (*Asplanchna brightwelli*) at 18 °C

Feeding interval (hr)	Longevity (days)	Reproductive period (days)	Offspring (number)
12	5.44	2.48	7
24	5.91	2.69	5
36	6.08	2.78	6
48	4.41	1.27	2
60	4.41	1.06	2
72	4.35	0.98	2

Table 3.3. Effect of temperature on lifespan and reproduction of the rotifer (*Asplanchna brightwelli*)

Temperature (°C)	Lifespan (days)	Length of reproduction (days)	Offspring (n)	Rate of reproduction (n/day)
15.0	5.62	1.02	2	1.96
17.5	5.91	3.19	7	2.19
20.0	3.98	1.38	4	2.90
23.0	3.35	1.15	4	3.48
25.0	3.04	0.83	2	2.41

(Figure 3.11). However, although the rate of increase in reproduction was constant from 18 to 23 °C, most of the decrease in lifespan occurred between 18 and 20 °C. The correlation between lifespan and rate of reproduction is clearly a negative one (Figure 3.12). On the whole, the rotifer work points to other factors, apart from reproductive competition being involved in the inverse relationship between lifespan and temperature. Unfortunately, oxygen uptake was not measured.

Drawing all of the invertebrate data together the overall situation is summarized in Figure 3.13. Food resources are used, broadly speaking, along three types of metabolic pathway; for activity (general or sexual), reproduction, and those general maintenance processes which extend longevity. Activity and reproduction inhibit maintenance by diverting resources elsewhere. Oxidative reactions generally have an adverse effect on the level of maintenance, either by inhibiting maintenance processes or increasing tissue damage.

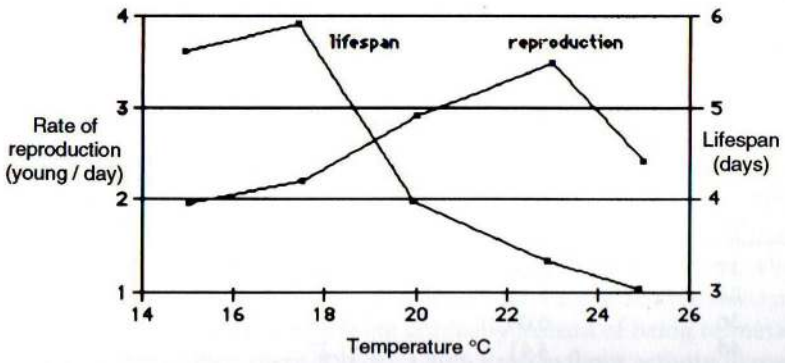


Figure 3.11. Relationship between lifespan and rate of reproduction of rotifers between 15 and 25 °C.

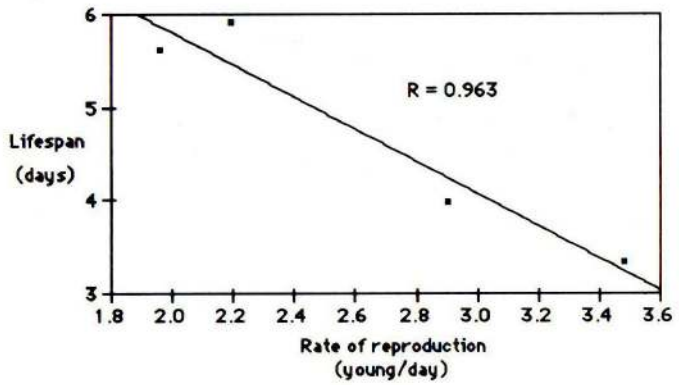


Figure 3.12. Relationship between lifespan and rate of reproduction in rotifers.

RESOURCE PARTITION

The evolution of lifespan is bound up with the selection of biochemical and physiological mechanisms to ensure the best system of resource allocation to the various parts of the body to promote survival. This resource partition takes place between the various competing cellular populations in organs, particularly between the biochemical systems governing cellular replication and repair (Figure 3.13).

From the structural and biochemical characteristics of modern prokaryotes we must assume that the cell has always had to invest in biochemical systems to abstract simple molecules from the environment to make highly organized granules, membranes and macromolecular complexes. Further we have to assume that this investment in developmental processes has always had to be balanced against the cost of protection against physicochemical damage and repair.

Materials and energy of the diet are used simultaneously for: cell division; the maintenance of organ function (by activating ion pumps and other membrane transport processes); replacement of denatured proteins and damaged nucleic acids; and, in mammals and birds, for heat production to maintain body temperature. These processes in the mature non-growing animal are collectively responsible for basal metabolic rate. Unfortunately it is not possible to determine the share demanded by each of these processes. The most important need is for cellular maintenance and, in particular, to detect and repair damage and correct errors, within cells. Ultimately, the failure of repair and error correction lies at the level of their nucleic acid biochemistry. DNA holds the genetic programme of both development and maintenance and it would be expected that resource allocation to maintain working genes, although

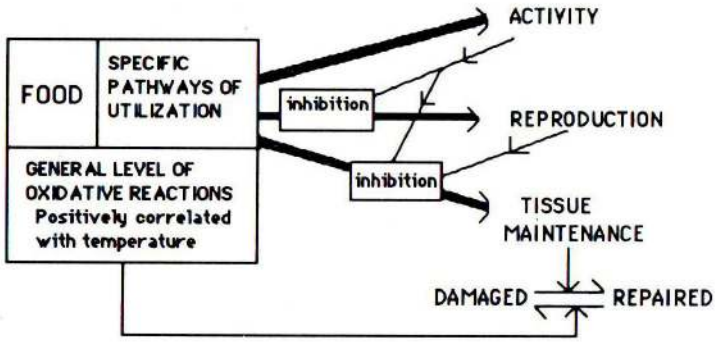


Figure 3.13. Partition of resources for growth, maintenance and molecular replacement and repair.

probably relatively low, compared with say, the needs of heat production, would have a high priority in evolution.

Error Accumulation

The first contribution to the decline in metabolic activity is a reduction in the preferential expansion of the body's cell population which is responsible for growth. After growth has ceased, the basal metabolic rate is a reflection, solely, of the needs for support and replacement of existing cells and their products. There is therefore no doubt that the allocation of resources in later life is not adequate to maintain the cellularity that had a survival value in youth. Does this reflect the operation of a sophisticated genetic programme selected to withdraw resources from the cell compartment? Or is it simply a legacy of error detection and correction of the outcomes of these errors, being set by evolution, below the levels required to sustain 100% biochemical efficiency throughout the ecological lifespan?

Two propositions are associated with the theory:

1. Ageing should result in somatic damage.

   Ageing is the result of somatic damage which could arise either internally from errors in the operation of biochemical and physiological systems or externally, from the random impact of physical and chemical factors in the environment. Special mechanisms exist to keep the germ line fully repaired

or to selectively destroy damaged cells in the germ line. Species with different longevities should exhibit corresponding differences in their levels of somatic maintenance and repair.

2. There should be an inverse relationship between longevity and fecundity

Theoretical Models

Metazoan development is usually complete about the time of sexual maturation. Before this period environmental mortality factors are likely to have taken a heavy toll. For those that survive to reproduce, unlike the modern human situation, environmental hazards ensure that they do not live to an age where loss of fitness becomes a major problem of coping with the day-to-day stresses of life.

Wild type selection works on metazoa at the species level to allow them to reach an age of maximum reproductive probability in the shortest possible time commensurate with the availability of food and shelter, and the chances of encountering a fatal impact of disease or predation. This wild type model is usually considered in relation to a population in equilibrium with its environmental hazards where death rate equals birth rate, and the total number of individuals in the population does not change with time. If no member of the population shows a loss of fitness with time, and there are no predation pressures related to age, all individuals will have an equal probability of being killed regardless of age. Young individuals would predominate in such a population because, regardless of the individual's age at death, it would always be replaced by a newborn individual. In this hypothetical population the number of individuals in a given age class decreases as its age increases. This is the 'teacup' model of Medawar. It represents the situation in a cafeteria where the major hazard to the crockery is through accidental breakages. Accidental mortality of organisms in the wild, even without a rise in the chances of dying with time, would reduce progressively the fraction of individuals surviving. The proportion of individuals that will be affected by a new gene having set time of onset will depend on whether it acts early or late. Since natural selection would have its maximum effects on advantageous traits present in large populations, the time of expression of good genes would be brought forward and the expression of bad genes would be delayed. Any bad gene which is expressed at a very late age, when the fraction of survivors is negligible will be subject to little or no selection at all. It is the accumulation of bad genes with late age-specific effects that causes ageing. No particular advantage is attached to ageing as a phenomenon and it results simply from the fall, due to random deaths of young individuals, of the corrective power of natural selection.

Williams introduced the pleiotropic gene into this ecological model to account for the termination of life by ageing. He recognized that the force of natural selection declines with age and attributed ageing to the effects of genes which were beneficial early in life but become harmful later. A pleiotropic gene is one which is selected for its beneficial effects on development in the wild, but turns out to have harmful effects later on if the individual survives beyond the maximum lifespan of the wild by being placed in a protective environment. The selection pressures to increase reproductive probability would ensure that any harmful effects of a gene would be delayed until sexual maturity began to decline. This relationship would be expressed as a continuous function, with selection against deleterious expressions of genes being proportional to the reproductive value of the organism.

Selection to increase lifespan at the population level involves the mortality rate, the chances of encountering environmental hazards and the reproductive capacity. In environments with a high mortality rate organisms would be selected for early sexual maturation, a species-specific period of reproduction, and would finally be killed by natural environmental hazards before there was significant decline of physiological functions through pleiotropic effects. Evolution of long-lived animals would only be possible by a change in the environment which reduced the chances of random deaths by starvation, predation and disease, so that development could take longer. On the Medawar model this would postpone the expression of pleiotropic genes.

Age-related deterioration is only found in animals that are under our protection. In the wild they rarely live to an age where they suffer a functional decline. The prediction that there should be an inverse correlation between the intensity of environmental mortality factors and lifespan is borne out by the field data, and, particularly with respect to birds, there is an inverse correlation between the ecological mortality rate and their maximum lifespan potentials in cages.

In part, the prematuration decline in cellular fitness within particular organs, which continues into old age, is a legacy of intrinsic genetic programmes selected to withdraw resources from systems that conferred survival value for only a limited period of development. It is also the result of error detection and correction being set by evolution below the levels required to sustain biochemical perfection throughout the short uncertain lifespan in the wild. From both points of view the body carries an ever-increasing burden of inefficiency.

Physical scientists viewing living organisms as systems for converting materials and energy have often remarked that cells do not operate at levels of efficiency that would be a first priority of an engineer-designer. From this developmental point of view the body is a balance of defects, the particular balance at any time being that which optimizes survival strategies against the uncertainties of accidentally encountering a lethal environmental situation.

Systems for increasing investment in growth and improving the efficiency of maintenance and repair will evolve so long as there is a return in increased survival of offspring. Intuitively it would be expected that genetic programmes would evolve to trade off resources between the body, which carries the genes, and the reproductive system, which maintains the gametes and ensures that they are used in reproduction. A genetic programme that fixed investment in the bodily systems above that necessary to give an adequate reproductive output, could disadvantage the organism as a whole when resources were in short supply. Survival of gametes could be improved in this situation by accepting a lower repair specification.

Conversely, an inadequate programme of investment in the systems that carry and support the reproductive organs could increase the chances of death before the organism had a chance to reproduce. This would favour those genotypes that devoted a higher fraction of their resources to maintenance. Therefore, natural selection can never set maintenance and repair at levels that would allow indefinite survival. The optimum level of investment in repair would be lower for species encountering a high rate of accidental mortality compared with one subject to a lower mortality rate. This competitive relationship between the needs of maintenance on the one hand and growth and reproduction on the other, has been formalized by Kirkwood and Cremer as the 'disposable soma' theory, the 'soma' being those tissues that support the germ line and its associated secondary sexual systems. Each species will have a particular value of this partition coefficient denoting the sharing of resources between maintenance and reproduction (Figure 3.14).

Kirkwood's theory makes an important connection between these ecological considerations and modern error theories of molecular ageing. The theory starts with the premises that:

1. The soma is that part of the body which carries and protects the germ cells and is off the main genetic line of evolution.
2. Organisms maximize their fitness by optimally allocating food resources among a variety of metabolic compartments.

The theory states that in the higher organisms which reproduce repeatedly, the optimum allocation of energy for somatic maintenance and repair is less that that required for the soma to last indefinitely. This means that unrepaired errors and somatic damage will accumulate through life, eventually causing senile degeneration and ultimately death in old age.

The argument starts with Medawar's view that even without being subject to ageing, given the continual hazard of accidental death, each individual soma would have a finite expectation of life. Death of the soma before reproduction is a waste of the resources invested in it. On the other hand, without investing

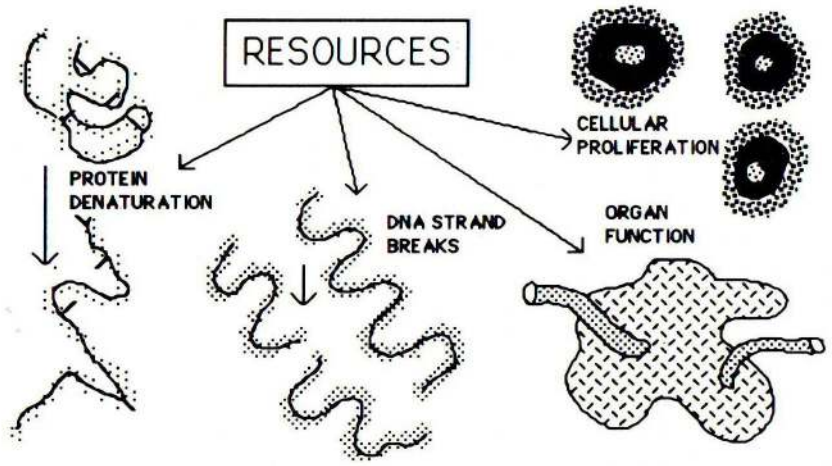


Figure 3.14. Flows of bodily resources which may be selected for or against according to availability in the environment.

resources the soma might disintegrate before it can reproduce. Therefore, too low an investment can bring a disadvantage, as can too high an investment because it is no advantage in repairing a soma so that it can last longer than the maximum time a species can expect to survive age-independent environmental hazards. In the latter case the excess resources would be better invested in a more rapid growth or greater reproductive output. Fitness, as the capacity to pass genes to the next generation, is maximized at a level of repair which is less than that which would be required for indefinite survival of the soma. This conclusion is independent of the total availability of environmental resources and is only concerned with the differential allocation of the resources, to support reproduction on the one hand, and maintenance and repair on the other.

A species subject to high environmental mortality will do better not to invest too heavily in each individual soma which will therefore age relatively soon, but should concentrate on more rapid and prolific reproduction. This may be called the 'mouse strategy'. Conversely, a species which experiences a low accidental mortality may profit by doing the reverse ('the elephant strategy'). In either case the level of investment in repair will be such that signs of ageing are not seen in natural populations. In using the two animals, mouse and elephant, to illustrate the strategies for 'high' and 'low' environmental mortality, an important relationship between longevity and development is highlighted. It appears that reduction of accidental mortality goes along with species adaptations for exploiting environments relatively rich in resources that enable them to attain a large body size.

The investment of resources in repair increases the chances of survival to reproduce. Eventually, a level of repair would be reached at which further investment would bring repair processes in competition with reproduction. Above this optimum level, an individual would begin to be at a disadvantage compared with organisms with a lower repair level. Evolution would therefore set investment in repair at a level lower than that necessary to keep all errors repaired. This optimum would be lower for species encountering a high rate of accidental mortality (species H) compared with one subject to a lower mortality rate (species L) (Figure 3.15).

SUMMARY

The best theoretical standpoint for discussing ageing in the context of evolution is that the basic cause of ageing is the accumulation of randomly acquired molecular damage. The processes of ageing are the outcomes of this accumulation of damage, which will vary according to the biochemical and physiological specialisms of the organ system. Damage is allowed to accumulate as a side-effect of traits selected for their positive contribution to fitness in early life, when the organism is programmed to develop, grow and reproduce. These adaptive traits are associated with suboptimal repair. This is not detrimental to fitness in the wild, partly because organisms stand a good chance of dying through accident disease or predation, but more importantly because it reduces the expense in energy and materials for complete repair through molecular and cellular turnover.

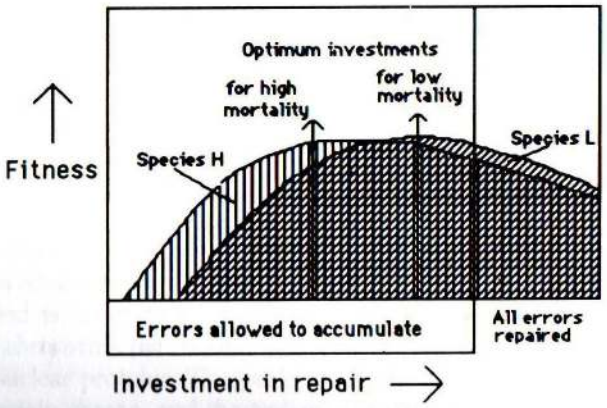
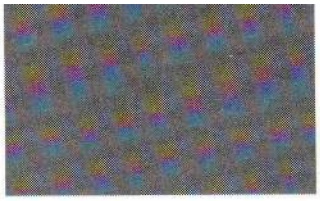


Figure 3.15. The Kirkwood-Cremer model of the "disposable soma".

According to this theoretical viewpoint ageing has evolved as a multifactorial by-product of pleiotropic genes. These genes optimize the body's allocation of resources among its many systems necessary for survival within an age-range when there is a high chance of individual survival. Degenerative diseases in old age are therefore side-effects of other processes which promote growth, survival during starvation, or suboptimal feeding, and reproduction. Ageing is expressed in an environment where the organism is protected from mortality factors of the wild. In this context germ cells are characterized by a high fidelity of surveillance and repair because extra resources are channelled into the gonads for this purpose, either during early development, at puberty, or as growth slows down.

In relation to non-adaptive theories of ageing, a very important area for future research is that concerned with resource partition. Hopefully, this will reveal the controls by which materials and energy are shared preferentially between cellular populations according to the survival value of this differential support at different times.



CHAPTER 4 Genetics and ageing

In the 70 years that have elapsed since the re-discovery of Mendel's laws, genetics has become not only a most important discipline in itself but also an integral part of every biological science. In particular, it is a keystone in human biology, for in the ultimate analysis the interaction between inherited constitutions and the environment are the sole biological determinants of all the characteristics of an individual and therefore of the population variability which is the prime concern of the human biologist. (Harrison 1977)

THE GENETIC SYSTEM

The genetic system may be studied from two distinct points of view. These viewpoints differ with regard to the kinds of methodology and the biological level of analysis:

1. 'Transmission genetics' is concerned with the ways in which patterns of inheritance, expressed in structure of chromosomes, and the routes by which behavioural, physiological and morphological phenotypes of individuals, enter populations and are maintained through mating and crossing.
2. 'Molecular genetics' deals with genes as nucleic acids and their biochemical expression as the DNA code.

Transmission genetics is important in gerontology because it is a significant factor in the demographic analysis of the clinical expressions of ageing. Molecular genetics is where the breakthrough will come in genetic manipulation of the diseases and longevity.

The approach to ageing through molecular genetics was pioneered by groups such as Kurtz, Russell and Sinex. The starting point is that a plot of thermal denaturation of isolated mouse brain DNA against temperature reveals up to 15 reproducible peaks. This phenomenon is termed the 'hyperchromicity of DNA', and is taken to be a reflection of the complexity of the internal structure of chromatin, particularly with regard to the structural links between DNA and nuclear proteins. The peaks probably reflect differences in protein bonding, protein charge, and the tertiary structure of the DNA. The type of result obtained is exemplified in Table 4.1.

An age effect is seen when the denaturation curves are divided into intervals

Table 4.1. Hyperchromicity and protein/DNA ratio as a function of age in mouse brain nuclei

	Age (months)			
	Young	Mature	Old	All
Percent hyperchromicity	3.4	11.6	18.7	23
Temperature °C				
50–60	5.5	14.5	5.1	8.2
64–72	18.0	23.5	15.1	22.0
72–78	25.5	19.9	26.2	20.4
78–92	51.0	42.0	53.6	49.5
DNA/Proteins				
DNA	1.00	1.00	1.00	1.00
Non-histones	2.19	0.85	2.50	1.85
Histones	1.39	1.45	1.23	1.35

between which minima occur (Table 4.1). Chromatin in young animals more closely resembles that in old animals than in mature animals. The ratios of non-histone to histone chromatin proteins show age changes taking place in the proportion of non-histone proteins. Also, there is a linear relationship between the content of non-histone protein and the amount of DNA denaturing between 78° and 92°. Apparently the structural association between non-histone protein and DNA raises the temperature at which denaturation is expressed.

It is concluded that the age-related variation observed in overall thermal stability is related to changes in the relationship between DNA and non-histone protein. This type of analysis therefore tells us about the molecular bonding of genes to a nuclear protein matrix. It is relevant to the working of genes because the formation and breakage of structural bonds between protein and DNA may govern the release of genetic information. Therefore, evidence for age changes in protein bonding to DNA supports ideas that the making and breaking of linkages between non-histone protein of chromatin is a part of the molecular switching of gene expression during development and ageing.

An example from transmission genetics is the research of Higginson and Oettle on combined influences of heredity and environment on demographic expressions of human degenerative diseases which was carried out during the late '50s (Table 4.2). The study is based on a survey of cancer incidence in Bantu peoples of the South African Transvaal. The results were compared with the expected incidence of the same types of tumour in American Whites, American Blacks, and Danish populations, all of corresponding age distribution. Compared with other populations, cancer of the colon, stomach and rectum were much less common in Bantu males, and cancer of the breast and body of the uterus were lower in Bantu females. In contrast, liver cancer was much more frequent in Bantu males. Both Bantu and American Negroes had low incidences of cancer of mouth, lips, and skin. This is probably attributable to pigmentation. Black

Table 4.2. Incidence of various forms of cancer in different human populations

Form of cancer	Bantu	White (USA)	Black (USA)	Danes
Males				
Buccal and lip	2	21.3	2.0	24.3
Colon	7	75.7	54.1	43.6
Liver	114	13.8	25.7	1.4
Lung	40	103.0	104.3	51.1
Oesophogagus	53	20.9	36.5	12.2
Penis	8	3.9	11.4	3.6
Rectum	5	67.8	45.7	61.9
Skin	14	212.7	18.2	49.5
Stomach	41	89.0	128.4	119.4
Tongue	8	12.9	9.3	
Females				
Breast	50	221.9	170.7	131.8
Cervix uteri	198	119.1	266.6	122.1
Corpus uteri	1	27.9	25.5	23.2

females generally had a high rate of cancer of the cervix compared with Whites. The study also revealed differences between American Whites and Danes in the frequencies of gastric, hepatic and mammary cancers.

This kind of study points to the importance of both genetic and ecological factors in ageing and their interaction which may be revealed in several ways:

1. The physical environment is an immediate and direct source of injury.
2. The biotic environment is a source of infectious disease from a range of pathogenic organisms.
3. The social environment produces limitations in food availability and deficiencies in food value.

Broadly speaking, information about the genetic system may be organized to answer six basic questions (Figure 4.1). This chapter deals with the problem to what extent and how are the morphological traits we define as degenerative diseases inherited.

DIFFICULTIES IN STUDYING THE HUMAN SYSTEM

Since records first began over 100 years ago it has been clear that the proportion of old people in the population is steadily increasing while that of the young is considerably decreasing. In the United Kingdom 100 years ago Victorian society was relatively young. Large families were commonplace and a

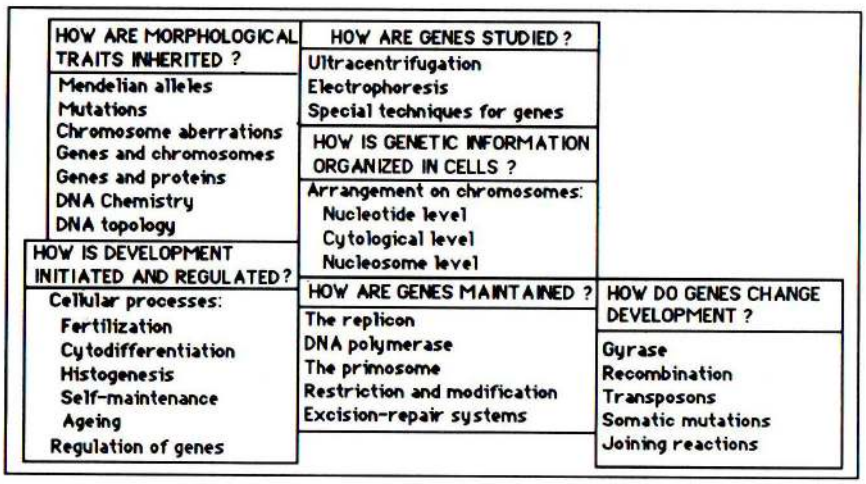


Figure 4.1. Six questions about the genetic system and the subject areas which provide the answers.

third of the population was under the age of 15 and only 5% over the age of 65. After 1900 the birth rate fell sharply as smaller families became socially desirable. Now, there are equal numbers of the very young and the very old in British society.

Whilst a lowering of family size has made a large contribution to the historical shift in balance in favour of the elderly, changes in mortality in different age categories has also played a part.

All descriptions of ageing at the species level begin with the tabulation of the numbers of individuals dying at different ages. For human populations the necessary data is obtained from the Government registry of death certificates. In its simplest form this is a table of the numbers of people that died in a particular year, separated into groups based on their age at death. For European and North American countries these lists tell us that most people die between the ages of 50 and 90. If this data is combined with information from a population census, which lists the numbers of people alive in a particular year with their ages, it is possible to calculate the percentages of people of each age class alive at the beginning of the year, who are likely to die during that year. This information can be used to calculate mortality rates at different ages and different times (Figure 4.2).

Assuming that this age-specific death rate has acted on a hypothetical population of say, 100 000, an important graphical representation of mortality may be constructed, derived from the tabulation of the number of survivors at different times after birth. Historical comparisons between modern and past populations shows that mortality rates have decreased in the very young and

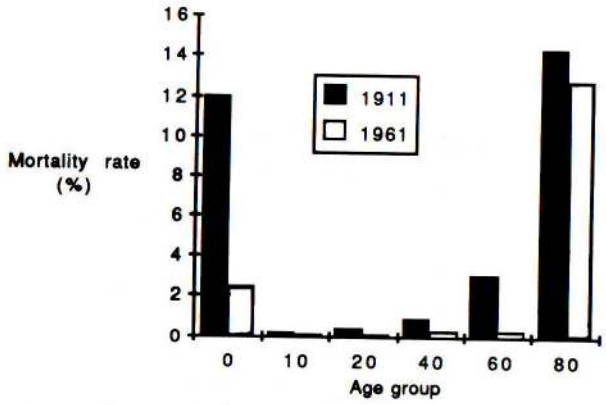


Figure 4.2. Historical mortality rates for United Kingdom populations.

the middle aged, although the maximum age at death has not increased significantly (Figure 4.3). These comparisons have shown that over the past century, mortality curves from all parts of the world have changed to take on a more rectangular shape. This has come about by a drop in infant mortality and a decreased death rate in the middle-aged group. These changes are attributable to improvements in nutrition, sanitation and medical care, reducing the impact of environmental hazards.

The application of the mathematics of probabilities to census data, which is available at 10-year intervals, enables these mortality curves to be used to predict the risks of insuring people of different ages against death. Since the cause of death of every person must be registered, mortality statistics can also be used to plot the frequency of each cause of death at every age.

Despite the statistical sophistication of the methods for calculating the risk of an individual of a certain age dying, the method cannot be used to determine the actual current trend of ageing in a population. This is because the environmental influences affecting the medical history of the youngest group in the census will not be the same as those that the oldest members of the censored population have already encountered. For example, the oldest members of all present day populations are likely to have suffered badly from infectious diseases compared with modern children, who can be protected by immunization, and rapidly cured by antibiotics. The residual effects of these early encounters with infectious diseases may well have produced a faster rate of ageing in their middle age compared with the present day middle-aged population. From the point of view of the present discussion it is likely that the differences in mortality factors acting on two historically different populations would also mean that the genetic baselines were also different because the populations would be equilibrated with different selection pressures.

Within these unknown but important limitations, Government vital statistics

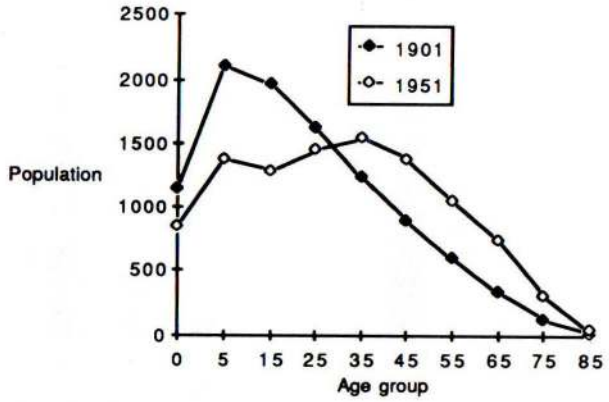


Figure 4.3. Historical comparisons of two United Kingdom populations by age categories per 10 000.

may be used to compare mortality rates between human populations, not only in historical time but also in geographical space. Although it may be said that causes of death now depend less on environmental risks, ongoing comparative studies on co-existing populations, between countries and regions, has shown that significant geographical differences in both death rates and causes of death still exist. However, human demographic data is too uncertain with regard to the balance between environment and genes so this approach cannot be used to investigate the genetic basis of human ageing. For example, it has been estimated that longevity is about 90% heritable. However many geneticists would say that the environmental or non-genetic element is much greater than 10% for most populations taken world-wide. Studies on the genetics of human longevity usually take the conventional viewpoint that there is a distinction between, on the one hand, genetic effects of one major mutant gene, following a single-factor type of inheritance which causes premature or pathological disturbances, and on the other, a more general genetic influence resulting from the interaction of many genes. The latter polygenic mode of inheritance is thought to be responsible for a large measure of variability in the lifespan in the human population, but in practice it is difficult to distinguish polygenic effects from single-factor pathological processes. With respect to the latter point, the frequency distribution and extent of pathological changes in human subjects are clearly genetically conditioned. For example, at the histological level generalized atheroma is considered almost a normal finding in the aortic arch for a person over the age of 60 yet is rarely found in the pulmonary artery of the same person. This points to the epigenetic basis of age-related diseases. With respect to genotype, twin studies indicate a greater concordance of cause of death from cancer and tuberculosis in one-egg twins compared with two-egg twins, and

this points to a genetic basis for variability in the times of onset and frequency distribution of degenerative diseases of ageing.

It has been established from many human studies that patterns of the ageing of individuals carrying an identical array of genes show greater similarity than the patterns of the ageing of individuals carrying different assortments of genes. In particular, ageing in every human population has many different phenotypic manifestations. However, these manifestations are not unlimited and most people will encounter the same battery of cosmetic changes and failures of physiological function. In particular, failing eyesight and wrinkling of facial skin for people over the age of 50 is commonplace, whilst loss of hair pigmentation and baldness are less uniform. The few people over 80 who are spritely and free of degenerative disease are the exception to the rule. The medical situation in the very old is illustrated by the story of the post-mortem of a male patient aged 102 which revealed generalized atherosclerosis; thrombosis of the left femoral, popliteal and tibial arteries, and of the accompanying veins; terminal bronchopneumonia, a carcinoid tumour of the ilium, enlargement of the thyroid and chronic emphysema; the cause of death was gangrene of the left foot!

To summarize, there is a wide range in the phenotypic expressions of age-related disease in the bulk of the population at all ages, although on average the main causes of death in old age are uniform throughout the world namely, heart disease, brain disease and cancer. To have long-lived parents does not guarantee long life, and there are examples of very long-lived individuals in every walk of life, social class and nutritional category.

HUMAN LIFESPAN GENETICS

The above features of the human race, together with outbreeding patterns of marriage, which give every person a unique genotype, make it impossible to study human populations directly, and the main lines of enquiry have sought to find general rules of inheritance of age-dependent phenotypic characters by studying differences and similarities between parents and offspring. In this context, the literature dealing with the problem of genetics and human lifespan may be divided into two types. There is the data derived from genealogical records involving large individual kinships and the collection of genealogies, and data taken from special subgroups of the population separated out according to occupation, size of family and so on. All of these studies may be criticized in terms of the weak statistical analysis and the unrepresentative nature of the chosen sample. There are also important differences with respect to the present day in that the early studies were carried out on populations that were subject to high probabilities of death from bacterial infections. Nevertheless, this kind of population analysis is in favour of the conclusion that in general

people with long-lived parents can expect to live longer than those with short-lived parents.

Lifespan is a species characteristic and this implies that genes exert an important and major biological influence on longevity and ageing. For humans the evidence is statistical and based on the analysis of pedigrees. One of the first gerontological investigations of collections of genealogies revealed this relationship. It was carried out in the 1920s by Bell using information on the descendants of William Hyde of Norwich, Connecticut who died in 1681. When classified into groups according to age at death a direct relationship was revealed between the longevity of parents and offspring. The average lifespan of children whose parents had both died before the age of 60 was about 20 years shorter than the average lifespan of offspring with parents living to more than 80 years of age. In another early study of a selected group of nonagenarians and centenarians, about half (between 46 and 57%) of all individual matings for two generations were found to be composed of two long-lived persons. In a control group drawn at random from the population, less than 37% had long-lived ancestors (range 12–37%). Surveys and correlations of this kind for all the offspring of octogenerians were gathered together by a group at the Johns Hopkins institute in the 1920s and '30s. The data was divided according to the age of the spouses of the octogenerians at the time of their death as follows:

1. In 448 cases the spouse lived to be over 80.
2. In 490 cases the spouse died between 60 and 80 years.
3. In 200 cases the spouse died between 40 and 60 years.

These groups provided a correlation between age at death of the spouse and longevity of children. It was a weak correlation but produced a statistical prediction that if both parents survived to be octogenerians their children could, at most, expect four years extra longevity above average.

Data on the heritability of human lifespan comes more directly and classically from studies on identical twins, who have a much smaller mean difference in age of death compared with fraternal twins (Figure 4.4). Longitudinal twin data show that all measurable differences are more pronounced for ageing two-egg twins than for identical twins arising from the same fertilized egg. The relevant parameters include physical features, mental abilities and psychological disturbances and social adjustments. This is also borne out by the smaller intrapair lifespan differences of identical twins. Also, one-egg twin partners are more than twice as similar in causes of death as two-egg pairs of the same or opposite sex. Additional support for strong genetic influences is that there are marked parental effects, in that ages at death of offspring are related to the parental age at death.

Work of this type is the best one can get scientifically to a genetic analysis of the human factors of longevity. It might be argued that the similarities between

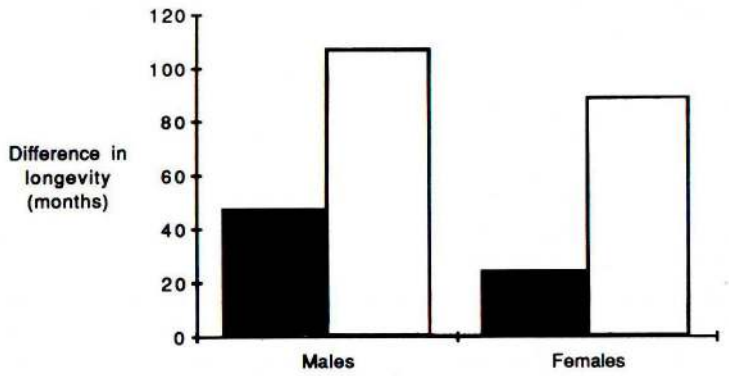


Figure 4.4. Intrapair differences in the lifespans of 58 twin pairs. Black = monozygotes; white = dizygotes.

ages at death in single-egg twins arises from the tendency of the pairs to seek similar environments, but on the whole the similarity in ageing patterns of monozygotic twins occurs even when each member of a pair has experienced a different physical and social environment. Where an identical twin has experienced a vastly different environment from the other it is sometimes possible to see that cosmetic effects of skin ageing depend greatly on environment. All of this may be summarized for the general population by the statement that statistically, the genetic element in the human lifespan is illustrated by the extra four years which is added to the mean lifespan by having all four grandparents surviving to 80 years of age. The environmental influence is indicated statistically by the average life expectancy in cities being five years shorter than that in a rural environment.

Even where there are differences in lifespan within populations due to genetic mechanisms, a difference in mean lifespan between populations need not be due to genetics. Differences in average lifespans between populations may have a genetic basis, even though there is no genetic basis for differences in the lifespans between individuals.

Generally, the correlation coefficients between lifespan of relatives are rather low, and it is possible that the correlation arises simply because genotype and environment vary together. This is particularly important in the study of data for human twins. On the other hand the studies often do not take full account of the proportions of deaths not due to ageing, such as infectious diseases, car accidents or suicides.

A critique of the problems of using family histories in work on the genetics of human longevity comes from the study of the fatal neurological disease called kuru. This disease was found to be a major cause of death in a tribe in western New Guinea. Initially the study of family genealogies indicated that kuru was

due to a dominant gene. Later work revealed that, rather, it is caused by a very slowly reproducing virus-like agent transmitted from parent to offspring through eating the brain and other parts of a deceased parent.

With respect to 'experiments of nature' in which an individual appears with the signs of accelerated ageing we can turn to the rare clinical expressions of premature human ageing, such as progeria and similar progeroid syndromes. Progeria was first described in 1884 by two British doctors Hutchinson and Gilford. It is a genetically dominant disease, occurring in all races and all countries, which affects about 1 in 6 million births, and is likely to be due to a structural gene mutation. The Hutchinson–Gilford syndrome first appears in children at about 2 years of age when they begin to lose their hair. They are emaciated with a lack of subcutaneous tissue and show age-related changes in skin pigment, and disproportionate development of facial features particularly in the jaw. They may develop limited motion at the joints due to an arthritic condition. The terminal digital bones of the fingers become eroded and they frequently develop hip dislocations in their early teens. By the average age of 13–14 they develop severe heart disease and die of atherosclerotic cardiovascular disease, and at autopsy they show severe arteriosclerosis.

Another condition of premature ageing is Werner's syndrome which affects adults. These patients tend to a grow normally in childhood but stop growing in their teens. The hair may turn white about the age of 12. If they survive to the age of 30 they often have severe atherosclerosis, with aged-skin pigmentation, and non-healing leg ulcers. Werner's syndrome is generally accepted to be caused by chromosome instability.

Another disease which may be connected with genetic changes in the rate of ageing is Down's syndrome, which occurs in about 1 in 1000 births. Childhood development is delayed and by early adulthood patients often have a premature aged appearance and a reduced life expectancy. Whereas, at the age of 40 most people might expect to live 30 more years, for Down's syndrome the average life expectancy is only about 8 years. They have a high proportion of histological changes in the brain which resemble those of Alzheimer's disease. The syndrome is due to an extra set of genes, there being three chromosomes 21 instead of two. The presence of the extra chromosome appears to stimulate the release of information from chromosome 21 genes. So far, about 16 genes have been traced to this chromosome, including the gene for the soluble superoxide dismutase which is thought to be important in protecting the cells against oxidative damage, but none of the genes has yet been clearly related to any particular pathological character. It has been said that Down's syndrome is a prime example of a genetic premature ageing disorder. This may be the explanation of the Alzheimer's change.

The evidence that Alzheimer's disease has important genetic aspects is based on observations that in certain families there have been individuals in successive generations with dementia and that the observed morbidity risk has been

Table 4.3. Down's syndrome in families of Alzheimer's disease probands.

Study	Probands	Relatives	Down's	Ratio (D/R)
1	125	3044	11	3.6:1000
2	74	329	0	
3	32	684	4	5.8:1000

approximately 50% in each generation. There have been over 50 reports of such pedigrees. There is also evidence for a familial association between Down's syndrome and Alzheimer's disease which rests on the finding of an excess of Down's syndrome in the relatives of Alzheimer probands (Table 4-1).

The breakthrough in our understanding of progeroid syndromes was the realization that they could be attributed to the action of one or a very few genes. This led to work with cultured cells from progeric patients. This work has revealed that the number of divisions that can be sustained in tissue culture is markedly reduced in cells from patients with Werner's syndrome. On the other hand, cells from patients with Huchinson–Gilford progeria have *in vitro* lifespans that are within those of tissues derived from normal people.

NON-HUMAN DEMOGRAPHIC MODELS

The major stumbling blocks in the study of human ageing, namely population heterogeneity and the difficulty of separating genetic from environmental influences on lifespan, can only be overcome by the use of non-human models. In this regard, the strongest evidence for a direct connection between genetics and lifespan comes from analysis of species-specific mortality. There is at least a fivefold range in lifespan across a wide variety of vertebrates, and breeding experiments have shown that there are also strain differences within various species.

It might be thought that it would be easier to obtain better demographic data for non-human populations. However, there are great problems of ageing individual animals accurately in the wild and ensuring that the sample is truly representative of the population. The dominant effect of environmental mortality in determining longevity would be expected to be expressed as an inverse relationship between the rate of natural selection in the wild and the lifespan that emerges in a protective environment. Unfortunately we have very little information on the natural population dynamics of the animals we protect from natural selection in our zoos, farms and the domestic environment. Records for captive animals are often suspect, there being a tendency, particularly in zoos, to overestimate the age of their inmates because of

popularity of old animals and their public relations value. Generally, it seems that the smaller mammals and birds that live, on average, for a few months in the wild, die of old age in a protective environment a few years after birth. It is the species genetic element which probably accounts for the thousandfold variation in the maximum lifespan between animal species in captivity.

Comparative ecological data is sparse and indicative of the ease with which the species may be studied. Taking all of the data together the overall range of animal lifespans, from the smallest metazoans, with a maximum longevity of a few days to man with a lifespan over 100 years is about 1–3500. This is about the same longevity range found in the higher plants. The existence of this well-defined pattern indicates that the variation in the characteristic lifespans of species depends on the genetic constitution selected in the course of evolution.

Wild type investigations of ageing depend on measurements of anatomical features that leave an annual 'mark' in individuals such as bands of different composition and changes in polymer structure in proteins of eye lens and connective tissue, and mineral deposition in teeth, bones and shells. On the other hand freshly killed specimens may be aged by the state of development of certain organs such as those connected with reproduction. For example, the number of ovarioles in the ovary of insects or the number of corpora lutea in mammalian ovaries may sometimes give a general assessment of age. Here the difficulties are connected with calibration of the feature to make sure that the parameters are related only to the passage of time and not to the rate of ageing.

The most commonly used practical methods of assessing age of wild animals is the mark–recapture technique. This method is of limited value. Where it has been carried out on a large scale, as in bird-ringing, it provides data on the maximum and minimum lifespans, but the small fraction of the actual population that can be ringed, together with the very low returns and ring loss from very old birds, make it difficult to obtain firm statistical data for the older cohorts. There is also the possibility that the stress of capture and marking may shorten lifespan of the birds after release. Also, animals that allow themselves to be caught may differ from those in the bulk of the population. For terrestrial species the method can only be used to advantage where it is certain that there is no movement of animals into and out of the experimental sampling area and the sample of animals trapped is a large fraction of the population, giving a representative cross-section.

Using data from ringing, adult birds are assumed to have a constant mortality rate in most bird population studies. However, the annual mortality rates reported for many bird species would result in exceedingly long potential lifespans. It seems more reasonable to assume that avian mortality is age-dependent with a minimum occurring in mid-adulthood and thereafter increasing. Fish are also believed to have an approximately constant adult mortality throughout life, but this conclusion is based on better data and more reasonable biological assumptions. Ages of most bony fish can be determined

directly by counts of the annular rings on their scales, and complete samples of all age classes of a fish population can often be obtained at an instant in time, or a cohort can be followed through several years of sampling.

Rodents, with their good laboratory base for experimental testing of ideas arising from ecological studies, offer the best potential models for making comparisons between ageing in the wild and laboratory environments. The data for the house mouse, living wild on the small offshore Welsh island of Skokholm is probably one of the best sets of continuous data for studies of wild-type lifespan in relation to the 'same' genotype kept under laboratory conditions (Table 4.4; Figure 4.5). The data was assembled by annual trapping for well over a decade, and involved genetic analysis of blood proteins and physiological investigations of metabolic rate of freshly caught specimens from live-traps.

In the wild, Skokholm mice have very short lifespans, during the summer months on average less than 100 days. Reproduction ceases in October and peak mortality comes in February, which correlates with the coldest average temperature. In March and April a small number of females overwintering from the previous year begin to expand the population once more. Over the years the population has oscillated from a few thousands at the annual peak to a few hundreds at the spring minimum on an island that is about a mile long, by about a quarter of a mile wide.

The population is carried from year to year by late autumn litters which overwinter to begin reproduction in March. There are strong selection pressures against the calculated Hardy–Weinberg equilibrium for a number of genes, and the magnitude, and direction, of selection varies month by month. With regard to predicting lifespan, about 25 individual morphological and

Table 4.4. Life tables for a 1 year cohort of the SK wild house mouse on Skokholm Island

	Life Expectancy (weeks)			
Month of birth	March	May	July	September
Month of sample				
May–June	13.9			
July–August	13.3	15.5		
Sept–Oct	12.5	14.3	14.3	
Nov–Dec	10.9	12.3	12.3	11.0
Jan–Feb	10.2	13.3	13.4	12.4
March–April	15.8	15.7	15.8	15.8
May–June	14.7	14.7	14.7	14.7
July–August	13.0	12.9	13.0	13.0
Sept–Oct	10.0	9.9	10.1	10.1
Nov–Dec	5.2	5.1	4.9	4.9
Jan–Feb	4.3	4.3	4.9	4.9
March–April			4.3	4.3

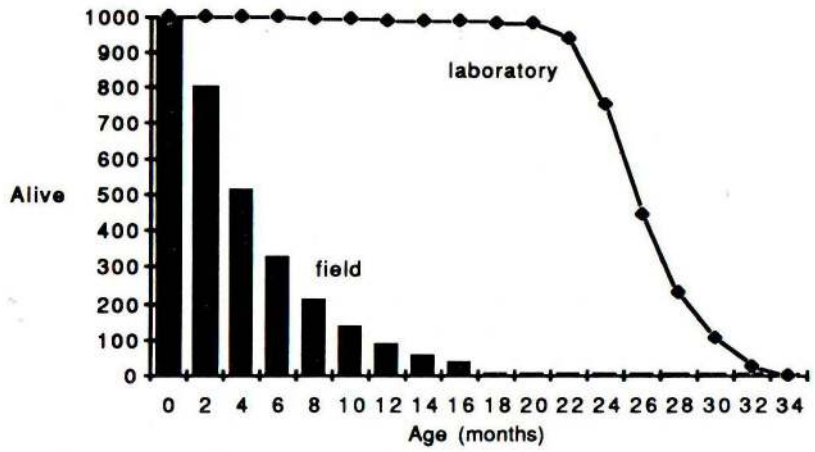


Figure 4.5. Longevity of Skokholm mice in the wild and in the laboratory.

biochemical measurements are required to get within a 70% probability of the actual age of an individual.

One of the methodological problems of trying to study the genetics of a population of small rodents by trapping live individuals is that only fit, healthy, and probably hungry individuals will enter the traps. Despite the very large numbers of specimens trapped on Skokholm over the years we are still ignorant of the causes of deaths of animals that disappear from one trapping to the next, although predation has been ruled out as a major factor.

Animals trapped on the island have been taken quickly into captivity and bred for several generations to obtain mortality curves. In terms of the mean longevity, the laboratory strain from the island is as fit as the fittest standard strains of mice. They die from the same causes with the same cellular pathologies. However, another methodological problem in interpreting this data is that there have been several points at which human selection has produced a different mean genotype from that which was studied by trapping in the wild. First, only those animals which entered traps could be taken from the island. Then some of them died of stress on the journey to the laboratory, and many did not breed under laboratory conditions!

Whilst this difference between wild and laboratory lifespans may be in part due to the selection of genotypes that have the potential to adapt to laboratory conditions, it is more likely due to the causes of death in the laboratory being different from those in the wild. In the wild, the major causes of death are predation, disease and possibly malnutrition. It is rare to find an elderly, unfit animal of any species in the natural world. Where large old animals are observed, as for example very old and infirm elephants in some of the African

game parks, this is because of human conservation measures. Under past evolutionary pressures most died as juveniles and the remainder during the period of active reproduction. In this respect it has been said that accidental deaths would limit life in the wild even if organisms were potentially immortal. On the other hand, laboratory animals, which are allowed to live longer in cages or parks, become senile and suffer from degenerative diseases similar to those which afflict the human population. This is not to say that animals in the wild do not have an ageing pathway, but rather, because early mortality is so high, they die before the degenerative conditions observed when under human protection become manifest.

THE GOMPERTZ–MAKEHAM EQUATION

Laboratory animals are usually of a uniform genotype and kept under controlled laboratory conditions. All members of the population have the same environmental history, but they do not die at the same instant of time. This variability in resistance to death, revealed in the spread of the mortality curve, which expresses the age-frequency distribution of the times of death, indicates that events must have occurred at random to produce different rates of ageing. Theories to explain the evolution of ageing address these two characteristics of longevity. A successful theory has to explain how lifespan has come to be connected with the genetically determined programme of development and therefore predictable as a species characteristic, yet it is unpredictable for each individual, even where many genes are shared with brothers and sisters.

The dual influence of age-dependent deaths and age-independent random environmental influences may be expressed mathematically in the Gompertz–Makeham equation. This dual equation contains an expression indicating that the age-specific death rate for humans beyond the age of 35 increases exponentially with age. At any age ux (the age-specific death rate), equals u0 (the intercept at birth of the straight line drawn through the logs of the mortality rates at different ages) to the power, a, (the slope constant). This is the Gompertz relationship.

$$ux = u0e^{ax}$$

The rise in the force of mortality is a characteristic of each population and a measure of the differences in age at death between members of the population. These differences are due to differences in genetic constitution and the environmental circumstances that they have each encountered from birth. The equation is a characteristic of the population and as such it may be used to describe differences in genetics, environmental circumstances and random encounters with mortality factors, between populations. Deaths in populations which approximate to the Gompertz function are age-dependent, but the

equation does not provide any information about the rates of ageing of individuals within populations or between populations.

This basic equation, due to Gompertz, was modified by Makeham, who made it a better fit to a wider range of data by adding a constant B, representing age-independent causes of death. Where environmental influences are dominant in bringing about mortality, the Gompertzian element of this equation makes a minor contribution. The combination of the intrinsic elements of Gompertz with the environmental term of Makeham enables all types of mortality curve encountered in the wild and in a protective environment, to be approximated with a single equation (Figure 4.6).

LABORATORY MODELS

The importance of genotype in determining longevity comes out more clearly from studies of inbred experimental animals where the environment can be controlled with relative precision. Direct evidence on the hereditary basis of ageing is provided by experiments involving the classical experimental techniques of transmission genetics such as inbreeding, crossbreeding, selection experiments and the analysis of lifespan differences related to sex. Other indirect data comes from studies which implicate DNA in the ageing process, both theoretically and experimentally.

Very few life-tables have been constructed for animal species other than man, and the animal data do not usually refer to breeding experiments. Most of the comparative information on generation life-tables comes from work on laboratory rodents and fruitflies, where it is convenient to follow relatively large numbers of separate cohorts repeatedly from birth to death. Such

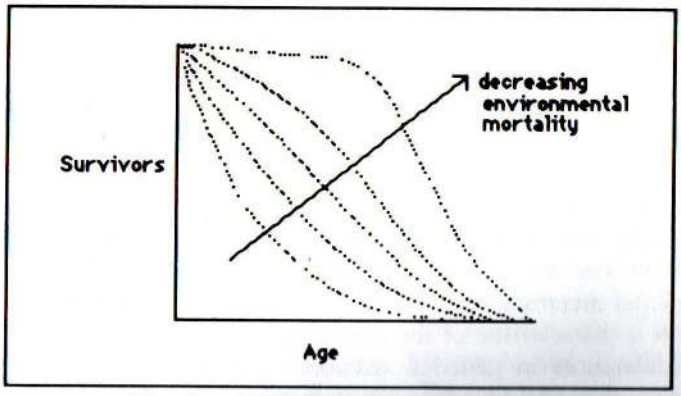


Figure 4.6. A family of Gompertz–Makeham mortality curves with different values of B.

experiments on selective breeding have produced short-lived and long-lived lines.

One of the first laboratory studies using *Drosophila* demonstrated great differences in the lifespans of five mutants with mutations all on the same chromosome (Table 4.5). Although this kind of work shows that genetic mutations occurring during life may influence lifespan it is difficult to relate such laboratory experiments to variations from a general point of view. However, the duration of life of various gene combinations is only rarely the average of the single mutations entering it.

Starting from the viewpoint that lifespan is genetically controlled, selection favouring reproduction late in life should increase longevity of offspring. An operational understanding of the ageing process therefore requires an experimental procedure for the creation of long-lived and short-lived strains. The first clear demonstration that lifespan can be increased through experimental selection was obtained using the fruitfly *Drosophila*. Selection was for early and late reproduction (i.e. short-lived and long-lived animals), and lifespan was measured every four generations for 21 generations. In one line, the larval density was controlled at low levels, whilst in the other, larval density was not controlled, and the larvae developed in crowded conditions. Where the larval density was controlled, selection for age-specific reproduction failed to generate any changes in lifespan. However, in the line where the larval density was not controlled, populations responded to selection for late reproduction with a 150-fold increase in the mean and maximum lifespan.

Larval density affects the expression of genes controlling ageing. Genes for short life are dominant at low densities, but display additive inheritance at high

Table 4.5. Longevity (days) in a wild strain and of homozygotic mutants and combinations of mutants of chromosome 11 of *D. melanogaster*

Strain	Female	Male
Mutants		
B	40.3	41.1
P	21.8	27.4
V	21.0	15.0
A	28.2	25.2
S	38.9	46.6
Combinations		
B–P	24.1	30.4
B–V	24.2	16.4
B–A	23.2	20.1
B–S	30.0	32.4
P–V	19.0	11.7
P–A	32.0	36.0
P–S	23.0	23.7
A–S	34.7	38.4
Wild type	40.6	38.1

densities. In this experiment parental age had no effect on the longevity of offspring. These studies also support Williams' pleotropic theory of ageing (see Chapter 3), in that lines selected for early reproduction had a shorter lifespan, but produced 24% more eggs early in life than the long-lived, late-reproducing lines (Figure 4.7).

Although there are some good reasons for accepting that lifespan is, to a certain extent, an hereditary trait it is difficult to carry out definitive experiments to prove this. Artificial selection has the aim of producing a permanent change in specific phenotype characters of a population by selection of parents with a desirable genetic trait. It is therefore is an obvious experiment to try. However to date there have been no unambiguous results from studies involving this kind of approach.

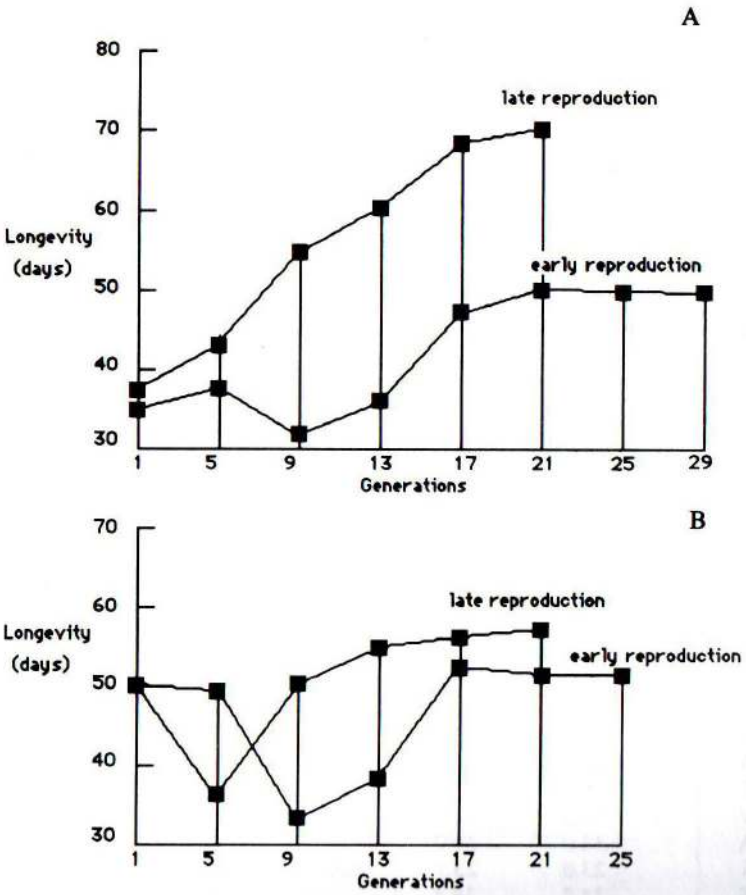


Figure 4.7. Selection of *Drosophila* for lifespan enhancement. A Larval density uncontrolled. B. Larval density controlled.

Reproduction through any mating system that encourages inbreeding affects the ongoing population in two ways. When it is imposed on a normally outbreeding population, that population becomes segregated into genetically distinct subgroups. Continued inbreeding is accompanied by an increased homozygosity which leads sometimes to a loss of fitness which is characteristic of the genotype and the number of genes fixed in the line. It is very easy to obtain short-lived strains of most laboratory animals but always the lifespan appears to be shortened because of the fixation of genes which promote abnormal physiological syndromes. In mammals these often have adverse effects on endocrine function and so reduce the efficiency of physiological homeostasis.

The usual approach is to look for lifespan differences when two healthy inbred lines are crossed together. Hybrids produced in this way are generally fitter than their parents with respect to one or more characters, and may even be fitter that the natural populations from which the inbred lines were derived. In one such study on *Drosophila*, using two lines previously inbred for 14 generations, and the hybrids obtained from reciprocal crosses between the lines, the expectation of life at emergence for the hybrid strains was approximately twice that of inbred flies. Similar results but on a small scale have been obtained in mice and cage birds. In one of the mouse experiments the mean longevity of two inbred strains was DBA, mean of 20.3 months, O mean of 22.4 months; and the mean longevity of the hybrid DBA/O strain was 24.1 months.

The reason for this phenomenon of hybrid vigour, termed heterosis, is not known. It could be due to the fact that at any given locus the heterozygous combination of two genes produces a phenotype superior to either of the homozygotes. Since a hybrid has relatively favourable dominant alleles at more different loci, another explanation is that heterosis is due to a new combination of favourable dominant genes and unfavourable recessives. The relevance of such experiments to the possible genetic control of lifespan in natural populations is unknown.

SOMATIC MUTATIONS

Another important line of research that has implicated chromosomes directly in the determination of lifespan has been the emphasis placed on mutation theories of ageing. In this context, a mutation is defined as a loss of one or more DNA information sequences, occurring one cell at a time, due to random impact of the internal or external environment. It is a logical approach based on the assumption that DNA, like all polymers, would be expected to have some level of intrinsic chemical instability, and there is a chance that this would manifest itself the longer organisms survived accidental death. Mutations in the somatic cells are losses of DNA integrity that take place in cells of the body rather than

reproductive cells. By occurring during the lifespan of a single individual they could contribute to ageing through the rise in the number of cells deficient in their capacity to process genetic information. If there was a loss of DNA critical to the overall integrity of the cell, that cell and its critically needed potential descendants may disappear. Alternatively, the mutated cell may persist and produce a line of defective cells which do not react appropriately to signals from other parts of the body, or which secrete products harmful to the body. The consequences would depend on where and when they occurred, but there is a good chance that the net effect of accumulated somatic mutations would be deleterious. The main proponent of this view was Curtis who in the 1960s listed seven main pieces of supporting evidence (Table 4.6). The general position has changed very little since then, although there has been no definitive identification of any particular mutation. The problem of testing the theory is that somatic mutations cannot be identified, as can germ line mutations, by breeding experiments to segregate the character.

The direct evidence for somatic mutations comes from histological observations on the frequency of structural chromosome aberrations in cells from regenerating livers of various mammalian species. Liver regeneration is a necessary part of the experimental system because normally very few hepatic cells divide, and the surgical removal of part of the tissue is necessary to visualize events that

Table 4.6. Curtis' main pieces of evidence for the somatic mutation theory of ageing in the 1960s

1. There seems little doubt that somatic cells undergo mutation at a very high rate and do so whether or not they undergo division. Further, in mice and presumably all mammals, mutations build up with age in the somatic cells until as many as 70% of the liver cells for example, will have grossly abnormal chromosomes.
2. Ionizing radiation shortens the lifespan of mammals in a manner closely resembling that of ageing so that the phenomenon is commonly referred to as radiation-induced ageing. Radiation also is a very potent mutagenic agent. The degree of acceleration of ageing produced by various kinds and regimens of radiation is proportional to the number of mutations produced by that treatment.
3. Pure strains of mice which have a short life-span develop somatic mutations at a high rate, and those that are long lived develop mutations much more slowly.
4. The more we find out about carcinogenesis the more it appears that mutation is at least one component of the process.
5. The loss of parenchymal cells with age seems reasonably well substantiated. One of the consequences of mutation is certainly cell death, when the mutation eliminates a gene responsible for an essential cellular function.
6. At least some of the vascular disorders associated with ageing may be due to mutations occurring in the intima of the vessels leading either to an abnormal cholesterol metabolism or a breakdown of the integrity of the vessel.
7. By far the most plausible explanation for the initiation of autoimmune disease is a mutation.

have occurred in interphase nuclei at the structural level of chromatin. The justification for relating endogenous, or intrinsic chromosome damage to the numbers of mutations comes from work with plants. In plants somatic cells eventually differentiate to form germinal cells whose mutations may be scored visually. Also, it is known that in ageing seeds, loss of chromosomes occurs during storage which can be identified, for example, by loss of chloroplast pigments in the early stages of germination and development.

Using the liver regeneration model, under a variety of conditions, there is an inverse relation between spontaneous chromosome aberration rate and normal life expectancy of mice, guinea-pigs, and dogs. In its simplest form, the mutation theory of ageing extrapolated from these experiments, postulates that the increase in mortality with age is caused by the gradual accumulation of spontaneous mutations in all the somatic cells of the body. This renders the organs inefficient, senescent and eventually leads the organism to death.

A theoretical starting point for this theoretical treatment of ageing was provided by Szilard who made the assumption that the rate at which chromosomes of a somatic cell suffered damaging 'hits' is a characteristic of the species. Experiments based on the scoring of abnormal chromosomes in regenerating liver show that mice accumulate aberrations 5–8 times faster than dogs, and on the Szilard hypothesis this should reflect the faster mutation rate in mice. However, there is no evidence of differences in the quantity and quality of chromatin between species that would support this idea.

In discussing this problem Lintz brought up another dilemma in that the data on the differential accumulation of chromosome aberrations, comparing mice with dogs, indicates that more aberrations are necessary to kill a mouse compared with a dog. He found it difficult to understand why dogs die at a relatively advanced age whilst the recorded amount of chromosomal aberrations remains almost constant during their entire lifespan. He felt that the mutation theory would be supported if ageing and death occur when approximately the same amount of chromosomal aberrations has been attained in all species.

With regard to the remainder of Curtis' points subsequent research has not clarified the issues. Radiation-induced shortening of lifespan is not now regarded as an example of accelerated ageing. One of the major pieces of evidence against it is that the pattern of tissue degeneration of irradiated animals which contribute to their deaths is quite different from that which occurs in controls (Figure 4.8). Also, relatively minor histopathological changes, such as amyloid infiltration of the heart, enlargement and infiltration by blood of the mesenteric nodes, and calcium deposition in the kidney, all of which are indicative of general cellular deterioration, are in no case accelerated by a level of x-irradiation which reduces mean lifespan by up to 50%.

A novel theoretical treatment of age-dependent disease based on somatic

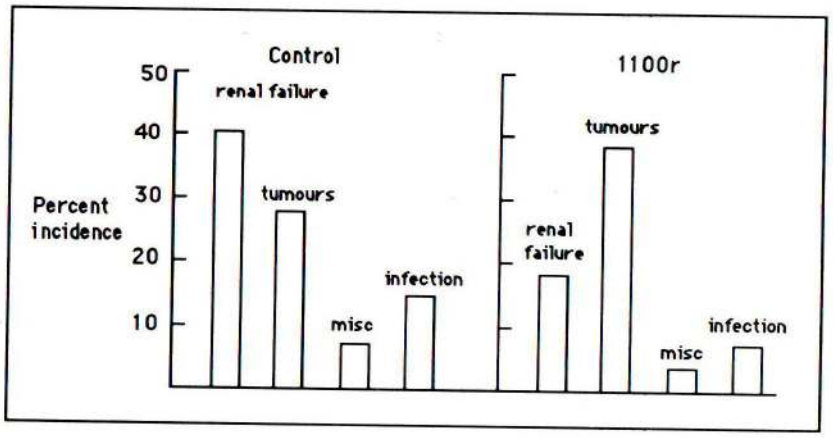


Figure 4.8. Factors contributing to the deaths of CBA mice given whole body X irradiation that reduced mean life span by about 50%.

mutations is the 'unified theory of growth, disease and ageing' which was proposed by Burch in the 1960s. In this model the pathogenesis of most age-dependent disorders can be divided into two principle phases: initiation and development. Initiation, a purely random phenomenon, is postulated to involve the occurrence of somatic gene mutations in stem cells of a hypothetical central system that normally regulates the growth and size of target tissue throughout the body. Each distinctive target tissue has its own central control element. A disease is initiated when one or more (r) somatic gene mutations have occurred in one or more (n) distinctive stem cells. A particular disorder is associated with particular values of r and n. When certain provisos are satisfied, the theory predicts that the sex-specific and age-specific prevalence (Pt), of a particular initiated chronic ageing condition, will be described by one version of the following stochastic equation.

$$Pt = S(1 - e - ktr)n$$

S represents the proportion of the population (specific for sex when there are sex differences) that is genetically predisposed to the condition. The kinetic constant k depends on the number (L) of stem cells in each of the n distinctive sets at somatic mutational risk, and the average rate (m) of somatic mutation at r specific genes. Thus, $k = Lmr$. Its value remains constant from around birth to the onset of the disease or ageing condition. It is unaffected by ordinary environments. To a good approximation, the initiation-age, t, can usually be measured from birth.

An interval, called the latent period, elapses between the completion of the initiation phase and the first detectable onset of the associated ageing condition.

During this development phase, one or n malfunctioning cells are propagated from one or n specifically mutated growth-control stem cells. The mutant cells of the clone, or their secreted humoral products, attack the target tissue whose growth and size they would normally regulate in their non-mutant form. When damage to the target tissue exceeds a certain threshold, symptoms and signs of the associated disorder become manifest. Burch call disorders of this general class, auto-agressive.

An example of Burch's mathematical analysis for the prevalence of arcus senilis (a white or grey arc or circle inside the peripheral margin of the human iris) according to this model is given in Figure 4.9.

Similar calculations were carried out for baldness and greying of hair, and all age distributions fit the statistical predictions of the model. Burch believed that if a few random somatic gene mutations, or indeed only one, can give rise to a widespread disorder affecting numerous target cells, in different anatomical locations—such as cells of the hair follicles—then some form of biochemical amplification must occur. Burnet's immunological idea of the forbidden clone satisfies this requirement, and so far no other explanation has been provided.

In the absence of any direct evidence to connect histologically defined chromosome damage with ageing, the arguments have shifted towards the

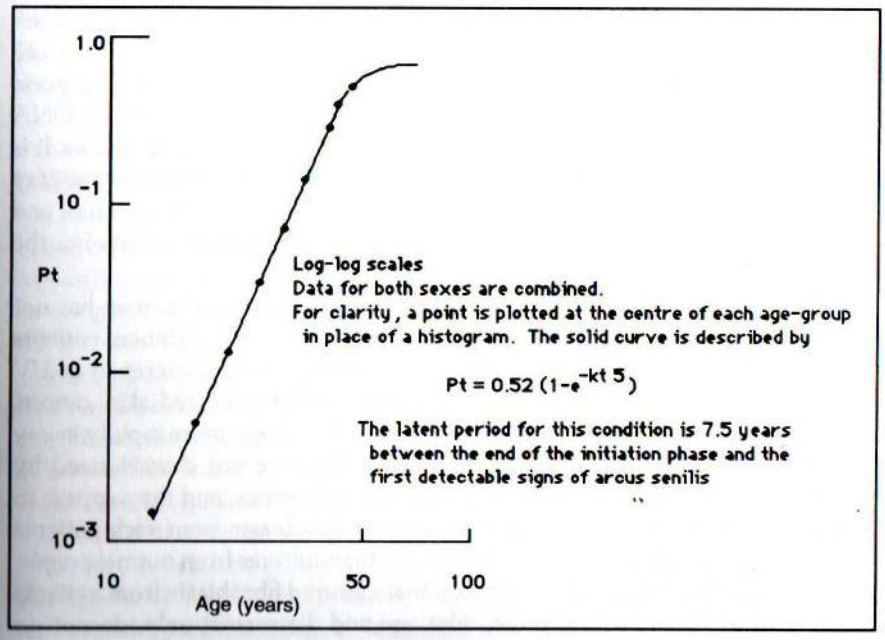


Figure 4.9. Age specific prevalence (Pt) of arcus senilis as found in York and Hartlepool, England, in relation to estimated age at initiation.

Table 4.7. Sorsa's evidence in favour of the somatic mutation theory in the 1980s

1. Most chemical carcinogens are mutagens.
2. Most chemical carcinogens are strong electrophilic reactants.
3. Known chemical carcinogens and ionizing radiation cause lesions in DNA.
4. Neoplastic transformation has been induced experimentally by direct perturbation of DNA.
5. Defects in DNA repair capacity seem to be correlated with a high risk of cancer.
6. A high chromosome aberration frequency is correlated with an increased risk of malignancy in several hereditary syndromes which are characterized by both spontaneous and induced chromosome breaks in blood lymphocytes.
7. Cell transformation by oncogenic viruses implies a change in DNA.
8. A malignant phenotype is inherited in the cell line.
9. Tumours are mostly monoclonal in origin.
10. Chromosomal changes are frequently found in tumours and seem to be non-random.
11. Cured cancer patients have an increased risk of a second malignancy.
12. Epidemiological correlations of increased cancer incidence and high chromosomal aberrations.

likelihood of damage which cannot be seen under the microscope, yet requires an active DNA repair system to cope with it (Table 4.7). This aspect was first studied in a comparative manner with cultured fibroblasts from the mammalian set of shrew, mouse, rat, hamster, cow, elephant and horse. Unscheduled DNA synthesis, i.e. the the synthesis of DNA during the non-S period of the cell cycle, was taken as a measure of excision repair. This work revealed a good correlation between both the rate, and the extent, of unscheduled DNA synthesis, and the logarithm of the lifespan of the set of mammalian species. It is possible, but not certain, that unscheduled DNA synthesis mimics what may happen in a cell as part of the repair process to cope with random chemical and physical damage to the genome. This mechanism is thought to involve the excision of any damaged sequence and its replacement.

However, a direct relationship between excision repair and lifespan has not been proved. Indirect evidence indicates the opposite. For instance, patients suffering from xeroderma pigmentosum are defective in excision repair of UV damage to the skin. Such individuals develop sunlight-induced skin cancers from which they would die if untreated; but they do not age more rapidly in any other way. From the evidence so far available they are not characterized by accelerated ageing, such as occurs in progeroid syndromes, and they appear to have normal lifespans. Furthermore, cultures of fibroblasts from such patients do not go through a fewer number of passages than cultures from normal people.

Another piece of negative evidence is that cultured fibroblasts from patients suffering from Werner's syndrome, who age and die prematurely, do not go through as many passages as do normal cells, but they show normal levels of excision repair. That last point is, however, equivocal since other authors

obtained data indicating that fibroblasts from individuals with progeroid symptoms were defective in their ability to repair single-strand breaks in their DNA.

On the other hand, in experiments on cell cultures where UV irradiation was used in an attempt to activate DNA repair late-passage cells showed less repair than early-passage cells, and many of them showed none. But the authors showed that there was a good correlation between cells that show scheduled synthesis and cells that show unscheduled synthesis. In other words, a cell that does not do scheduled synthesis is unlikely to do unscheduled synthesis. Furthermore, the decrease of unscheduled synthesis with passage number is slower than of scheduled synthesis. The authors, therefore, interpret their results as indicating that failure of repair is not a causal event in the failure of late passage cells to divide.

A major objection to the mutation theory of ageing comes from research using the wasp *Habrobracon serinopae*. This insect produces both haploid and diploid males. X-irradiation of haploid and diploid males decreases lifespan and this is related to the amount of damage to the chromosomes brought about by irradiation; the decrease observed in haploids is larger than that observed in diploids. However, when the lifespans of normal insects are examined, haploid and diploid males have identical lifespans. Since the somatic mutation theory implies that in non-irradiated animals haploid males should have a shorter lifespan than diploids, because the haploids lack the genetic redundancy of the diploids, the theory if it holds at all, does not apply generally throughout the animal kingdom.

LABORATORY RODENTS

There are distinct practical and economic advantages in studying longevity in laboratory models. Nevertheless, few species meet the necessary criteria for carrying out repeatable experiments. Some of the most important specifications are listed in Table 4.8.

It is probably true today that most experiments are deficient with respect to most, or all, of these criteria. This deficiency is not only serious in the choice of non-mammalian models, it also applies to the strains of laboratory rodents used as mammalian models, and the conditions under which they are kept.

Intraspecific genetic differences in ageing have repeatedly been demonstrated in studies of the lifespans of rats and mice from different inbred strains raised in laboratory conditions (Table 4.9). Inbred strains for this purpose are produced through many successive generations of single-pair brother-sister matings and maintained by continuation of identified sibling matings. However, this does not guarantee complete homozygosity but only that they are very much alike with respect to the genes that are selected for in the management programme,

Table 4.8. Criteria for selecting whole animal laboratory models for ageing

1. Life-tables must be available.
2. The species must have a lifespan that is short in relation to the logistical problems of organizing long-term research.
3. Facilities should be available for maintaining animals free of infectious diseases throughout their lifespan.
4. The genealogy must be well known and the genotype should be stable.
5. The environmental conditions must be specified and controlled to narrow limits.
6. The main degenerative diseases of ageing must be described.
7. Breeding maintenance of the colony should be a routine procedure and food should be readily available to a standard specification with regard to macro and micro nutrients.
8. The model should be defined with biochemical and physiological 'markers' so that results may be readily applied to human situations.

and that the members of a particular inbred stain share a unique genome, containing a particular array of homozygous gene pairs. Therefore, significant differences in mean and range of lifespans between two inbred populations studied together must be attributed to genetic factors.

Heterogeneity Between Strains

Although it may appear that inbred stains offer the advantage of genetic purity with minimum variability, their use poses problems because strains often suffer from a single major disease process which may shorten life in an idiosyncratic way. There is no such thing as a species lifespan for 'the laboratory rat' or 'the laboratory mouse'. For the laboratory mouse, genetic variants can be obtained that differ by a factor of about three in maximum lifespans between strains. Hybrids of the F_1 generation have been shown to have a mean lifespan that is greater than that of either of the parent inbred lines.

Differences in longevity of laboratory populations are associated with differences in the rate of decline in physiological vigour and incidence of pathologies. With respect to the latter point, the frequency distribution and extent of pathological changes in human subjects are clearly genetically conditioned. Also, cause of death in mouse strains is strain specific. Deaths from cancer in the CBA mouse result mainly from hepatomas; in the AKR/J mouse, lymphoid leukaemia is more common and mammary tumours have a high frequency in CBA female mice. Removal of one major cause of death by selection can be expected to unmask another major cause with a high incidence at a greater chronological age.

The classical study in this area was carried out in the 1940s and 50s of mice from 10 different strains by Russel and her collaborators. This research showed

Table 4.9. Mean lifespans (days) of several strains of laboratory rodents

Strain	Females	Males
A	558	512
AKR	312	350
A2G	644	640
BALB/c	561	509
CBA	825	486
CE	703	498
C3H	676	590
C57BL	580	645
C57BR/cd	660	577
C57L	604	473
DBA/1	686	487
DBA/2	719	629
NZB	441	459
NZW	733	802
129/RrJ	666	699
LACA	664	660
LACG	617	536
WA	749	645
P	782	729

that in inbred strains the mean lifespan of females in the earliest-dying strain ((AKRIJ), mice die very young with lymphatic leukaemia) was only 287 days, while that of females in the longest-lived strain (C57BL/6J) was 576 days. Thus, genetic differences were important contributors to ageing but the demonstration of genetic influences did not preclude the possibility of environmental effects. Later work showed mean lifespans of 794 and 889 days for C57BL/6 females, gains of 200 to 300 days over the earlier observations. Compared with the initial studies, improvements in rodent husbandry had eliminated many intercurrent infections. This, together with improved nutrition, allowed almost all the mice in an inbred strain to survive to their normal old age. Thus, environmental, as well as genetic, factors can influence the lifespans of laboratory mice.

Despite problems of standardizing conditions, laboratory research involving many individuals from each of several different genetically defined inbred strains has been important in demonstrating both genetic and environmental effects. The use of inbred mice also provides other advantages for research on ageing. Investigators in other research areas, such as cancer, developmental biology, physiology, endocrinology, pathology, immunology, and haematology, have used inbred mice very extensively.

As a result of this type of research, the scientific literature is full of

information on many characteristics of mice from a great variety of inbred strains. The investigator can not only work with many copies of the same experimental object, he can also know before beginning his experiment a great deal about the potential of that object. He can find in the literature evidence that levels of certain circulating hormones are considerably higher in some inbred strains than in others; that the normal numbers of both red and white blood cells are higher in some than in other strains, and that mice of one strain will become obese on a diet which does not result in fat deposition in others. Immune responses differ markedly among strains, and also change with age. These and many other strain differences at all levels of organization could have effects on patterns of ageing. One kind of mouse may be very likely to develop mammary cancer, another lung tumours, still others kidney disease or leukaemia. This should also make investigators cautious about making general claims about ageing of mice without studying animals from a variety of different inbred strains.

Fewer rat than mouse inbred strains have been developed, and data on genetically controlled differences in rat lifespans are only now being collected. Two commonly used types are the Sprague-Dawley and Wistar. The major difference between them is that Sprague-Dawley rats continue to increase in size throughout their lives, while Wistar rats stop growing by the age of 1 year. The difference in size is partly due to growth of the skeleton but is also associated with increased volume of individual fat cells in the Sprague-Dawley strains. The latter strain also shows a very high incidence of mammary tumours.

Additional evidence for genetic diversity in the processes of ageing comes from long-lived, vigorous mice produced in crosses between parents from two different inbred strains. All the offspring of the first generation from such a cross are alike genetically but differ from either parent because they are heterozygous for each of the many gene pairs which differ between the two homozygous parental genotypes. Not all of the most favourable alleles can be fixed by chance in any one strain during inbreeding. It is clear that some of the gene pairs fixed in any one inbred strain are slightly deleterious in their potential effects on lifespan, since interstrain F_1 hybrid mice almost always live longer, and eventually develop a different array of pathological lesions, than do mice of either parental strain. In general both mean and maximum lifespans are longer in hybrid mice than in mice of parental strains. A greater diversity of tumours has been observed in hybrids than in mice of parental strains, although the incidence of a specific type of neoplasm could be either higher or lower in the hybrid.

An efficient research tool for tracing the pathway of action of a specific single-gene difference is the congenic line, which carries an identified 'foreign' gene segregating against a known uniform genetic background. Mendelian monohybrid crosses yield offspring which are essentially identical except at the single known segregating locus.

Many congenic lines have now been bred for research. The 'foreign' gene

may be a mutant allele with quite deleterious effects, or it may be a different, presumed normal, allele which has been transferred by repeated crosses from the strain of origin to another inbred strain.

Comparisons made between mice from an inbred line and a specific 'partner' congenic line with an identifiable single-gene difference, have the advantage that any differences observed between them are attributable to the specific gene difference.

This approach has value in answering questions about the influence of inherited differences in the immune system on longevity. For example, mice from congenic lines, identical except for known differences at specific loci which determine tissue compatibility, have slightly different lifespans. Thus, some genetic differences in ageing may be due to single gene differences in the balance between immune cell populations. The genes concerned may act at the stem cell level. More evidence for a single gene effect on a single tissue producing a change in lifespan comes from work on macrocytic anaemia in mice. This condition can result from the substitution of a particular deleterious allele for its normal counterpart. Anaemic mice have a short lifespan which can be restored to normal with a bone marrow implant. Presumably this supplants the abnormal stem-cell system of the anaemic host.

Heterogeneity Between Laboratories

The likely environmental differences must be taken into account when, as frequently happens, different results come from two different laboratories using the same strains. For example, two cohorts of C57/BL/6J American male mice, one maintained in New York City and the other in Maine, both derived from the same colony, differed in average longevity, maximum longevity and age at which mortality rate began to increase; 50% survival occurred at about 18 months in the Maine laboratory and at about 30 months in New York. It must be concluded that diet and general laboratory conditions were responsible for these large differences.

Diet is in fact a major problem in trying to standardize laboratory management of rodents to make valid cross-laboratory comparisons. Very few commercial diets are standardized with respect to both major and minor components. More importantly it can be argued that the high protein diets now used universally for breeding and maintaining rats and mice is far removed from their ecological diet in the wild. Where studies have been made it appears that *Rattus* and *Mus* are mainly seed eaters. Grass seeds were found to be the main dietary components in mice on Skokholm island. In fact their dentition tells us this, yet, starting with 'Purina Chow', countless generations of rats and mice have been provided with an inappropriate carnivore diet. This together with farmer's and livestock breeders' methods of selecting for rapid growth and

reproduction using young mothers for only one or two pregnancies has produced a very odd genotype with respect to the zoological realities of rodent evolution. In fact, even the most highly inbred lines can be induced to breed on low protein/high fibre vegetarian diets (12% protein as against 18% to 25% protein normally provided commercially). Diet appears to be the major determinant of ageing and pattern of diseases in laboratory animals. Since quite small differences in composition can produce relatively large differences in longevity and the type and incidence of several degenerative histopathologies, research is urgently required to define an 'eco-gerontological diet' for rats and mice.

Whilst there can be little doubt that many pathologies in old age result from the decreased resistance of microbial diseases, studies on germ-free mice have shown that infections are not an important fundamental aspect of ageing. Ageing germ-free mice show the same pattern of non-infectious pathological conditions as do conventionally maintained animals. This is particularly the case for kidney glomerulosclerosis—a thickening of the glomerular basement membrane associated with hyaline deposits in the wall of the glomerulus—which has been described as the most prevalent lesion of the vascular system of laboratory mice. The incidence and severity of this lesion is the same in germ-free and conventional mice. This is also the case for the incidence of muscular degeneration which occurs within a narrower age-range than glomerulosclerosis. There is also clear evidence that the introduction of germ-free conditions to a laboratory where rodent strains are already well managed by conventional methods does not increase lifespan; in some experiments germ-free conditions have actually reduced longevity!

Heterogeneity Between Species

Although the good correlation between lifespan and body size in the mammals points to growth rate being an important evolved variable, differences in body size and rate of maturation are not always such a good fit. For example, the maximum laboratory lifespans in the house mouse and rat are between 2.5 and 3.5 years. Laboratory maintained deer mice (*Peromyscus leucopus*), which are approximately the same size, and mature at the same age as do house mice, have a minimum lifespan of about 6 years in the laboratory, and many survive for 8 years. It has been pointed out that the genes responsible for intraspecific differences in longevity may often have specific effects, largely in particular kinds of cells and tissues, rather than general effects common to all cells.

In the face of so many exceptions to the statistical rules across species which have no special explanation we have no general genetic theory to account for evolution of longevity. Some thoughts in this direction came from Medvedev who suggested that molecular repetition of genetic information, which is evident in the redundancy of some types of genes, could be an evolutionary

strategy for delaying ageing and increasing longevity. However, apart from rRNA, histones, and tRNA genes, which have key roles in development, the redundancy of important genes has never been clearly demonstrated. In a theoretical context arguments can be developed for and against the idea that redundancy of genes is a good thing. On the one hand it can be related to the advantages of having multiple copies of information available in case of loss or damage. On the other hand, genetic redundancy, instead of being a protective device, could present a severe load. That is to say the more a gene is repeated, the more chances there are that one or the other of the repetitions is mutated. It may be that genes affecting general rate of ageing differ more frequently between than within species.

THE GENETIC SYSTEM OF HUMAN EVOLUTION

In principle, it is accepted that genetic differences are responsible for all differences in longevity between species. Longevity is therefore an integrated function of the total array of species genes which were presumably selected to fit with a particular ecological niche. Most comparative studies have dealt with the mammalian series whose members obviously age at different rates. It is assumed that the similar patterns of old-age debilities such as neoplasia, arthritis, and circulatory problems, which occur at greatly different chronological ages in different mammalian species, are also under a strong genetic influence.

Interspecific differences in ageing suggest, but do not prove, the existence of genes whose specific role is to set the general rate of ageing. As yet, we have no proven ideas as to how many such genes there would be and how they might act. The current balance of opinion is that very few genetic loci may be involved, and that these act to aid survival during the reproductive span of a species. That is to say the traits they control affect the frequency with which genes will be carried into the next generation.

An additional mechanism may be important in social animals where, for example, caring activities of post-reproductive female elders for juveniles may promote the continuity of the gene combinations of their close relatives. This appears to be the situation with foxes and elephants where non-reproductive 'aunts' play an important role in protecting the young of their 'sisters'. This type of mechanism may have also have been responsible for the evolution of the human menopause with its attendant cosmetic changes, which could have effectively placed old, yet fit, females in a non-competitive sexual situation, where they assume the role of caring for their grandchildren.

The existence of the human menopause presents theoretical problems to the biologist and ethical problems to the doctor. Degenerative diseases appear at random and are not found in all individuals. The menopause occurs in all females at a very precise time. This timing mechanism seems to be set by the

steady loss of eggs in the ovary, a process which begins before birth. When the number of eggs remaining reaches a certain threshold the neuroendocrine support to the ovary ceases. In other words, the menopause is part of programmed development. It is not associated with an immediate increase in mortality, and the post-reproductive survival period may be one third of the total lifespan. This poses a dilemma to doctors who are asked to treat the menopause with the missing hormones as a deficiency disease. Hormone replacement has been shown to have beneficial effects. The lack of hormones in some women may lead eventually to brittle bones which fracture easily, and in such women hormone therapy can strengthen the bones by stimulating calcium deposition and inhibiting calcium loss. Other problems can be relieved such as restoring the ability of the uterus to support a child, albeit conceived outside the body by the union of sperm with a donor ovum. There are also effects in alleviating physiological discomfort due to the loss of oestrogens such as hot flushes, and bouts of anxiety, and positive cosmetic effects on skin condition.

The menopause may have appeared at the stage in our evolution when females first began to be protected socially from random mortality factors. Humans are unique in both the slow development of children, and in the extent to which ageing is a feature of our social organization. When large numbers of women began to survive into late middle age it may have been more advantageous for them to switch off reproduction in the sixth decade to avoid the increased hazards of pregnancy and have time to care for their children and grandchildren. Both of these activities would result in an increased evolutionary fitness of their particular set of genes. It is probable therefore that the menopause is a comparatively recent evolutionary innovation.

It is generally assumed that in our still rapidly evolving situation it is virtually impossible to draw conclusions about the evolution of ageing over the last two million years, which essentially represents our entire period of evolution. Yet the age frequency assessed from skeletal remains in most cemetery investigations bears no similarities to modern populations in that death is seldom recorded for individuals over 40. Methodological difficulties appear to be responsible for the lack of the 70-plus age group in paleodemographic studies. Anthropologists have yet to agree on the significance of ageing indicators. In particular, it becomes more difficult to calibrate the morphometric skeletal features once a cohort has left middle age. Even in this younger group a death assemblage can tell us very little about the living population of which it was a part. The age structure is distorted by burial practices which could exclude some groups, and augment others, by status, and cause of death, and therefore, on both counts, by age. Whilst it is accepted that the expectation of life has been increasing for thousands of years, it is also accepted that the maximum lifespan has not increased by genetic selection, but that the number of people dying before the eighth decade has been reduced.

CHAPTER 5 Tissue homeostasis and ageing

Although in the normal ageing central nervous system, loss of nerve cells is not inevitable, e.g. unchanged population in the cochlear nucleus, in certain areas substantial decrease in number occurs particularly of large neurons. There is, for example, a reduction, by the age of 70 years, of about half the number of neurons in the locus coeruleus. In other areas, such as the hippocampus, a steady decrease in the number of pyramidal cells has been reported. As a result of nerve cell loss, the elderly subject is therefore potentially more susceptible to neuronal dysfunction than the younger person with a full complement of healthy nerve cells, as a loss or damage to neurons has to reach a critical level before functional signs are seen. (Davison 1987)

HOMEOSTASIS

The commonplace observation behind the concept of homeostasis is that living things maintain their own stability. As an idea it was first articulated by the Greeks, particularly in relation to their concepts of disease. For example, the idea that disease could be cured by natural powers, i.e. that the body could detect and regulate its physiological health was held by Hippocrates. This implies the existence of agencies ready to operate correctively when the normal state of the organism is upset.

The fluid matrix of cells and tissues is the central feature of all stabilizing mechanisms, and as organisms have evolved, they have done so by the selection of physiological systems to become more independent of changes in the outer world. The concept of self-regulation was first articulated in relation to the preservation of their own inner world, the fluid matrix, as a steady state, independently of shifts of outer circumstances. The physiological action by which body fluid stability is maintained by physiological actions is termed homeostasis. The term homeostasis was invented by Walter Cannon in the mid-1920s. It comes from 'homeo' meaning 'same' and 'stasis' meaning 'condition'. The central concept of homeostasis is that internal steady states are produced by the action of metabolic forces triggered by any tendency for them to change.

The internal states are defined by 'norms' and 'regulators'. A norm is a desired state, and a regulator is a component of the organism that detects changes in the norm and adjusts the organism to maintain it.

Major steady states of homeostasis are:

1. Blood glucose.
2. Osmotic pressure.
3. pH.
4. Body heat.

Major material supplies for homeostasis are:

1. Food substances for energy, growth and repair, i.e. carbohydrates, proteins and fats.
2. Water.
3. Inorganic constituents.
4. Oxygen.

In the mammalian body, homeostasis works to very fine tolerances. For example, a fall in the level of human blood glucose below 45 mg% brings on convulsions, possibly coma and death. An increase above 180 mg% results in glucose loss in the urine. Too much water results in water intoxication, characterized by headache, nausea and dizziness. Too little water results in a decrease in blood volume and increase in blood viscosity and a rise in body temperature. The latter point indicates that homeostatic systems are interconnected.

The inorganic constituents of body fluids are also regulated homeostatically. If the percentage of NaCl in blood rises above 0.3%, water is drawn from the lymph and cells and fever may result. A fall in concentration below 0.3% produces a marked reflex irritability followed by muscular weakness.

The principles of homeostasis are as follows:

1. Constancy of unstable material in a changing environment is evidence of homeostasis.
2. Tendency to change is met by increased effectiveness of factors opposing change.
3. Factors maintaining a steady state in one direction do not act to change the norm in the opposite direction.
4. Homeostasis may comprise co-operating factors acting simultaneously or successively.

Although Cannon coined the term homeostasis with respect to physiological norms it has since been applied to many other levels of biological organization, using the central cybernetic idea of negative feedback. The idea has been

applied to the regulation of the course of development under the term homeorhesis.

Generally, a potentially harmful disturbance of physiology, behaviour, tissue structure, or the course of development, signals the disturbance to a controller or regulator, which diverts resources to bring the disturbance under control. The controller or regulator holds the specification of normality, and the restoration of normality cuts off the signal which activated it.

From this broad homeostatic viewpoint ageing is defined as a loss of tissues and functional reserves. This implies that the specifications of normality are either gradually destroyed, shut off, or become faulty.

TISSUE HOMEOSTASIS

The bodies of multicellular organisms are organized on the basis of tissues, organs and systems. A tissue is composed of many cells, usually similar in both structure and function, that are bound together by intercellular material. An organ, in turn is composed of various tissues (not necessarily similar) grouped together into a structural and functional unit. A system is a group of interacting organs that 'co-operate as a functional complex in the life of the organism'. In fact some form of co-operation or integration is necessary for the creation and maintenance of tissues, not only to support their own structures in time and space, but also to keep the organ intact. Therefore it can be postulated that homeostatic systems will exist to maintain the characteristic histological features which we use to define both tissues and organs morphologically. It would be expected that these tissue homeostatic systems would act to maintain a dynamic equilibrium at the biochemical level.

Although as yet, we know very little about the regulatory mechanisms behind tissue and organ maintenance, it is likely that both elements have local homeostatic systems which govern the use of resources to maintain the morphology and disposition of fibres, cells, blood vessels and nerves. From this point of view any age-related deteriorations in tissue and organ structure are likely, to some degree, to be a reflection of failures in homeostasis at the tissue level. For example, the multifocal proliferations of myointimal cells are early characteristics of atherosclerosis. There is a failure of feedback controls on the mitotic stem cells in the arterial wall which could involve a local decrease in the production of cell division inhibitors. One theoretical model for this failure in tissue homeostasis is presented diagramatically in Figure 5.1. Cell division inhibitors are produced by cells in the inner smooth muscle wall of the aorta. This normally maintains the balance between differentiated smooth muscle cells and their mother stem cells. By diffusion into the outer zone of myointimal cells it also regulates division of myointimal stem cells, so controlling the overall thickness of the arterial wall The inhibitor level in the arterial wall is

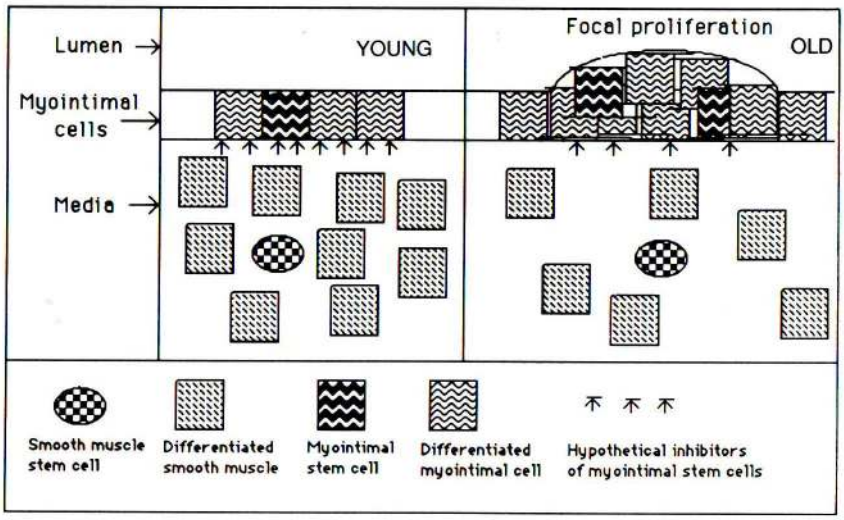


Figure 5.1. Tissue homeostasis model for age-related proliferations of myointimal cells of the aorta.

proportional to the density of smooth muscle cells. If the concentration of inhibitor is reduced by local accelerated death of smooth muscle cells, or by failure of inhibitor production, differentiated myointimal cells will increase causing a focal thickening of the outer region of the artery, which becomes the starting point for the accumulation of an atherosclerotic plaque.

The evidence for this theory is indirect. Arterial multifocal proliferations occur side by side with tissue atrophy of the medial region, in which there is a loss of smooth muscle cells. The technique of clonal assay of cells in mouse medial/intimal fragments digested with the enzyme elastase has shown that the replicative potential of vascular smooth muscle cells decreases linearly as a function of donor age. This phenomenon is shown with primary and secondary cloning which is strong evidence for a negative linear regression of mitotic potential with age. The tissue homeostasis model came from efforts to reconcile these observations of low cell density, and low mitogenic potential in the medial region, alongside an increased proliferation in the arterial lining.

These substances are purely hypothetical. However, it is known that substances do exist, distinct from circulating hormones, that regulate the growth and differentiation of cells, and also play a role in cytoprotection and repair. These substances fall into a general category of 'growth and maturation factors' (Table 5.1). Several have been identified chemically but so far there is no information of their significance with respect to ageing.

Table 5.1 Definition of growth and maturation factors involved in tissue homeostasis

General definition:
Growth and maturation factors are substances that stimulate cell division in post-embryonic tissues with, or without, differentation of the daughter cells

Roles:
- Maintenance of cell turnover
- General growth
- Specific stimulation of stem cell proliferation
- Local trophic coupling (e.g. nerves and muscles)

Model systems:
- For stem cell regulation
  - *Erythropoietin and its target cells
  - *Epidermal growth factor
  - *T.-cell growth factor
- For inter-organ integration
  - *Sciatin muscle trophic factor
- For general mitogens
  - *Prostaglandins and cultured animal cells
  - *Growth promoting effects of insulin and insulin-like growth factors
  - *Tumour-derived transforming factors

CONNECTIVE TISSUE HOMOEOSTASIS

Animal cells are embedded in an extensive intercellular matrix termed connective tissue. Much of the total volume of connective tissue is matrix, the cells themselves often being widely separated. The matrix may be liquid, semisolid or solid. Vertebrate connective tissue is conventionally divided into four main types:

1. Blood and lymph.
2. Connective tissue proper, which is more logically described as the 'pericellular matrix'.
3. Cartilage.
4. Bone.

These last three are sometimes collectively defined as supporting tissues. With respect to ageing, well-defined losses of morphological and functional integrity have been found in all four categories.

Most gerontological research into problems of ageing connective tissue has involved the chemical and structural organization of the pericellular matrix. Connective tissue research from the medical point of view has concentrated on bone and the arterial wall, where for example, osteoporosis and atherosclerosis, respectively are important age-related diseases.

Glimpses of the special features of tissue homeostasis that may be relevant to ageing are beginning to emerge from studies in developmental biology. This research is particularly important in that it broadens the base for the study of ageing of the pericellular matrix. For example, the acquisition of form and function in the embryo involves several types of cell behaviour such as proliferation, movement, shape-change and adhesion, as well as expressions of specific molecular products. A comparison of neuroendocrine homeostasis with tissue homeostasis is given in Figure 5.2.

In a standard neuroendocrine mediated regulation, such as heat balance, the negative feedback components involve a combined action of circulating hormones and nerve links working jointly in response to changes in a relatively simple physicochemical property of the body fluids. In tissue homeostasis the norm is likely to be a three-dimensional structure, with a feedback mechanism involving local growth factors. Furthermore, the regulatory events of tissue homeostasis take place within a matrix of cells and pericellular substances and structures. The cells would be expected to interact with the three major classes

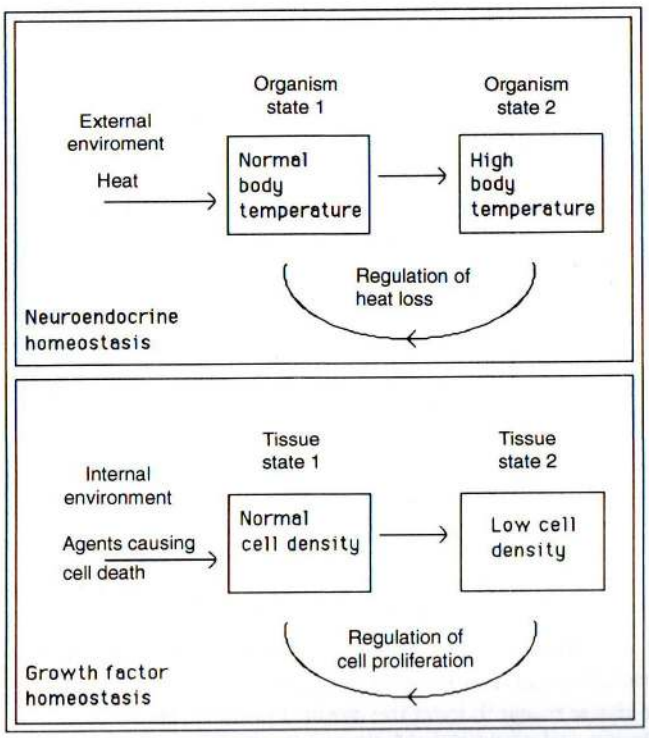


Figure 5.2. Relationship between homeostasis of organism and tissues.

of pericellular substances—collagens, proteoglycans and glycoproteins.

In early development the various extracellular components are highly interactive, both with one another and with the surfaces of the cells which they surround or abut. The composition of embryonic connective tissue varies greatly in relation to its locale, and the stage of development of the particular organ or tissue. Over the past decade it has become apparent that these extracellular macromolecules, and the fibrous matrix in which they live, together play an important role. They not only maintain the physical integrity and structural properties of the emerging anatomical features, but just as importantly, they also regulate the behaviour of their constituent cells of organs, in the mature organs. From this point of view it is to be expected that future research into the gerontological aspects of connective tissue will extend the methods and concepts of research into the control and regulation of early development to define the following:

1. The detailed molecular mechanisms of interaction between the pericellular matrix components.
2. The regulation of synthesis and degradation of individual components in relation to changes in the pericellular matrix.
3. The relationship of a given set of interactions to the superstructure of specific pericellular matrices and the exact spatial localization of the chemical and cellular components in relation to one another and to the cell surface.
4. The role of a given pericellular matrix in specific aspects of cell behaviour *in vitro* and *in vivo*, and analysis of the contribution of individual components.

Ageing of the Pericellular Matrix

The pericellular matrix provides a framework for organs and parenchymatous tissues. It is a kind of dynamic packing material to fill the spaces between parenchymatous elements, and also a protective cushion layer for these elements. It is through this tissue the cells receive their nutrition, and have their products removed. The ground substance plays an important and active part in the production of the fibrous elements of the connective tissue, and, possibly in some other physicochemical processes, is concerned with the maintenance of its water and electrolyte content, and biochemical signalling at cell membranes. In this respect, connective tissue fibres play an important part in morphogenesis to orientate migrating cells to form the axial features such as notocord and muscle somites. It is therefore a mistake to think of collagen simply as an inert filler of spaces between cells. In those organs which have a high level of structural differentiation and zonation, such as the kidney and intestinal mucosa, the

connective tissue scaffold is highly ordered, and there is evidence from regeneration experiments that the orientation of fibroblasts sets a structural pattern of fibres to which other cells, making up the specialized functional units, orientate. Primary failures in fibroblasts to construct this scaffold to the specifications of youth may be the major feature in the deterioration of the cellular matrix, and of function, in some of the major organ systems.

The pericellular matrix contains three major types of fibres, consisting of collagen, elastin and reticulin respectively.

Collagenous fibres

Collagenous fibres, or white fibres are very common and composed of numerous fine fibrils of collagen a protein that constitutes a very high percentage of the total protein in the animal body. Such fibres are flexible, but resist stretching and confer considerable strength on the tissue containing them. They are nonbranching and cross-striated with 640-650 Å periodicity. They are digested by collagenase and pepsin, but not by trypsin. Medium heat contracts these fibres, while high temperature (boiling) gelatinizes them. They swell, or are dissolved, in weak acids and alkalis.

Reticular fibres

Reticular fibres, as the term reticular indicates, branch and interlace to form complex networks. They are important points where connective tissues and other tissues join, e.g. they are common in the basement membrane between epithelial cells and connective tissue. Reticular fibres are in some respects similar to the collagenous ones: they are cross-striated with the same periodicity of 640-650 Å, resist the action of trypsin, but are rapidly lysed by collagenase and pepsin. There are, however, considerable differences: reticular fibres, besides their branching characteristic, stain black with silver (collagen yellow-brownish), bright red with PAS (collagen faint red), and do not stain well with fuchsin in Van Gieson's stain (collagen-bright red). While collagenous fibres are found throughout the pericellular environment, the reticular fibres form very thin layers at the sites wherever connective tissue is bounded by other tissues (e.g. epithelial layers, glandular basement membranes, around muscle fibres, under the epithelium of blood or lymph vessels, etc.). There is evidence for a continuous and gradual transition of reticular into collagen fibres.

Elastic fibres

Elastic fibres (or yellow fibres) can, as their name implies, easily be stretched. When the stretching force ceases, the fibres return to their former length. Elastic

fibres are often much thinner than collagenous fibres. They are composed predominantly of the protein elastin. Elastin at electron-microscope examination appears as non-striated branching fibres, or fenestrated laminae: they possess rubber-like elasticity, and resist heat (even boiling), do not swell in dilute acids or alkalis. They are resistant to tryptic digestion, but are hydrolyzed by elastase. Also, the amino acid content of elastic tissue differs from that of collagenous fibres. In spite of all these marked differences there is strong experimental evidence that at least a part of elastic tissue is derived from collagen. Experimental transformation of collagen fibres into elastic-like fibres has been obtained by various means: heat, alkali, enzymes, potassium iodate, etc. The fact that the transformed collagen acquires properties of elastin has been confirmed by its degradation with elastase.

Cellular Elements

Several kinds of cells are generally found in the pericellular matrix. They perform a variety of functions, which indicates that they are an integral part of it, and that this tissue may be defined as a functional unit at the cellular level. The main cells that can be identified histologically are:

1. Fibroblasts; spindle-shaped cells that secrete the proteins from which fibres form.
2. Macrophages; irregularly shaped cells, particularly common near blood vessels, which become mobilized when there is an inflammation; they can move by amoeboid motion and actively engulf particles such as dead red cells and foreign material such as bacteria.
3. Mast cells; produce heparin that tends to prevent blood clotting and histamine both of which are important in the inflammatory reaction, which is based to a large extent in connective tissue.
4. Fat cells; highly specialized for fat storage; when they are very numerous in a region of connective tissue the tissue is often called adipose tissue.
5. Various kinds of white cells; help fight infection and some can move easily between the blood or lymph and the pericellular matrix, a clear demonstration of the close interrelationship between these tissues.

Both cells and fibres are embedded in a rather amorphous ground substance, which is a mixture of water, proteins, carbohydrates and lipids. Associated with the ground substance is the tissue fluid, a liquid derived from the blood.

Analysis at the Histological Level

Ageing changes in the skin were first clearly described by Unna in 1896. He found that 'senile' collagen fibres take up neutral and acid dyes less readily, and more readily basic stains. On elastic fibres irregular thickenings appear. Both types of fibres at first show some swelling, and later several of them break into small-sized fragments and granules. At the same time the degenerating collagen fibres acquire staining properties of the elastic type. These changes were found chiefly in the exposed parts of the skin, on the face and hands. Protected regions of the skin are, comparatively, unaffected in this way. Therefore, Unna considered that the pronounced 'senile' degeneration of the connective tissue in the exposed skin is actually due mainly to environmental stress and injuries (cold, rain, snow, wind, sunshine, etc.) and not to old age. Nevertheless, he considered a general atrophy of the skin to be a specific old-age change. This is supported basically by evidence that skin ages by the action of external and internal forces.

Age-atrophy and granular breakdown takes place in collagenous and elastic fibres of the human skin. Changes in the chemical composition are inferred since specific dyes stain old collagen weakly, and old elastin in somewhat different colours. There is also local thickening of elastic fibres. Elastic tissue exists in two varieties, namely, as fibres or as lump-like condensations. The latter become more predominant in older persons, and are seen by electron microscopy as fenestrated laminae of irregular form and different size. Electron-microscopical studies established that the width of collagen fibres becomes enlarged with ageing.

In young persons the bundles are dense, interwoven in various directions, and with no gaps between. With age, collagen fibres become atrophic, rarefied, and arranged in a parallel direction to the surface of the skin, with several gaps between the bundles. Under the electron microscope they lose the sharpness of their striations and appear to bind amorphous pericellular material. Specific collagen dyes do not stain these degraded collagen fibres typically, but metachromatically. Exposed parts of the skin show various regressive changes in the elastic fibres; splitting, dispersed nodular thickenings, atrophic and degenerative changes, in particular granular and fatty degenerations of the fibres. Simultaneously, however, regenerative changes have been observed, namely, a transformation of the 'connective tissue fibres' (presumably collagen fibres) into elastic-like fibres.

The effect of environmental exposure to injurious effects of the environment is evident from differences between exposed and non-exposed skin. Examinations of histologically unexposed regions of the skin in persons whose age varies from infancy to the eighth decade reveal no changes in the elastic or collagen fibres which have any consistent relation to age. However, the pegs (rete ridge) of the epidermis show atrophic changes with advancing years. Thus, there appears to be an endogenous failure in morphological maintenance of a skin

structure that is vital to its protective and elastic properties.

A count of elastic fibres in a standard area of the unexposed skin has shown that on the average the number of the fibres decreases with ageing, but the range of variations is considerable. For instance, one study in male infants yielded an average value of 46, with a range of 60–35: the respective values in men aged 17–80 years was 36, with a range of 44–25.

In children up to the age of 5 years elastic fibres are numerous, large and well-formed. In young persons aged 15–23 years the number of elastic fibres starts to decrease, and at the age of 30–40 years slight irregularities appear in the fibres. In old persons over 65 years of age regressive changes can be observed: rough irregular thickenings on the fibres, fragmentation of their ends and occasional aggregates of elastic fibres forming irregular masses. There are changes in orientation towards stretched fibres being arranged in a parallel direction to the surface of the skin; the latter change probably is caused by the same arrangement of collagen fibres. In people aged 78–90 years there is a definite decrease in the number of elastic fibres and their splitting into component fibrils occurs.

Physicochemical Ageing

An *in vitro* approach to the possible chemical and physiological significance of connective tissue ageing was opened up by Verzar in the mid-1950s. He studied the effect of temperatures of between 60–70° C on collagen fibres taken from rat tail tendons of various ages. This temperature converts them into transparent, elastic rubber-like fibres, which become contracted. In old animals this contraction can be inhibited, but with considerably larger weights than in younger age groups of animals; and the weight which cannot be lifted by contracting 'young' fibres still permits a strong contraction of 'old' fibres.

Verzar considered that the deteriorating effect of heat may operate even at the level of body temperature. From his own, and other experiments, he interpolated the time which, theoretically, would be necessary for the above-mentioned transformation of collagen fibres, at various temperatures, to the elastic state: at 63° C only 4.3 minutes are necessary for this, at 53° C about 1.9 days, at 43° C about 5.8 years would be required, and at 38° C about 215 years. A degree of heat, which is sufficient to convert 'young' collagen fibres into the elastic state, gelatinizes 'old' collagen fibres.

Contraction of rat tendons may also be brought about by immersing them in 40% potassium iodate solution. The time necessary for the contraction of these fibres, obtained from rats aged, 4, 19 and 28 months, was 134, 360 and 420 seconds respectively; in order to elongate the respective contracted fibres the weights of 2, 19 and 42 g were necessary; in order to break the fibres 9, 134 and 260 g have to be attached to the respective fibres. It is also very typical for age

differences in this kind of experiment that after contraction, collagen fibres from young rats relax quickly to original length; the more aged the rats the more slow this spontaneous relaxation becomes. The fibres from old animals (e.g. aged 24–30 months) do not relax spontaneously at all. When collagen fibres are warmed to about 60° C they rapidly contract and become elastic.

All of these phenomena are thought to depend on changes in the alignment of component fibrils and their juxtaposition. This alters the crystalline zones in the fibres which can be discerned by X-ray analysis. The main reactions are thought to be the breakage of hydrogen bonds, and the formation of cross-links by oxidation of the amino acid residues that project from the main polypeptide chain. The form of contraction measured by applying weights to prevent it is also thought to be dependent upon the number of cross-links in the sample (Figures 5.3 and 5.4).

Much has been made of cross-links in discussions of collagen ageing. Attempts have been made to measure them by X-ray crystallography (Figure 5.4) and by the degree of swelling in water. The latter approach depends on the fact that many cross-linked polymers will imbibe water and swell under certain conditions. One form of swelling is the so called osmotic, or Donnan equilibrium type. Such swelling occurs maximally in sodium chloride solutions at certain pH levels. In the case of collagen in HCl solutions, H^+ and Cl^- ions diffuse into the tissue, where H^+ ions neutralise carboxyl ions in the free amino acid residues. At pH 2.5 a Donnan equilibrium is established such that there is a maximal excess of diffusible Cl^- ions held electrostatically to the insoluble protein inside the tissue over Cl^- ions in the external medium. Chloride ions

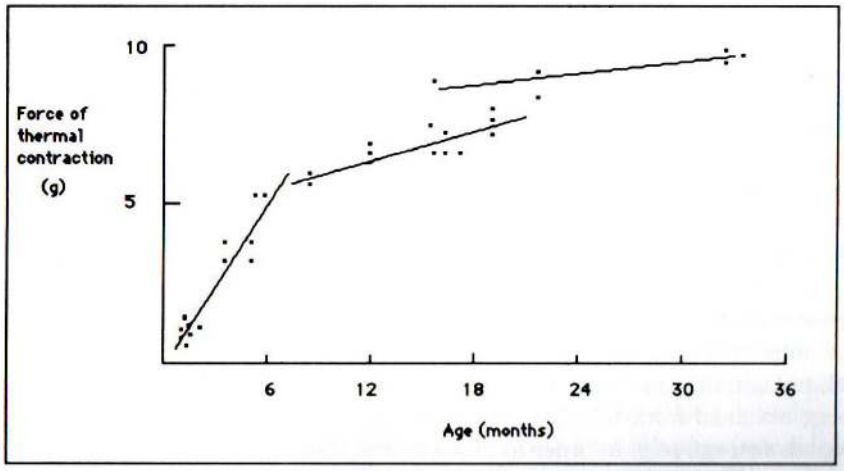


Figure 5.3. Thermal contraction of rat tail collagen at different ages.

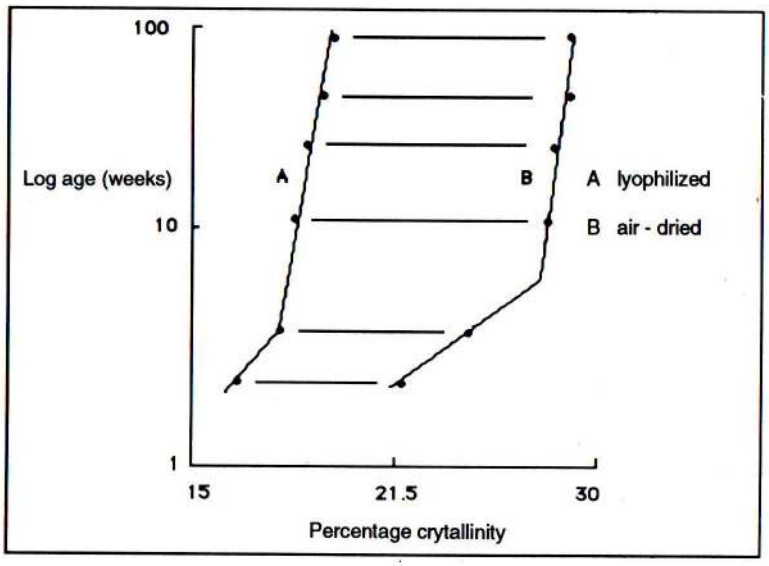


Figure 5.4. Crystallinity of rat tail collagen in relation to chronological age.

inside cannot diffuse out to equilibrate the chloride concentration in the medium, and will therefore exert an osmotic pressure, causing water to enter the tissue. The degree of expansion of the tissue due to the intake of water is actually a measure of the bulk modulus. Since swelling is restricted by stable cross-links the amount of swelling would be inversely related to the proportion of such links.

Human tendon has been used as an experimental material to test this idea (Figure 5.5). There is essentially no difference in osmotic swelling before maturity. Between the ages of 30 and 50 years there is a rather abrupt decline in swelling ability. After 50 years of age, swelling ability continues to decrease at a slower rate.

Swelling of collagenous tissue is also caused by thermal denaturation below the temperature at which contraction occurs. This is probably due to unfolding of partly coiled polypeptide chains held in place by hydrogen bonds. With increasing age of tissues there is an increase in the amount of time required for thermal swelling, and a decrease in the extent of swelling. Thermal denaturation and osmotic swelling experiments indicate that several different stages of increased cross-linking occur over the lifespan.

Based on the ideas that chemical oxidation was also responsible for the loss of the desirable properties of collagen, experiments were devised to age the fibres *in vitro*. Ageing of young fibres may be brought about by means of oxidizing substances (e.g. by $KMnO_4$) at alkaline or neutral reaction; and, on the

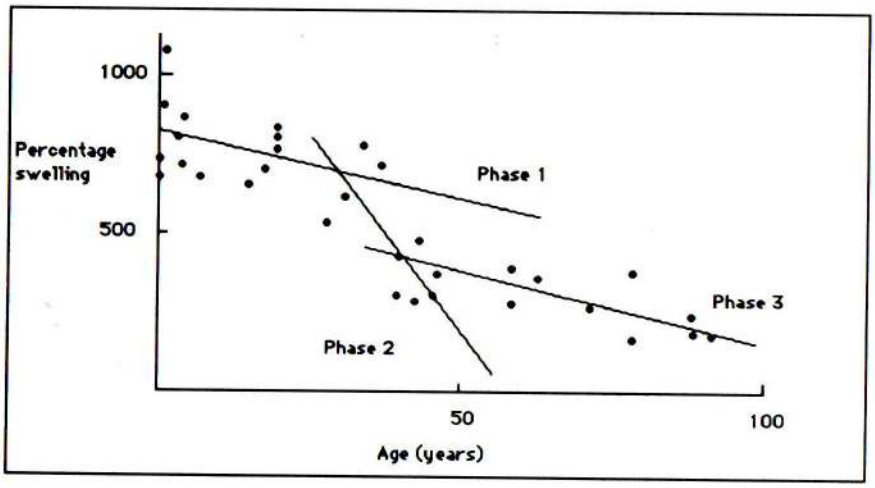


Figure 5.5. Osmotic swelling of human tendon in relation to age.

other hand, 'rejuvenation' of 'old' fibres may be carried out with reducing substances at acid pH; of these 'rejuvenating' compounds the most striking effect has been induced by ascorbic acid, which acts even at M/1000 concentration. This kind of result suggests that oxidation may be involved in forming cross-links in the body. An idea of the type of reaction that might be involved comes from test-tube experiments on lysine, the major amino acid in collagen, and its derivatives (Figure 5.6).

Collagen metabolism may be studied using glycine labelled with ^{14}C administered intraperitoneally. The 'metabolic activity' of collagen, as assayed by radioactivity, was high in young rats, very low in 'old' animals, and intermediate in adults. This indicates that even in adult rats, collagen fibres are continually, but very slowly, replaced: and it is likely that in young rats this change is somewhat faster. Whatever the nature of the changes it appears that they cannot be reversed *in vivo* because of the very low turnover of collagen. In this context, connective tissue proteins are for the most part irreplaceable.

Because of its high level of collagen and elastin, and its bearing on problems of human ageing such as cosmetic changes of wrinkling skin, non-healing ulceration, and skin cancer, skin tissue has been used frequently to investigate connective tissue ageing by *in vitro* experimentation.

With regard to general elasticity, the experimental extension of skin can be divided into three phases. In the first, rapid extension occurs, the second consists of a slower extension phase, and the third is a yielding phase tending to rupture. The first phase is ascribed to a straightening out of the weave of collagen bundles and an extension of the few elastic fibres of skin. The second phase is due to an extension of elastin and can be expressed as;

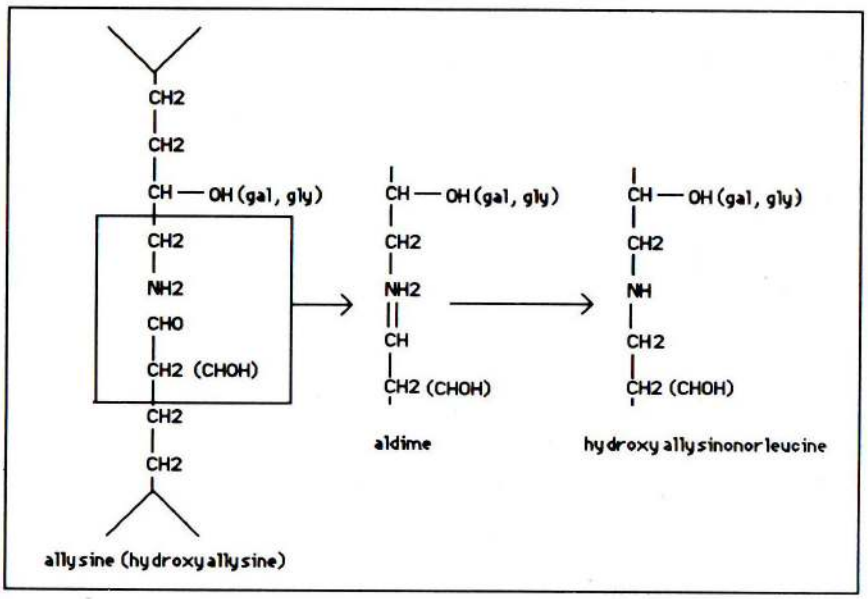


Figure 5.6. Possible covalent links that could be formed by oxidation involving lysine and hydroxylysine residues of collagen by way of aldimes.

$$E + C + kL\,b$$

The constant b represents the relationship between stress and strain—an increase indicates a 'stiffening' of the structure and a decrease shows a 'loosening' effect Figure 5.7).

It would therefore appear desirable to divide the ageing process in skin into two separate phenomena. The first, which may be regarded as a process of maturation, entails the formation of an increasing number of covalent cross-links. The second is a degeneration phase, when there is an increased aggregation of fibre proteins, and a loss of elasticity which at the surface results in wrinkles appearing where a pair of muscles exert a push-pull action.

Using collagenase on human skin, it can be shown that the hydrolysis of collagen decreases considerably during the first 20 years of age, slowly in the next three decades, and became comparatively constant in the following decades of life (Figure 5.8). This is indicative of a general closing up or masking of the fibrous core, but the exact molecular interpretation is unknown.

Results concerning the solubility of the skin are summarized in Table 5.2. These data are noteworthy in that, although in the majority of cases the solubility of collagen decreases with age, in the older group there are still 4% of individuals with the highest grade of collagen solubility. Most of the research

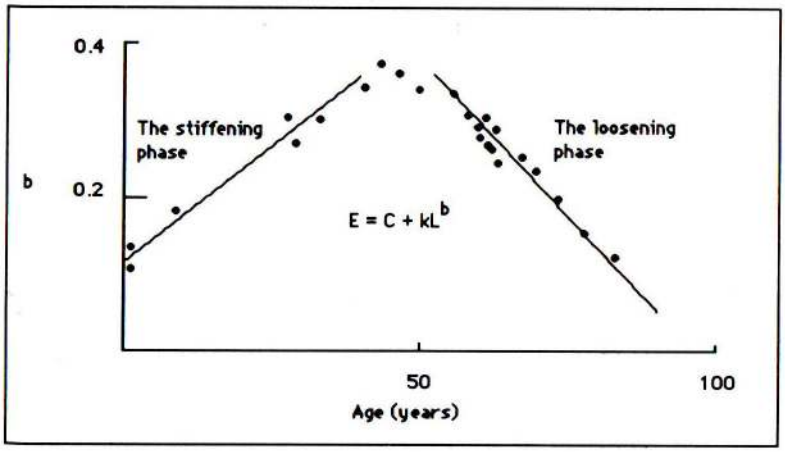


Figure 5.7. Age changes in the stiffness of human male abdominal skin.

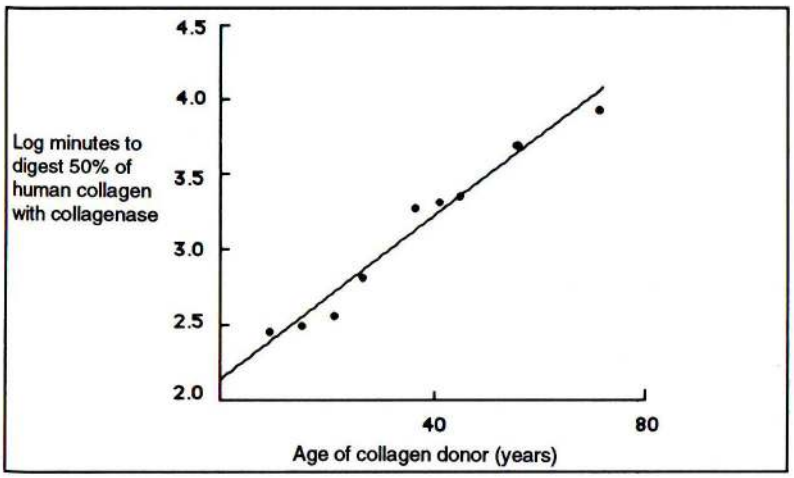


Figure 5.8. Time required to digest human collagen with collagenase in relation to age of collagen donor.

has so far concentrated on changes in morphology and chemical integrity of the various fibrous elements of the pericellular matrix. Soluble collagen prepared by extracting rat tail tendon with weak acetic acid may be precipitated from this solution, by addition of 0.01–1.0% of sodium chloride, when it forms collagen-like fibres. This provoked ideas that one of the factors causing sclerosis of organs and tissues with ageing, may be chemical processes alone, without any direct formation of the fibres by fibroblasts.

Table 5.2. Changes in solubility of human collagen with age

		Percentage distribution of individuals according to their collagen solubility			
Solubility*		Grade 1	Grade 2	Grade 3	Grade 4
Age groups	n				
1 year	16	6	19	25	50
1–30 years	20	20	30	35	15
30 years	83	35	31	30	4

* Grade 1 least soluble; Grade 4 most soluble

There is evidence pointing to the probable formation of elastin from collagen in the organism. For example, in infancy elastic fibres are produced by the activity of fibroblasts. These 'young' elastic fibres are relatively resistant to chemical and enzymatic action, and have the amino acid composition which has come to be regarded as most typical of elastin. In early adulthood, in the course of normal catabolism, collagen fibres begin to degrade and to form material deficient in hydroxyproline; this material, by combining with polysaccharides of the ground substance, constitutes a new type of elastic fibre, which in contradistinction to young elastic fibres, are more susceptible to enzymatic and chemical attacks. In old age the above-mentioned catabolic process continues, but the insufficiency of the ground substance prevents the formation of elastic fibres, while enhanced elastase activity results in the degradation of the already formed elastic fibres. Because of the importance of elasticity to the normal function of blood vessels this aspect has been studied in relation to the loss of elasticity of vessel walls. Experiments on human aorta show that homogenized elastic tissue from the aorta of old people is 'heavy' in terms of its sedimentation characteristics. It can be readily packed to the bottom of the centrifuge tube; while elastin from 'young' aortas is 'light' and remains to the surface. Amino acid composition is also different in these two elastins; the 'old' elastin contains more of aspartic and glutamic acids, possibly also more of some other amino acids; but less of glycine and valine; and probably less of proline and alanine.

Ground substance, even at electron-microscope magnifications, appears to be amorphous in structure. Chemically, it is characterized by considerable amounts of mucopolysaccharides. These compounds are combined in the ground substance with proteins. So far the following mucopolysaccharides have been isolated from various types of connective tissue, the main ones being hyaluronic acid, chondroitin sulphates, and various types of chondroitin and keratosulphate. Histologically, the proportion of fibrous elements increases with ageing, while that of the ground substance decreases; in electron-microscope photographs of this matrix the collagen fibres in younger individuals are seen to be covered with a dense veil of amorphous ground substance, while in old age

this does not occur. Chemically, changes in the proportion of the ground substance to collagen have been studied in the skin of rats, rabbits, pigeons, squabs and human subjects, and in the femurs of rats and guinea-pigs. The amount of ground substance was assayed by hexosamine content, since the latter is a constant constituent of mucopolysaccharides of the ground substance. During the growth period collagenous and elastic fibres are produced at a faster rate than the gel of the ground substance. In the case of squabs the period of growth ceases early, and at the same time the decline of this H/C ratio ceases as well. The changes in old age are not so dramatic in the ground substance as those in the fibre tracts, the integrity of ground substance may be equally significant in that the ground substance provides the basis for migration routes for white blood cells and macrophages.

ATROPHY OF ORGANS AND TISSUES

Two universal aspects of the failures of tissue homeostasis throughout the animal kingdom, and the features evident in every tissue of the body, are the age-related replacement of cells by unstructured extracellular matrix, and the slow, and often inadequate, responses of homeostatic systems to changes in environment. In this respect, ageing clearly involves reductions in the structural and physiological specifications of organs. The decline of structural specification is manifest as a loss of cells and an increased number of aberrant, intracellular macromolecular structures in those that remain, and this is a fundamental principle of gerontology. The spaces previously occupied by cells is filled by the development of unstructured pericellular matrix.

In organs that undergo a dramatic age-involution such as the thymus and kidney, a large proportion of the mass becomes connective tissue. However, even liver, which does not show involution accumulates collagen. This seems to take place because collagen is laid down continuously throughout life whereas cellular proliferation stops at maturity. The hepatic balance between collagen and hepatocytes can be altered by diet. In particular, collagen deposition appears to be more sensitive to dietary restriction than cellular growth (Figure 5.9).

Experimental gerontology at the cellular level deals with the following three propositions and their connections with the loss of adaptability to environment:

1. Ageing results in an increasing number of bad cells.
2. Ageing results in less cells.
3. Ageing results in the failure of communication between cells.

There is no doubt that old people contain less cells than at the peak of maturation. This cell loss is shown by indirect measurements of the volume of

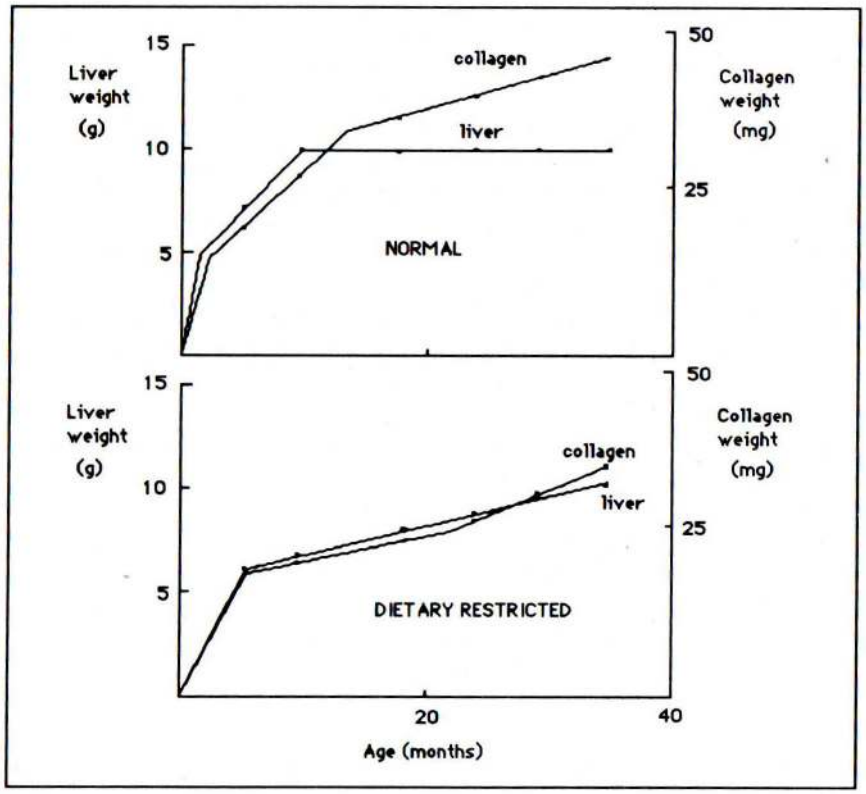


Figure 5.9. Collagen accumulation in rat liver in relation to liver growth in normal and dietary restricted rats.

the intracellular compartment of the body, and directly by the decreased actual and relative weights of organs, described as atrophy or involution. Organ involution occurs in a great majority of tissues, being least or absent in the heart, liver and prostate, and more or less pronounced in skeletal muscles, gonads, spleen, kidneys and bone. From their magnitude and obvious disruptive effects on organ structure these changes would, by themselves, be expected to account for the loss of adaptability to environment characteristic of old age.

Atrophic changes of organs and tissues were recognized by the early histologists as the most typical and universal feature of ageing. This feature led Warthin in 1929 to define old age as a 'major involution of the living organism', and he drew attention to the early onset of involution and the rapidity with

which it developed. He stressed that, 'each single line of involution once well initiated, may through the weakening or loss of the given function initiate or strengthen retrogressive changes in other organs'.

Involution is defined as the decreased actual and relative weight of organs and tissues. Experimentally and clinically there appears to be a high degree of cellular redundancy in that large volumes of cells can be lost by accident or disease with little or no loss of overall function. However, there may be possible latent deficiencies in this kind of massive cell loss, which become manifest in cases of increased and prolonged functional requirements, or in various physiological emergencies in the organism.

Korenchevsky was the first to collect data on changes in the weight of human organs with ageing; he recalculated and tabulated them, and also studied the problem of involution by experiments on rats. Most of the human data were obtained from hospitalized patients who died from various diseases, and there is much uncertainty whether these data represent normal organ weights. Therefore he also collected data from accident cases (suicide, murder, road or work accidents, etc.) which were assumed to be relevant to healthy persons (Table 5.3). The changes were the same in men and women, and surprisingly did not differ essentially in hospitalized and accident cases. As judged by actual weights, it appears that the growth of human organs (e.g. the heart, liver, testes) continues into the fifth decade or even later. During this 'late' period the growth of organs (if it occurs) is slow, in contrast to the vigorous growth during the 'early period' from birth to maturity. A definite decline of actual weights is usually observed in the sixth and seventh and especially in the eighth decades, but in some cases it starts as early as after the third decade. This senile decline of

Table 5.3. Average weights of organs in human males

	Weight of organs (g)									
Organ*	Te	Th	Ad	He*	Li*	Ki*	Sp*	Br*	Lu*	Mu*
Age										
Birth	1.1	4.7	6.2	2.3	14	2.4	1.1	39.7	5.0	
6–10	2.4	6.2	4.6	7.6	46	8.6	4.2	113	20.6	
11–15	3.3	9.0	6.6	13.0	64	12.0	58.0	130		14
16–20	7.6	2.1	8.6	19.0	104	20.0	11.0	140		23
21–30	36.0	34.0	14.0	26.0	147.0	16.0	14.0	136	67.0	42
31–40	41.0	33.0	15.0	31.0	156.0	29.0	16.0	139	81.0	54
41–50	41.0	33.0	15.0	31.0	156.0	29.0	16.0	139	81.0	54
51–60	42.0	33.0	13.0	33.0	160.0	27.0	14.0	136	79.0	54
61–70	34.0	30.0	13.0	34.0	152.0	28.0	15.0	134	82.0	48
70+	37.0	25.0	13.0	34.0	150.0	26.0	12.0	131	79.0	52
(%70+)**	−20	−17	−14	2	−15	−18	−50	−13	−7	−15

*Organs: Te, testes; Th, thyroid; Ad, adrenals; He, heart; Li, liver; Ki, kidney; Sp, spleen; Br, brain; Lu, lungs; Mu, triceps muscle.
**(%70+) = Percentage loss or gain of weight over the age of 70

organs and tissues is especially clear when the changes in the 'over-70 group' are compared with the weight values at the prime of life of the '21—30' group. Such comparison shows that the degree of senile involution is different in different organs, being least or absent in the heart, and more or less pronounced in the skeletal muscles, gonads, spleen, kidneys and liver.

Relative weights follow a different pattern in that the 'relative' involution with ageing starts in most organs after birth, and is greatest in childhood. Involution slows down considerably, especially after the third decade of life. In the last decades of life the relative weight of the heart and in some cases of a few other organs (the thyroid, brain, lungs) might rise again. The sex organs (testes, prostate, seminal vesicles, ovaries, uterus) and skeletal muscles, increase not only in their actual weights, but also in relative value, from birth to maturity. Only after the latter has been reached does their growth stop and sooner or later involution begins.

Examining organ weights in this way underestimates the likely functional alterations with ageing because a loss of parenchymatous cells may be compensated by an augmentation of connective and fatty tissues; or by an abnormal increase in extracellular water and mineral deposits producing abnormal composition of organs and tissues. However, taking this into consideration, changes in actual weight indicate that involution in most human organs and tissues appears to start between the fifth and seventh decades. Similar changes occur in rats between the 600th and 700th day of their life.

The heart is exceptional in it shows little weight change in either rats or humans. Korenchevsky felt that this was evidence of little functional change since most of the data were obtained from patients who died from grave diseases, i.e. the heart was resistant to the simultaneous effects of disease as well as normal involution.

The interpretation of morphological, chemical and functional changes in old people is extremely difficult. Pathological complications are most probably always present, and the number of these complications and the degree of their development is likely to vary greatly between individuals. These variations will be evident in morphological, chemical and functional changes in cells, tissues and organs. Therefore in most instances it is difficult to say whether any changes observed are due to the physiological causes of ageing or to its pathological complications. In this respect, the findings in two first-class research laboratories may be different, even opposed to each other.

Decrease in Proportion of Cells

Fibrous tissue cannot replace the highly specialized function of postmitotic cells, and Metchnikoff was the first to emphasize that in the main, morphological ageing is the process of replacing the highly differentiated parenchymatous cells by fibrous tissue elements, devoid of any highly specialized function.

The first indications pointing to the shifting balance of the fluid compartments of the body come from studies of young, growing experimental animals. In the chick embryo, for example, between the sixth and 18th days of life, while the dry body weight increases over two-fold, the chloride content drops by 25%. Chloride is mainly an extracellular anion and such a fall in the proportion of extracellular fluid indicated by the drop in chloride is probably common to all vertebrate embryos.

Postnatal development is characterized by a reversal of this embryonic trend. In rats, the cellular water of the individual fibres of skeletal muscle remains constant during the first year of life, whereas the proportion of extra-fibre water decreases. A similar change occurs in skeletal muscle of rats during the first 6 months of age. There is also an increase in size of the individual muscle fibres and a relative decrease in the size of the spaces between the fibres. Similar, but less marked changes in the extracellular fluid occur in liver and brain of growing cats with no change in the fluid content per cell; mature cats show just over a 10% decrease in extracellular fluid. Cardiac muscle of dogs falls into the same category in that the proportion of fibres increases significantly from neonate to adult. Detailed investigations on the postnatal development of the cerebral cortex in the guinea-pig shows that extracellular fluid, calculated from the chloride content, rises slightly at an early period when the nerve cells are becoming more spaced out, and then falls by about 50% by the time of adulthood. This kind of work provides the basis for the view that the early phase of life is concerned not only with building up the numbers of cells but also with increasing their density.

In contrast, after maturity there is a major alteration in the body's water compartments in the opposite direction. This appears to be connected with a decrease in the proportion of body water (Figure 5.10), a trend which seems to start at birth. Late in life by far the greatest part of the decrease is due to cell loss rather than cellular dehydration, indicated by a decline in the proportion of water contained in the cellular compartment of the body. By the ninth human decade this trend results in up to a 30% decrease in the volume of cellular water.

Sodium and potassium are the main cations responsible for maintaining osmotic pressure of the body fluids and the active conformation of enzymes. The amounts of these ions in the human body can be measured using isotopic exchange methods. These methods show that the decline in body water is linked with the loss of a third of the body's exchangeable sodium, which is mainly an extracellular ion (Table 5.4). Between the second and ninth decades there is a loss of 60% of the exchangeable potassium, most of which is in the cells. Although this change in the dynamics of cellular potassium proceeds at a steady rate there is evidence that the body has a transient gain of sodium during the seventh decade, indicating a selective enlargement of the extracellular fluid compartment. When expressed on the basis of total body water the changes in exchangeable cations are in the same direction but are not so marked. This

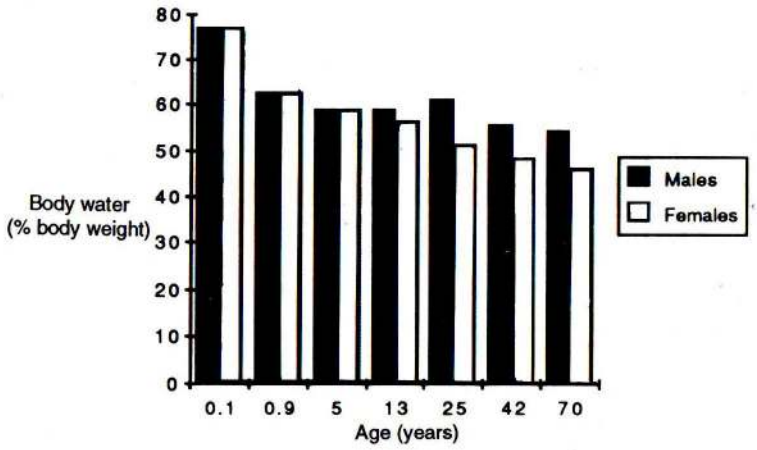


Figure 5.10. Changes in total body water of normal people.

Table 5.4. Effect of age on sodium and potassium in the human body

Age range	Total exchangeable ions		Ratio of ions to dry body weight	
	Potassium	Sodium	Potassium	Sodium
27–40	3271	3197	130.8	127.6
41–50	2933	2728	92.2	84.8
51–60	2348	2497	93.1	102.0
61–71	1992	2803	82.9	122.1
71–80	1679	2473	64.1	94.7
81–90	1363	2425	59.6	105.0
90–100	1214	2250	47.3	86.9

provides further evidence that they are not due to a decrease in the proportion of water in each cell or to major shifts in the dynamic balance of potassium to sodium across cell membranes, but to a decrease in the body's population of cells (Table 5.5). Most of the loss is accounted for by the loss of skeletal muscle.

It is important to note that the rate of loss of body potassium, and therefore the death of cells hardly changes from the third to the ninth decade of life (Figure 5.11). This indicates that the process of cell deletion originates long before the Gompertzian rise in mortality rate, and mortality is not related to cell death in a simple or direct way.

Age-involution is partly due to the loss of cells but is also associated with their partial replacement by non-cellular, connective tissue, a change that may be assessed in individual organs by quantitative histochemical measurement as

Table 5.5. Changes in the concentrations of sodium and potassium expressed on the basis of total body water

Age range	Potassium	Sodium	K/Na
27–40	56.5	55.3	1.02
41–50	54.9	50.3	1.09
51–60	48.3	53.2	0.91
61–70	42.0	60.7	0.69
71–80	40.5	60.5	0.67
81–90	35.2	63.5	0.55
91–100	33.5	63.5	0.56

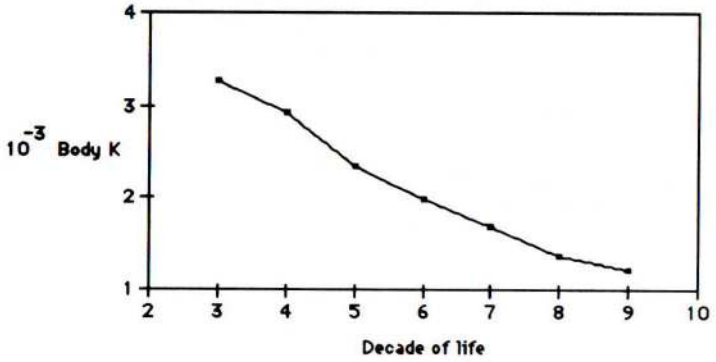


Figure 5.11. Loss of total body potassium.

well as by a rise in the proportion of extracellular fluid. These histological procedures are described as micromorphometrics.

In the brain the decrease in cellularity is evident from the decrease in brain size. There is also a shift towards a larger proportion of extracellular fluid, which has been followed using modern techniques of X-ray scanning, a procedure known as 'computed tomography'. The 'atrophy index' of the whole brain measured by tomography is the ratio of the volume of extracellular fluid to the volume of the bony cranial cavity, expressed as a percentage (Figure 5.12). This index increases consistently from the third decade.

THE KIDNEY MODEL

In the investigation of physiological ageing of the human kidneys the same great difficulty arises as in studying physiological processes of ageing in any other organ or tissue: experiments are nearly always performed on easily obtainable and controlled material, namely on hospitalized patients suffering

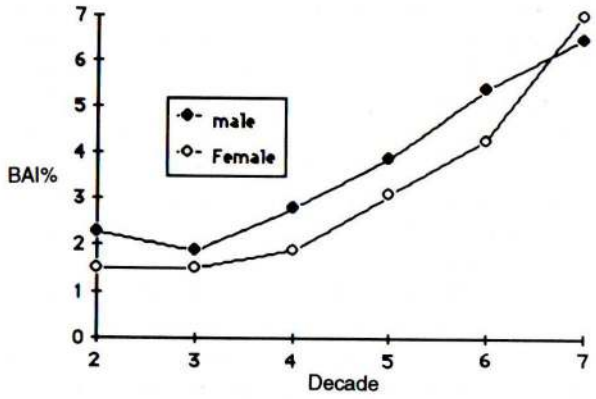


Figure 5.12. Rise in the 'atrophy index' (BAI%) of a cohort of people with no brain disease.

from various diseases and dying frequently in a state of exhaustion or from some highly pathological condition. Their organs are used for post-mortem morphological and chemical examination. It is the fact of using such pathological material, and not the difference or errors in the experimental technique, that is responsible for the wide range of variations usually observed in the results obtained.

This can be clearly seen in investigations dealing with the physiological ageing of the kidneys, and especially in the two main theories about the aetiology of this process. One of these theories explains the ageing changes in the kidneys not by the physiologically normal causes of ageing but by a definitely secondary and pathological cause of arteriosclerosis. In this respect, it has been considered that the ageing of the human kidney is only a special case of the ageing of the whole vascular system; and if a primary senescent involution of the kidney exists, its effects are overshadowed by the changes produced by arteriosclerosis.

The second theory acknowledges the primary ageing involution of parenchymatous elements of the kidney. Kaufman describes this process as a simple senile atrophy of the whole organ, diminution in the size of the tubular epithelium, and hyaline degeneration or total atrophy of several glomeruli. The atrophic changes in senile kidneys are defined as a typical senile process. This pure senile involution of the kidneys occurs in the minority of old people without any concomitant arteriosclerosis; while renal arteriosclerosis afflicts the overwhelming majority.

Not being, however, a constant concomitant of senile changes in the kidneys, renal arteriosclerosis obviously cannot be regarded as their primary cause. This contention is borne out by the fact that senile regressive changes occur in the kidneys of the dog and the rat which usually do not suffer from spontaneous arteriosclerosis. The primary simple atrophic, and secondary arteriosclerotic senile kidneys have been observed in about three-quarters of human cases.

On the average, in the great majority of cases, the involution of the human kidneys, as shown by decrease in their weight, usually starts from the sixth or seventh decade of life. This decrease is more pronounced in the actual than in the relative weights. On the other hand, in the minority of cases the highest figures in the range of variations of the kidney weights in seniles over 70 are equal (males) or very close (females) to the respective values of persons aged 21–30 years. This fact indicates that the involution of the kidneys as shown by weight decrease is not a constant concomitant feature of ageing. Similarly, in 300 post-mortem examinations of the kidneys in old subjects over 65 years of age, between 40 and 50% showed no gross structural changes.

Histologically in the great majority of cases, a wide range of variations in the severity of the atrophic and degenerative changes has been observed in senile human kidneys. Observations by histological examination, and microdissection of whole nephrons reveal all these changes in several aspects. A comparative absence of inflammatory changes is considered to be especially typical for pure forms of senile kidneys. The atrophic reduction of the nephron diameters may be so great that, at microdissection, about 30 atrophic nephrons may occupy the space usually filled by three normal ones.

In ageing rats chronic nephrosis occurs frequently in severe forms. In an average colony up to 30% of animals could suffer from between 300 to 500–600 days of age. High protein diet increases the incidence of 'senile' nephrosis, while this disease was exceptional in growth-retarded rats.

The variations in incidence between colonies is exemplified by the observations by Korenchevsky on his special gerontological colony established in the 1950s. He was unable to find this disease in senescent male and female rats aged 22–27 months. Only slight pathological changes were recorded in the senescent animals: from one to three minute nests of round cell infiltrations, and one or two edematous glomeruli and hyaline casts were found in some of the kidney cross-sections. These slight changes occurred in a considerable number of senescent rats: cell infiltrations in about 50–70%, oedematous glomeruli and casts in about 15–41%. But these slight abnormalities were not predominant features in the kidneys of senescent animals; on the contrary, progressive favourable changes were prevalent, namely compensatory hypertrophies of cells in renal convoluted tubules.

Compensatory hypertrophy of the cells in convoluted renal tubules with ageing has been observed both in man and rats, chiefly in the proximal convoluted tubules; such a hypertrophy may increase the functional tubular tissue by about 12 times.

Hypertrophy of the cellular mass of the convoluted tubules in ageing male rats as measured by the paper replica method, indicated that the weight of the tubules increased by about 35% at the age of 635 days; and of the size of the lumen by about 50%.

While (as the average figures show) the cytoplasm in the great majority of

the tubular cells hypertrophies, and the tubular lumen considerably enlarges with ageing, the size of the nuclei in cells remains comparatively constant. These results in the decrease of the nuclear-cytoplasmic ratio with ageing once more confirm Minot's generalizations about age changes in the ratio of nucleus to cytoplasm.

Thus, with ageing two opposite main processes, atrophic and hypertrophic, are simultaneously active in the tubular and glomerular tissues of the kidneys. It is very remarkable indeed that with ageing (in particular in the period of senescence) progressive hypertrophic processes predominate over regressive atrophic ones. This is especially clearly shown by the progressive increase in the average size of convoluted renal tubules with ageing. The results obtained do not justify defining the physiological ageing of the kidneys as an 'atrophic' or even as a 'mainly atrophic and regressive' process. This conclusion accords with the previous interpretation of the changes in the weight of the kidneys with ageing.

Experimentally produced hypertrophy of the kidneys shows that the rate of the hypertrophy of the remaining kidney decreases with ageing, but still remains considerable. After unilateral nephrectomy the quotient of the hypertrophy obtained in young animals (aged 1–6 months), adult and senescent rats (aged 15–26 months) and in old rats (29–31 months) was 140–142%, 122–125% and 138% respectively. Therefore, the compensatory hypertrophy spontaneously developing with ageing in renal tissues, and that produced experimentally, both show that the compensatory growth response is preserved in old kidneys.

Changes have been found consistently with ageing in the renal glomeruli of man and rat. In all these investigations it was found that the number of the glomeruli decreases with ageing. This number in human senile kidneys may become equal to two thirds or even one half of that in the kidneys of young adults. In rats about 10 700 glomeruli were counted at birth, 31 000 at maturity, and about 20 000 in senescent rats. The size of the glomeruli may remain normal, or become enlarged or atrophic; and some of them may finally perish. The range of variations in the size of glomeruli is considerably greater in the old rats than in the young. Apparently in older animals, the smaller sizes indicate more pronounced atrophic ageing changes, while the larger sizes express a compensatory hypertrophy of the remaining normal glomeruli. Some normal structures of the renal glomeruli may be observed in extreme old age: e.g. 78–83% of histologically normal glomeruli were found in three of the investigated human subjects aged 86–90 years. The number of renal mitoses decreases with ageing as it does in all other organs.

Further details of the decline in cellularity of the kidney are revealed by quantitative histology. For example, there is an overall decrease in the size of the human kidneys after the third decade due to a loss of nephrons. There is also a decrease in the cellularity of the remaining structural components and the density of tubule cells falls steadily from the first decade along with a drop in the

density of the glomeruli and the number of cells per glomerular tuft (Table 5.6).

The size of the malpighian corpuscles and their glomerular tufts increase in size from an early age indicating that the whole structural specification of the renal tubules is a dynamic system, which is constantly changing throughout life (Figure 5.13). As time passes, the frequency of atrophic glomeruli and tubules increases. These results raise questions as to the way in which the unit structures of organs, such as the nephron, are maintained, particularly with respect to the interconnections between the cell populations making up the different anatomical components.

Even where there is no evidence for a major loss of cells in terms of total weight of the organ, as for example in the small intestine, careful measurements of histological sections reveals that older animals have a different specification of unit structures from younger ones. As an example, the epithelium of the

Table 5.6. Changes in the cell density of human kidney tubules

Age	Cell density	Glomerulus density	Cells/glomerulus
1–10	338	74.9	163
11–20	319	54.1	156
21–30	310	49.2	154
31–40	290	44.8	145
41–50	292	43.7	137
51–60	284	41.3	131
61–70	270	39.1	123

All densities are cells/14×10^4 μM^2

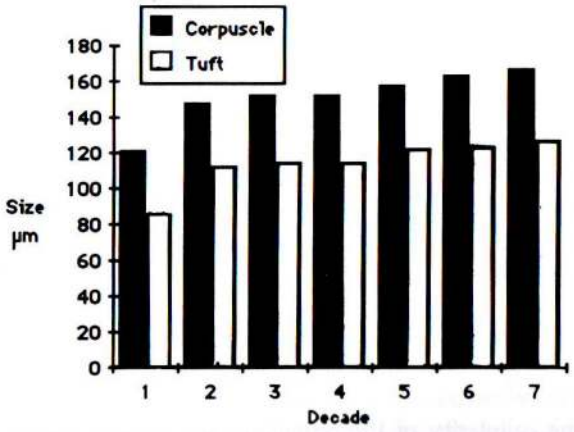


Figure 5.13. Changes in size of human kidney corpuscles and glomerular tuft.

jejunum of old mice is very similar to that of young animals except for a 27% decrease in the depth of the crypts (Table 5.7). Such a difference could impair absorption and may be associated with defects in the cellular replacement/repair processes of the intestinal mucosa of the older animals.

VARIABILITY IN STRUCTURAL SPECIFICATIONS

Most medical assessments of ageing are carried out on individuals taken from populations that have either been divided into age cohorts before selection, or that are taken at random and subsequently classified into cohorts based on decades. This type of selection is called cross-sectional sampling, and was discussed in Chapter 2. It has the disadvantage that different cohorts have probably encountered different environments as they have aged, and so are not strictly comparable. Nevertheless, cross-sectional analysis is more often used because it is easier to organize than the better, but more complicated method of longitudinal sampling, where a particular parameter is followed in the same individual over several years.

Cross-sectional measurements on people categorized in decades often show an increase in the variability of the parameter being measured, even when the mean value does not change with the age of the cohort. An example of this type of result is given for the decline in body potassium and rise in sodium already discussed (Figure 5.14).

Variability is commonly expressed as the standard deviation divided by the mean value for the cohort, expressed as a percentage. This index, called the coefficient of variability, provides a measure of the spread of the hypothetical normal distribution curve for all values for individual members of the cohort, corrected for any difference in the mean value. For both cations in the body fluids there is a drop in variability between individuals in the fourth decade, followed by a rise to maxima in the seventh decade for potassium, and the

Table 5.7. Effect of ageing on the structure of the rat jejunum

Structure	Linear dimension (μ)	
	5 months	27 months
Villus–crypt length	6000.0	646.0
Villus height	472.0	500.0
Crypt depth	120.0	153.0
Height/depth	4.0	3.3
Villus width		
Upper	102.0	111.0
Lower	105.0	110.0
Villus core thickness	30.0	34.8

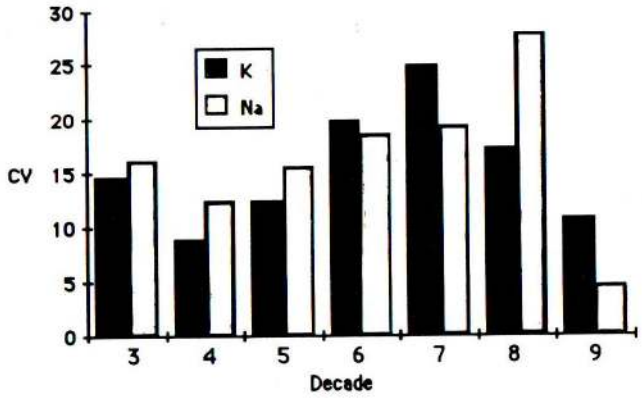


Figure 5.14. Changes in the coefficient of variability (CV) of total exchangeable sodium and potassium related to total body water.

eighth decade for sodium. The subsequent fall in the variability of both ions brings the population variability in the ninth decade close to the minimum of the fourth decade. This age-related pattern of variability may be taken to mean that ageing results in random drifts in individuals away from an earlier more precise specification of body electrolytes. The existence of a small population of very old people with low electrolyte variability may well be the outcome of selection against the more vulnerable members of the population who, for whatever reason, departed too much from the youthful specification.

Similar tendencies for physiological variability to increase within the sampled population are also seen in the size of cells, and in the dimensions of cellular organelles such as the nucleus (Figure 5.15). Variability in both of these parameters begins to increase in the human kidney during the first decade of life. Here the variability is due not only to increasing differences between individuals but also to variations between individual cells and organelles within each individual. Thus, there is actually an age-related increased variability in the coefficient of variation of hepatic cell nuclei (Figure 5.16).

CLOCKS OF INVOLUTION

Current ideas on the initiation of involution involve the concept of a physiological clock, set mainly by chronological age, but which may be speeded up or slowed down by the plane of nutrition and temperature. Broadly speaking research is organized into two schools; one group of researchers regards ageing as being governed by a central 'head clock'; the other postulates the existence of many individual organ clocks.

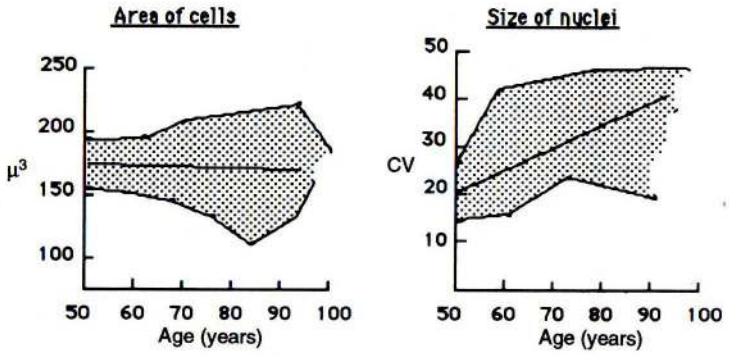


Figure 5.15. Changes in area of liver cells and variation in size of hepatic nuclei in livers of 74 Japanese autopsy cases (scatter of points and regression lines).

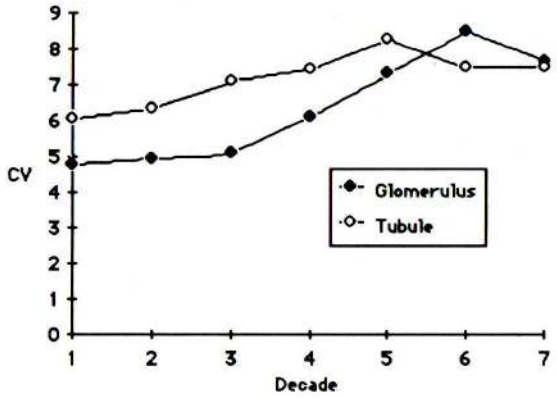


Figure 5.16. Changes in the variability of nuclear dimensions in cells of human renal glomeruli and tubules.

Investigation of cellular clocks of ageing requires the careful choice of a suitable organ as a model. Ideally the model should:

1. Consist of an organ or part of an organ system.
2. Show well-defined temporal changes in structure and function.
3. Be readily accessible.
4. Consist of a uniform population of cells.
5. Be capable of experimental manipulation both *in vitro* and *in vivo*.
6. Not be a vital organ to give scope for *in vivo* experimentation.

From all of these points of view the rodent thymus gland has long been regarded as a suitable model for studying age involution. The organ is composed mainly of two cell types, the epithelial cells, which govern the overall shape and size of the gland, and the lymphocytes which are under the control of the epithelial cells. The bulk of the mature organ is composed of lymphocytes characterized by a high rate of cellular turnover, with about 10% of the longer-lived epithelial cells. The thymus matures very early in life, subsequently showing a rapid decline in the total numbers and density of its lymphocytes. This change is evident by a large drop in organ weight which begins at about the time of sexual maturation. The precise timing of involution indicates the value of the thymus as a clock model. As well as losing cells the remaining tissue is infiltrated by connective tissue and the gland loses the characteristic bilobed shape of maturity. From the age of 55 days the rat thymus drops in weight from about 250 mg per 100 g body weight to about 25 mg per 100 g body weight at 700 days. Most of this loss occurs in the first 300 days.

It is possible to reduce the number of cells in the thymus by treatment of intact animals with corticosteroid hormones. After hormone injection the cell number is rapidly restored to normal, a response which involves shifts in the relationship between cell division and cell death offering insights into the factors controlling the cellular composition of organs. Although this experimental manipulation is very useful in defining the powers of thymic regeneration the effects of corticosteroids probably have very little relevance to age involution.

Another important advantage of the thymic model is that whole organ grafts may be made successfully at almost any site of the body offering possibilities of examining the interplay between intrinsic and extrinsic ageing factors.

Work on the thymus as a model for age-involution has provided answers to the following questions of principle.

1. Is age involution controlled by peripheral factors?
2. What is it that shifts the balance between cells and extracellular components?

Is Age Involution Controlled by Peripheral Factors?

The observation that thymic involution begins at about the time of sexual maturation led many workers to believe that involution was triggered by sex hormones and was under the control of the reproductive system. Although organ size may be reduced by sex hormones, this is reversible. Furthermore, although in castrated animals of any age the size of the gland is higher than in animals with normal levels of circulating sex hormones, the initiation of involution and the timing of the steady loss of weight after maturity into old age are not affected (Figure 5.17).

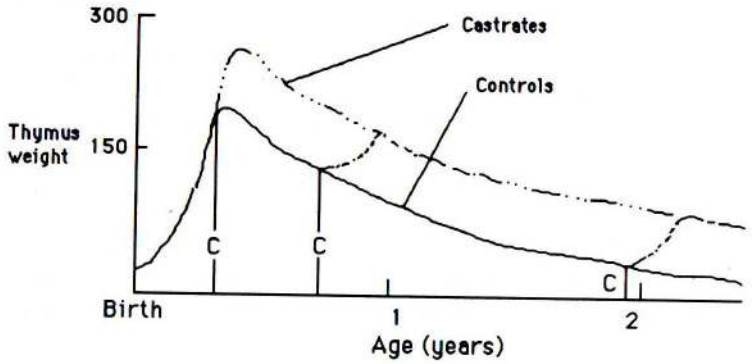


Figure 5.17. Age-involution of the rat thymus and effects of castration (C) at different ages.

Definitive evidence that the 'clock' of involution is intrinsic to the thymus and is not activated peripherally from some kind of central 'ageing clock' is that transplants obtained from very young animals always begin to involute at about 9 weeks irrespective of the age of the host. Furthermore, multiple transplants in the same host always grow to the same maximum size before involution no matter how many are transplanted at one time. These experiments argue against the thymic cell population being governed by diffusible self-inhibiting molecules and again point to growth and involution being regulated from within the gland. The epithelial cells, being the stable element of the gland, are the most probable site of the 'growth/ ageing clock'.

The Balance Between Cells and Extracellular Components

Although lymphocytes make up about 90% of the total mass of the young thymus, other cell types are present, such as epithelial cells, fibroblasts and macrophages, and extracellular tissue dominated by collagen. With age, the proportion of lymphocytes decreases with a rise in the density of fibroblasts and macrophages. The proportion of collagen also increases until it occupies most of the volume of the thymus in senile animals. In both young and old animals the turnover of thymic lymphocytes is very high. In a young rat about 2% of its thymic lymphocytes are in division at any one time giving an average lifespan of about 18 hours. Cell division balances the continuous loss of lymphocytes which involves migration from the gland and death in the gland.

Pieces of thymus may be maintained under tissue culture conditions for several days. During this time the lymphocytes continue to die. Dead cells that appear in the explant are ingested by macrophages, which increase in number,

but the lost lymphocytes are not replaced through lymphocyte division. As the number of lymphocytes declines fibroblasts begin to proliferate and eventually they dominate the tissue explant. Although no definite mechanism of inter-dependent regulation has been established between the two types of cells this inverse relationship may be taken to mean that fibroblast proliferation is normally inhibited by adjacent lymphocytes. Fibroblasts and the collagen they synthesize occupy space left by the dead lymphocytes. This series of events in thymic explants is similar to that occurring during wound healing and indicates that the prime event in involution is cell death exceeding division in mitotic tissues, and cell deletion in tissues consisting of postmitotic cells. Pericellular connective tissue accumulates with age as a secondary response to fill the space formerly occupied by cells.

CHAPTER 6 Organ homeostasis

Chronobiology (a section of biology) represents the objective description of biological time structure—the sum total of nonrandom, and thus predictable temporal aspects of organismic behaviour including with the spectrum of biologic rhythms, developmental changes, growth and age trends.

It is now recognized that biological time structure characterizes individuals and their subdivisions (organ systems, organs, tissues, cells and intracellular elements including electron-microscope and molecular ultrastucture) as well as groups of populations of organisms. It has now also become apparent that a complete study of ageing must be concerned with the broad spectrum of rhythms not merely because it represents a confusing source of variability but rather because it may gauge sensitively (otherwise unattainable features) in the dynamics of senescence and senility. (Halberg 1982)

HOMEOSTASIS

The maintenance of a steady state or homeostasis is a general characteristic of all organisms. It is a characteristic of cells, their component organelles, the tissues of which they are the building blocks and the entire organism which is the outcome of co-operation between its various tissues.

The life of each cell is a balance between processes of construction and destruction, and each cell is itself a steady-state system, maintained precariously between these opposing tendencies. The important feature of any homeostatic system is that it detects changes in the working conditions and provides information as feedback for making the appropriate corrective response out of a number of possible actions. An animal or plant thereby depends on a series of biological decisions or choices from moment to moment as it lives in an ever-changing environment. Night follows day, a cloud obscures the sun. There may be alternatives to attack or to retreat. The question may be in what way an oxygen or sugar molecule should be combined with existing substances in the body.

In every homeostatic response, at every level of complexity, is it possible to identify the same basic components? There are receptors which receive information as a coded representation of the course of the system. A comparator measures the difference between the actual code received and the code of instruction that the machine is supposed to follow. Compensatory changes are made from moment to moment to eliminate any difference between

the instructions and the actual course of action. Where the course of action is to maintain a fixed state, i.e a constant concentration, or a precise, repeatable sequence of neuromuscular activity, we can define the norms of the steady state. Where there is a progressive, irreversible change in size or shape as in development, homeostasis keeps the changes within species limits which define the normal trajectory of change. All norms have the possibility of drift due to errors in operation. These may arise from delay or inertia, i.e. not following the code of command closely enough, or they may arise from overcorrection during adjustment, and give rise to some form of disturbance, which may be irreversible. At all levels of complexity ageing is a failure of homeostasis but it is at the level of control and regulation of organ function that it is most easily perceived biologically, socially and medically.

BEHAVIOURAL AND PHYSIOLOGICAL NORMS

It is probably safe to state that the ability of every organ, or organ system, declines functionally and progressively with age. The task is to knit together experimental results at all levels of analysis and provide, for each organ, a total explanation for the decline in physiological efficiency. Needless to say we are far from achieving this kind of explanation for even the simplest organ.

The major task is to relate changes in physiological norms to the systems which set them, the controllers, and the systems which maintain them, the regulators. This involves studying the short-term regulation of behavioural and physiological norms in response to demands of the environment, which is mainly the task of the central nervous and endocrine systems. But it is also necessary to integrate this work with the longer-term endogenous controls and regulators which maintain endogenous rhythms (biological rhythms) in baseline function.

Norms are not only set for body fluid homeostasis but also for our bodily proportions, and the complex behavioural patterns which characterize the ways we walk, talk and think. Anthropomorphic data clearly indicate that the elderly are subject to change in a wide spectrum of norms, from those governing gait and posture to those responsible for maintaining general body proportions (Table 6.1). Height and its associated body and limb lengths, and biacromial and bideltoid diameters, decrease steadily with age. This is interpreted as showing the secular trend towards increased body size in the population, with individual shrinkage after the sixth decade. Functionally, grip strength falls steadily. Weight and triceps skinfold remain fairly constant until the sixth decade then fall. Subscapular skinfold increases slightly from the third to the fifth decade, remains constant to the seventh and then falls to its initial value. Bi-iliac diameter, chest and abdominal depth, abdominal circumference, and nose and ear lengths increase. Head, face, hand and foot measurements are similar at all ages.

Table 6.1. Percentage decrease in norms of physique in 2015 medically screened healthy white North American males aged between 22 and 82 years of age.

Weight	5.9	Sitting height	3.7
Height	3.5	Trunk height	2.4
Span	2.1	Knee height	3.6
Forward arm reach	1.0	Popliteal height	4.1
Span akimbo	1.6	Thigh height	5.8
Biacromial	3.3	Buttock–knee	2.6
Bideltoid	5.3	Buttock–popliteal	2.6
Skinfold triceps	22.0	Seat breadth	2.0
Cirumference upper arm	5.7	Elbow–mid finger	2.1
Circumference calf	6.1	Grip right	29.5
Circumference waist	1.6	Grip left	28.2

Changes in a range of behavioural norms can also be measured. These are probably associated with differences in both bodily proportions and changes in sensory/motor functions. For example, when moving, old men take a shorter stride with a diminished vertical excursion of the head. They also show decreases in excursions of the lower limbs during the swing phase and decreases in their knee flexion and peak heel elevation.

In long-term responses involving locomotor activities the impairment in ventilation and gas exchange appears to underlie the age deterioration. However, in short-term reflex delays in the aged it is suggested that the increased reaction time is due to decreased excitatory influences from higher levels. A specific component of the change may be the loss of motor end plates.

Norms for cognitive behaviour also change with age. Although there are serious methodological problems in carrying out tests on people there is general agreement that both ontogenetic and generational changes can be detected in a range of tests. Where tests have included measurements of verbal meaning, space, reasoning, number and world fluency it appears that generational changes are more important than the ontogenetic components.

Research has also been carried out on behavioural norms of maze learning in laboratory rats. Where mazes of varying complexity have been used it has been found that age differences are found for learning tasks with a high degree of complexity rather than for relatively simple tasks. For example, these differences are revealed using T-mazes with a large number of choices. This situation also reveals a great deal of variation between learning in older animals which may often be divided into two or more groups on the basis of the speed of learning and the level of errors at the termination of the experiment (Figure 6.1).

Clark in the late 50s investigated a gamut of behavioural changes in an effort to establish a general theory and establish a minimum number of parameters that could establish a single dimension along which ageing occurs. He worked

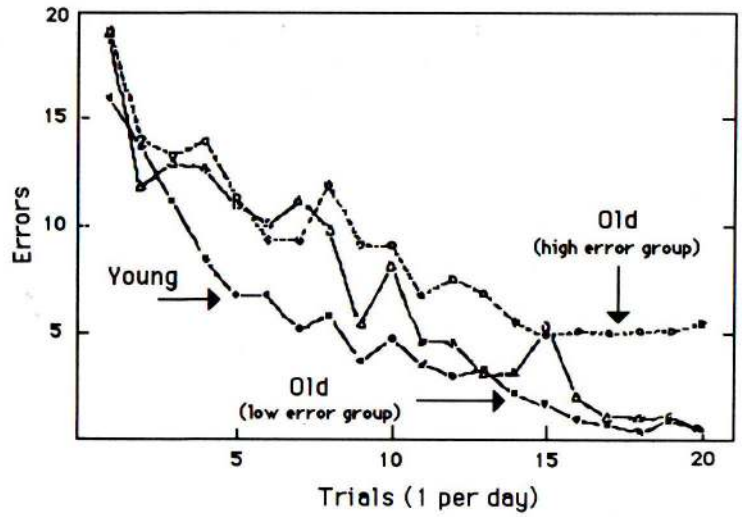


Figure 6.1. Maze learning in a 14 unit T-maze by 8 and 24 month old male rats.

with 10 males and 10 females in each decade over an age range from 20–70 years. The following variables were assessed:

> age, sex, spatial ability, reasoning, reading preference, aspiration level responsiveness, letter comparison speed, critical aggressiveness, accuracy, associations, emotional recall, attitude to religion, systolic blood pressure, attitude toward old age, hand strength, reaction time, auditory threshold, lens accommodation, word association latency, fear of death, maze errors, maze time and socioeconomic status.

Clark's analysis revealed that the effects of age occur along a single dimension and are best measured by systolic blood pressure, lens accommodation at near point of vision, and sound threshold (3000 Hz). A single factor with a loading of 19 of the 25 measures could account for almost all of the variance of age.

In contrast to analysis of ageing at the behavioural level, changes in physiological norms are not so readily revealed. If the composition of the blood is taken as an indicator of the overall state of homeostasis, there appears to be no impairment of functional capacity. For example, acid-base balance of the blood in resting humans varies little between the ages of 25 and 85 and there are no significant changes in the carbon dioxide tension, total carbon dioxide content and bicarbonate concentration. In agreement with this the pH only changes by about 0.06 units between the second and eighth decades of life. Organic constituents also show a similar stability. The general constancy of the ionic composition of the body is also borne out by studies on individual tissues.

The liver is particularly significant in this context in that it is responsible for metabolic homeostasis and no major changes are observed in total water, fat, potassium and sodium content. Similarly, in muscle, the intracellular water, nitrogen, potassium and phosphorus decrease only between 5 and 8%. There is no change in muscle lactic acid and creatinine. Analysis of human aortas taken over an age range from 2–69 years reveals no significant alterations in either total nitrogen or sulphur; elastin and creatine also remain relatively constant.

The relative constancy of blood chemistry is also borne out by long-term studies of laboratory rats (Table 6.2).

The failures to detect age changes indicative of a large scale deterioration in blood homeostasis are perhaps surprising when placed alongside the gradual general increased cytological atrophy.

Table 6.2. Blood chemistry of male Wistar rats of different ages

Age (months)	10	20	24
% Survival	90+	80	69
Alanine transferase[5]	55.0	55.0	55.0
Albumin[3]	3.7	3.7	3.7
Alkaline phosphatase[2]	204.0	196.0	231.0
Aspartate transaminase[5]	128.0	128.0	128.0
Bilirubin[1]	1.0	1.0	1.0
Blood urea N[1]	14.0	14.0	14.0
Calcium[1]	9.8	9.8	9.8
Chloride[4]	107.0	107.0	107.0
Cholesterol[1]	94.0	116.0	142.0
CO2 content[4]	24.1	24.1	24.1
Creatinine[1]	0.44	0.44	0.44
Gamma-glutamyl transferase[5]	1.8	1.8	1.8
Globulin[3]	2.6	2.4	2.7
Glucose[1]	131.0	160.0	163.0
Inorganic P[1]	5.8	5.6	5.1
Potassium[4]	5.5	5.5	5.5
Prolactin[8]	29.0	43.0	52.0
Sodium[4]	145.0	145.0	145.0
Testosterone[6]	3.2	3.2	1.5
Thyroxine[6]	4.5	4.1	3.4
Total protein[3]	6.2	6.2	6.3
Triglycerides[1]	126.0	118.0	167.0
Triiodothyronine[7]	86.0	99.0	94.0
TSH[8]	2.1	1.7	1.7
Uric acid[1]	1.4	1.4	1.4

[1]mg/100ml; [2]IU/l; [3]g/100ml; [4]mEQ/l; [5]IU/ml; [6]ug/100ml; [7]ng/100ml; [8]ng/ml.

BLOOD HOMEOSTASIS: THE GLOBULIN MODEL

On the face of it blood chemistry offers a model for investigating the reasons for changes in norms in that the plasma concentration of a substance is a balance between its input and output from this fluid compartment (Figure 6.2). Globulin is synthesized in liver cell nuclei from the local pool of amino acids. It enters the blood where it has a characteristic half-life before most of it is broken down again to amino acids. At any one time the concentration of plasma globulin is, in the main, a balance between synthesis and degradation. There is a control which sets the permitted range of variation and any departure from this triggers regulatory mechanisms which either remove or add globulin to the plasma to maintain the norm.

Over the years there have been many reports dealing with different electrophoretic patterns of serum proteins from young and old people. There is now general agreement that the relative concentration of albumin decreases and the relative concentration of globulins increases with age. A similar phenomenon occurs in rats, and in both rats and people it has been found that the increase in globulins is mainly in the alpha globulin fraction (Table 6.3).

A simple experimental approach to investigate this age-related change in the steady state of blood proteins is to measure the rate of synthesis using a radioactive isotope. The results of such an experiment are presented in Table 6.4.

If valid comparisons are to be made of proteins synthesized in the liver it is necessary to determine the specific activity of amino acid precursor in liver cells for both groups of rats. This data was obtained by measuring the specific activity of free leucine in liver nuclei at various times after injection of a labelled amino acid. In making this measurement the assumption is made that there is rapid equilibration of small molecules between nucleus and cytoplasm. There is

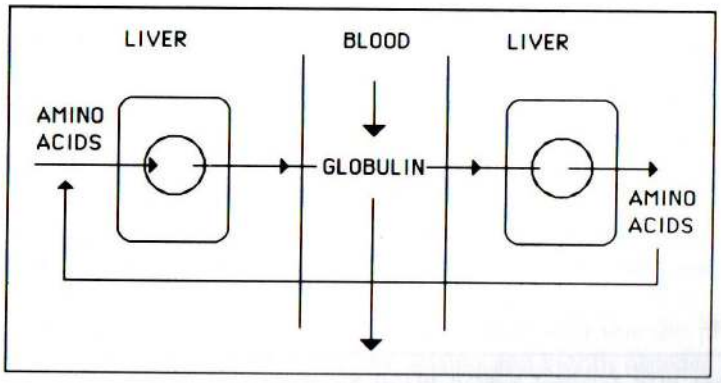


Figure 6.2. Model of the dynamics of plasma globulin.

Table 6.3. Albumin and globulin in young and old rat sera (n = 15)

	Protein concentration (mean and range; mg/ml)			
	Young rats 6 weeks		Old rats 20–24 months	
Serum protein	56.5	(50.0–65.4)	73.9	(68.0–84.5)
Serum albumin	29.9	(27.1–36.2)	31.3	(26.8–35.0)
Serum globulin	23.2	(20.0–28.3)	39.1	(33.0–46.4)

Table 6.4. Amino acid pool size and synthesis of rat plasma globulins using leucine labelled with ^{14}C and ^{3}H *in vivo*.

	Pool size (cpm/mg protein)			Alpha globulins (cpm/μmole leucine)		
Time (min)	Y	O	Y/O	Y	O	Y/O
0				1.1	0	—
1.0	160000	61500	2.6	16.7	7.5	2.23
2.0	194000	84250	2.3	34.1	15.6	2.18
5.0	82000	46300	1.8	113.0	62.0	1.83

about a twofold difference in pool size between young and old rats and the same difference in the apparent rate of protein synthesis. Since the globulin in old rats was synthesized from a pool of leucine that was twice that in young rats the radioactivity would be diluted by a factor of two and the apparent rate of synthesis would be half as much as in the young rats with half the pool size and twice the concentration of radioactivity per leucine molecule.

Synthesis is measured by the appearance of label in serum proteins in circulating blood. Cyclohexamide is given at various times after injection of the radioactive leucine to prevent further protein synthesis to obtain numbers which represent synthesis during short pulse times. Differences in the rates of incorporation and differences in pool size between young and old rats are the same. The conclusion is that there is no difference in the rate of globulin synthesis. There is a substantial increase in the half lives of total globulins and alpha globulin in old rats so it may well be that the rise in these proteins is due to a slower rate of their degradation.

This leaves the following questions about prime causation unanswered:

1. Does the degradation regulator adjust?
2. Is the higher level of globulins the result of a failure of a synthesis regulator to detect a rise of protein concentration?
3. Is there a failure of a stimulatory regulator of protein degradation?

4. Is a new route for protein degradation opened up?
5. Is there a connection between age changes in amino acid pool size and changes in the norms for circulating globulins?
6. Is there a connection with changes in the immune response?

In leaving these fundamental questions of principle unanswered the blood protein model stands generally for the difficulties in opening up complex closed loop feedback systems to define the origins and physiological implications of age-related changes in the concentrations of important metabolites.

NORMS IN TISSUE STRUCTURE

It is virtually impossible to work backwards from changes in the norms of tissue structure. For example, at the tissue level, only moderate signs of deterioration are found in the histochemistry of liver and there are no obvious large-scale functional deficiencies in the brain. Kidney which is marked by having the largest age-alterations in histological structure does not show physiological deterioration on the same scale.

Despite this evidence in favour of an overall stability of tissue function despite large changes in structure norms, age changes in composition are frequently observed, although they do not fall into any obvious physiological or biochemical pattern.

Where changes are found they are not uniform between tissues. For instance, although there is no variation in the riboflavin content of human brain, heart and skeletal muscle over seven decades, aortic tissue is characterized by a 60% drop in the riboflavin content over the same period. Also, in contrast to ventricular tissue, the foliar tissue of the cerebellum loses 30% of its RNA throughout the lifespan.

Some of the largest alterations in tissue composition have been found in the lipid fraction. It has already been pointed out that a common feature of ageing in many types of postmitotic cells is the progressive accumulation of age pigment. This is a yellow-brown intracellular material with the properties of a highly oxidized lipid. In the brain of old rodents the amounts range from 0.6% in the granular cells of the cerebellum to 17% in the Purkinje cells. Cholesterol in the blood of healthy women increases twofold in concentration from the age of 20–60. There are also substantial increases in the concentration of tissue elastin, collagen, mucopolysaccharides and calcium. However, these changes, although a general feature of ageing in many tissues, are not universal in their magnitude between both tissues and subjects.

Therefore, taking the available evidence on the gross composition of tissues, it appears that on the whole, age changes are not very marked, or general, in those intracellular components that are concerned with fundamental cellular

organization. On the other hand there are marked changes in the chemistry of the extracellular compartment, notably in the ground substance. These changes are probably a reflection of the general tendency for there to be an age-dependent shift in the balance of cell populations. For example, the eosinophil count in rats decreases progressively with age, there being a 50% drop between the ages of 300 and 800 days. In ageing cattle, it has been observed that a decrease in blood lymphocytes is accompanied by a rise in the proportion of blood neutrophils and a fall in the total leukocyte counts. These cellular changes indicate shifts in the stem cell production system, but the outcome of these shifts cannot be related to any failure of function of, for example, the inflammatory response.

STRUCTURAL HOMEOSTASIS: THE THYMIC MODEL

It has already been pointed out that changes in homeostasis are seen most dramatically at the histological level. Tissue involution is a universal aspect of all organs and tissues indicative of a failure to maintain a balance between different cell types and their extracellular matrix. This is a well-established and obvious feature of the thymus and lymphoid tissues. In the thymus the loss of cells is accompanied by shifts in the ratios of lymphocytes to macrophages and fibroblasts (Table 6.5). In particular there is a rise in the proportion of fibroblasts relative to lymphocytes. This is also a feature of the thymic cell population when lymphocytes are destroyed selectively with cortisol injections. This indicates some kind of negative feedback regulation between the two cell types which is related to the concentration of circulating adrenocortical hormones of the cortisol type.

Table 6.5. Effect of age and cortisol treatment on the cellular composition of the cortex of mouse thymus (grid area 80μm × 80μm)

Cell type	Young	CV	Old	CV	Young (cortisol)	CV
Lymphocytes	296.0	0.2	137.0	0.4	82.0	0.7
Epithelial cells	19.0	0.6	13.0	1.0	13.0	0.5
Macrophages	1.9	0.7	18.0	0.6	22.0	0.4
Fibroblasts	0.4	1.6	6.1	1.0	5.2	0.7
Plasma cells	0.5	1.3	1.4	1.2	1.0	1.4
Mitotic index	1.5		0.9		1.3	
Total cells	319.0	0.2	186.0	0.3	124.0	1.3

CV = coefficient of variation

THE WHOLE BODY CELLULAR NORM

Age involution is also a feature of skeletal muscle, and some rats show a 30% loss of thigh muscle between the first and second years of life. Taking body potassium as a measure of cell mass, the number of cells per unit body weight shows a steady decline in humans from the late teens. Some studies have shown little change in extracellular space, although total body water diminishes slightly. Intracellular water calculated as a difference between total body water and extracellular water has a significant age regression (Table 6.6).

In general the norm for total body potassium in old men is 77% of that in young men and in women the comparative figure is 82%. At all ages men have significantly greater amounts of body potassium than women. The fat norm increases in men from 18% to 36% whilst fat-free mass and cellular mass decreases concomitantly from 82% to 64% and from 45% to 36% respectively. In women fat increases with age from 33% to 45%, and fat-free mass and cellular mass decrease from 67% to 55% and from 38% to 31%.

Although there are great changes in relative cellular masses of some organs with age this is not reflected in the regulation of . intracellular electrolytes. This suggests that electrolyte homeostasis is controlled and regulated independently from the cellular mass of the organs which take part in governing electrolyte balance (Table 6.7).

Unanswered, and possibly unanswerable, questions remain. How does the body 'know' how many cells it should have? Does it count cells, measure cellular potassium, or assess the concentration of special cell and extracellular fluid markers?

RESPONSES TO PHYSIOLOGICAL DEMAND

None of the studies on changing cytological and chemical homeostasis has

Table 6.6. Total body potassium, potassium concentration, and cell mass, in 215 men (M) and 305 women (F) aged between 18 and 85 years determined from naturally radioactive ^{40}K.

	Potassium		Cell mass	
	M and F		M	F
Age	mEq	mEq/kg	kg	kg
18–25	4.05	56.1	33.7	21.3
25–35	4.13	53.5	34.4	21.9
35–45	4.11	52.7	34.2	22.1
45–55	3.78	49.5	31.4	21.4
55–65	3.61	47.8	30.0	20.0
65–85	3.17	43.4	26.4	19.6

Table 6.7. Electrolyte content of tissues in young (Y = 2 month) and old (O = 18 month) male C57 mice

Age	Kidney		Muscle		Brain	
	Y	O	Y	O	Y	O
Potassium[1]	93	88	89	79	99	93
Sodium[1]	78	88	21	25	50	48
Water[2]	75	76	72	73	79	79

[1]mequiv/g wet wt; [2]% wet wt.

thrown light on the reasons for the increased chances of mortality as time passes. There is no consistent trend that can be definitely interpreted as a deterioration in function, and apart from tissue involution through the loss of cells, there seems to be no major change in functional organization as expressed as mean values for the population. On the other hand the means mask a much greater variability between old subjects. Physiological parameters characteristic of homeostatic systems in a resting state, such as ionic composition and blood glucose, hardly change with age, but there is an increased tendency for there to be short-term departures from youthful norms. These may be measured as increased variability between and within individuals. In particular, many old individuals exhibit characters found in young adults, but there the range of values within a particular age group is extended.

This kind of increased variability is also found in experimental animals. Systolic blood pressure of mice under controlled laboratory conditions remains within the range 126 ± 12 mmHg throughout the lifespan for two and a half years, but the normal distribution curve becomes biphasic with age. When the rats pass the age of 600 days, about 30% of males and 20% of females become hypertensive with marked renal disease. The latter condition is probably the main cause of the hypertension. This points to mortality being related to individual departures from homeostatic chemical, physiological and structural norms of the young.

An important generalization is that whole body physiological measurements made on people under basic or non-stressful conditions do not reveal any general trend of changes related to age. This is particularly the case with respect to the composition of blood. However, changing temperature, food availability or setting conditions which require the body to consume resources above the level required in the customary environment always reveals age-related failures, and the chances of a particular failure having a fatal outcome increases exponentially with age.

Ageing is most clearly seen when physiological mechanisms for regulation and control of norms are activated to respond to internal or external demands for increased work or metabolic or glandular products.

Failures to respond to the endogenous signals for reproduction is evidenced

by the decline in overall fertility of domesticated mammals (Figure 6.3). After 14 months the litter size of hamsters, rats and mice is greatly reduced and the length of gestation increases. This occurs well before death from old age, and is associated with a failure to conceive again immediately after parturition. There is also a failure of maternal behaviour and lactation.

Although reproduction must come to an end when the ovaries no longer contain oocytes, it appears that animals stop producing litters a considerable time before they exhaust their complement of germ cells. The available evidence indicates that ovulation and conceptions may proceed as normal well into old age but the embryos fail to develop normally, and are usually aborted in the middle of pregnancy.

In the mouse, parental age influences the length of the period of fertilization plus gestation, the litter size and the stillborn mortality rate. Resorption during the late phase of gestation is thought to be a contributory factor in the reduction of litter size in senescent females. It is also possible that the basic cellular cycles of reproduction change with age. In this connection it is known that the cell cycles of epithelial mitosis in the reproductive tract gradually increase in length. Changes have been observed in the trophic responses involving the mobilization of cellular reserves and activation of mitosis.

Food restriction, which is likely to be a common stress encountered by animals in the wild, may be examined conveniently in the laboratory. There have been few investigations of mobilization of bodily resources as a response to starvation in relation to age, but one study failed to reveal age differences in either the rate of weight loss in long-term starvation or the survival time after food deprivation. Greater attention has been devoted to the effects of dietary restriction in an effort to understand the mechanisms by which it extends lifespan in laboratory rodents. Here, free access to food by animals previously

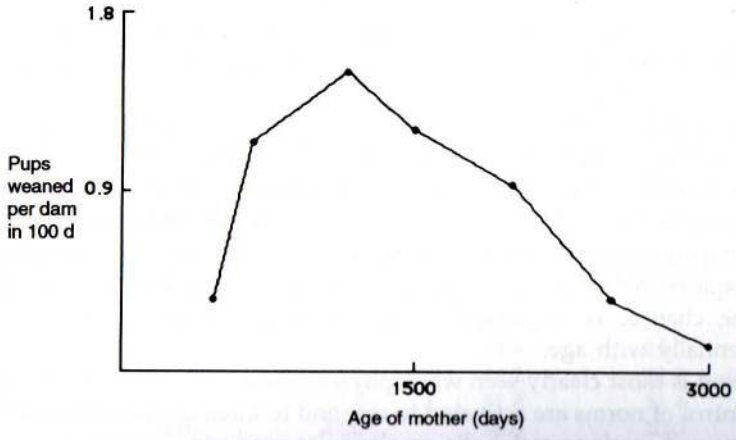


Figure 6.3. Fertility of a laboratory colony of beagle bitches in relation to age.

held at a growth-inhibiting food intake level for a large proportion of their lifespan produces an immediate growth response which is the same or greater than that in youth.

Data for humans do not support the concept that older individuals have less chance of surviving traumatic injury. On the other hand, this conclusion relates only to traumatic abdominal injuries. Regeneration of the liver of rats after partial hepatectomy occurs to an equal extent in the young and old, but old animals take more time to form the requisite number of new cells. Following unilateral nephrectomy, senescent rats have the same capacity for compensatory growth as young ones. However, as is the case for liver regeneration, the kidney of old animals responds more slowly. There is no evidence that new cells produced in response to unilateral nephrectomy by old rats are biochemically different from those in young rats. Despite this finding, the chronic hypertrophy of kidneys in rats given a pathogenic diet is associated in old animals with a decreased capacity of mitochondrial oxidative phosphorylation.

Responses to drugs, anaesthetics and toxic agents have also been used to measure age changes in adaptation. Increased age is often a factor which is responsible for a loss of drug responsiveness. For example, patients with arthritis are more responsive to steroid therapy in the third decade than in later years. This difference is not related to the duration of the disease. Hexabarbital anaesthesia is prolonged in old rats compared with young ones. Alongside this age change there is an increased mortality attributable to the drug. The endocrine status and immunocompetence are both thought to be important aspects of this rise in toxicity with age, but neurophysiological and neuropathological factors may well be involved as well. In this connection, the toxic action of uranium salts is manifest locally by specific changes in the kidney tubule, which are more marked in old animals. These histological changes in the epithelial cells go in parallel with the loss of functional capacity of the kidney.

MODELS OF STRESS RESISTANCE

Simms was the first to make a model of stress resistance with which to examine the increased chances of dying with generation time. The stress was bleeding and he quantified the mortality of rats to different degrees of haemorrhage in relation to their chronological age. As the animals grew older they began to die at levels of blood loss that were not fatal to younger animals. This increased sensitivity to stress was expressed as a linear decrease in the amount of blood required to kill a given percentage of animals as they grew older (Figure 6.4). At each age tested the overall sensitivity of the population to stress was given by the spread in individual values of blood loss required to kill all of the animals. The figures for blood loss were plotted as distribution curves for each age tested. The area under the distribution curve between any two levels of probability also increased with age. A steady decline in resistance to death from

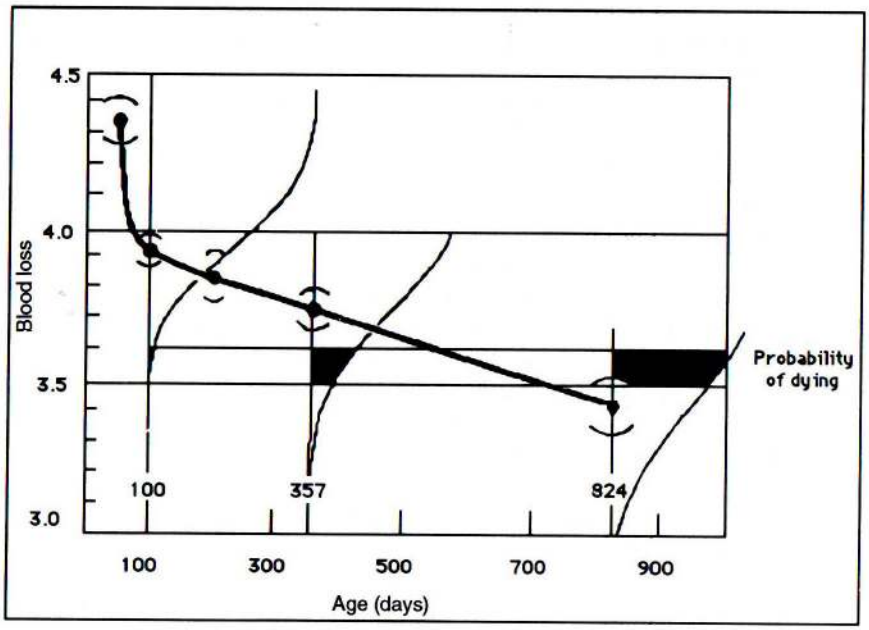


Figure 6.4. Amount of blood loss (% of body weight) required to kill rats of different ages

experimental haemorrhage of only 12% per decade, when coupled with an increased variability in resistance, results in an exponential rise in the chances of death occurring at a particular blood loss; the decline in resistance to death from haemorrhage matches the mortality curve for normal rats.

Simms' model is a measure of how severe an injury of a given type is required to kill a population as it ages. The results are consistent with our knowledge of the effects of infectious diseases, and milder stresses such as surgery and hospitalization. As individuals age they are killed by progressively less tissue damage. The blood loss model demonstrates two general principles of homeostasis in ageing:

1. There is an increased susceptibility to stress.
2. There is an increased variability between individuals to the effects of stress.

THE SYSTEMS APPROACH

Ageing is manifest in a progressive loss of vigour and the lowering of resistance to the many and varied onslaughts of the environment. Such changes may

produce chronic illness, or an acute failure to respond to a change in the physical environment. Failures of homeostasis obviously contribute greatly to the problems of old age but only when the heart stops can we say definitely that there has been an ultimate failure in homeostasis. We can therefore only analyse each of the major homeostatic systems of the body for age-related weaknesses, particularly in relation to the objective of discovering pharmacological strategies to reinforce limiting factors. It is virtually impossible to trace any particular failure, in man or experimental animals, to the point of death.

Homeostatic systems evolve in relation to the problems of organisms facing a varied unpredictable environment from which they have to obtain resources in order to grow, mature and reproduce. Because of the relatively small amount of information on other animals, discussion of homeostasis in relation to ageing tends to concentrate on man and other mammals. The major systems in mammals which support development are presented in (Figure 6.5).

The central feature of ageing of any cybernetic system is the loss of feedback regulation. From this point of view, growth and reproduction which depend upon the integrity of several neuroendocrine feedback loops, which link the brain with endogenous and environmentally triggered rhythms. The former rhythms, timed on scales of weeks or months provide the physiological clock-like baselines which ensure that reproduction occurs at the correct time in relation to the availability of environmental resources. This integral relationship of brain and endocrine homeostasis with the processes of growth and reproduction, indicates that the rate of ageing may be connected with the rate of development. Therefore it is important to consider growth and reproduction in the context of failures of age-related changes in homeostasis.

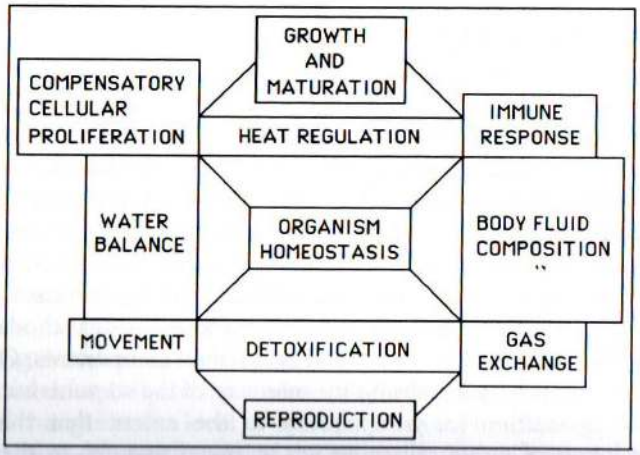


Figure 6.5. Diagram of major systems and states involved in organism homeostasis

Failure in the homeostatic systems of the body with age may be visualized at three levels:

1. Receptors tend to show a higher stimulus threshold.
2. The central nervous system handles information at a slower rate.
3. Effectors become less efficient in making the appropriate response.

Therefore ageing is a failure of systems. Systems can either be studied as black boxes, by investigating the overall function in terms of only the inputs and outputs, or by separating the system into its component parts, the processes and elements, each of which could then be investigated in isolation. In practice both of these strategies are followed by gerontologists.

Many physiological functions deteriorate with age, any one of which may be equated with a loss of vigour. Whole body behaviour is therefore the highest level expression of ageing. For example, a considerable amount of statistical evidence attests that on average the ability to exercise and carry out physical work declines with age. Recovery from a bout of physical activity is also affected, and between the ages of 30 and 70 there is a twofold increase in the time it takes the body to re-equilibrate after exercise. This may be related to changes in many factors, such as oxygen uptake, pulmonary ventilation, diffusing capacity of lungs, work pulse and clearance of blood lactate.

An important aspect of physical fitness, and many adaptive responses to other physical demands, is the ability to make rapid neurophysiological adjustments. Generally this kind of adaptation becomes slower in old people There are age-dependent losses in the ability to respond rapidly in response to environmental changes.

SENSORY PROCESSES

Since behavioural responses are triggered by events which occur in various receptor systems is is important to determine which sensory processes are involved and what kinds of modifications occur. Studies of losses in adaptability begin at the sensory processes and in this area most of the research has been carried out on human subjects in the six areas of vision, audition, gustation, olfaction, touch and vibration, and pain (Table 6.8). In all areas there is in general a decline in sensory and perception functions but the age of onset and rate of functional decline differ markedly between various sensory modalities. The changes involve a mixture of neural and non-neural components. Quite often, the latter can be offset by increasing the intensity of the stimulus but the neural changes are insensitive to manipulation to the extent that they involve irreversible losses of nerve cells.

On balance, visual and auditory impairment play a primary role in mediating

Table 6.8. Measurements of human sensory perception which reveal marked changes with age

Vision	Audition
Absolute threshold	Absolute thresholds
Visual acuity	Speech discrimination
Accommodation	Pitch discrimination
Dark adaptation	Discrimination threshold
Colour matching	Auditory retention
Critical flicker frequency	
Figural aftereffects	
Visual perception	
Gustation	**Olfaction**
Taste sensitivity	Atrophy of cellular elements
Taste bud atrophy	
Touch	
Touch and Vibration	
Tactile sensitivity	
Vibratory sensitivity	

the poor performance of old subjects in psychological tests. This suggests that any general theory of ageing and behaviour should incorporate the following:

1. The ability to process information generated by the physical and social environment.
2. Non-sensory factors which affect the performance of old people such as learning, memory and personal attitudes.

Examples of changes in these sensory processes are given below.

With age there are certain structural modifications in the eye which affect visual functions. Major changes occur in the crystalline lens which eventually shows a gradual and progressive accumulation of inert tissue at the centre. Nuclei and cell membranes become dehydrated and compressed making the central region of the lens less transparent. The thickness of the lens increases and the capsule becomes thicker and less permeable.

These changes interfere with two primary functions of the lens to transmit and refract light. They produce an increase in the absolute threshold for vision (Figure 6.6). There is a consistent decline in the capacity to see at low light intensities, and an increased limitation upon the extent of dark adaptation, both of which can be traced to ageing of the postmitotic tissues of the eye.

Increasing evidence seems to suggest that the fall in visual acuity in old age is not solely due to opacity of the cornea and the lens but is in part dependent on age-related changes at the level of optic pathways. These changes involve loss of nerve cells in different layers of the retina, the visual cortex and the optic nerve of old rats (Table 6.9). Only those nerve fibres with a diameter less than

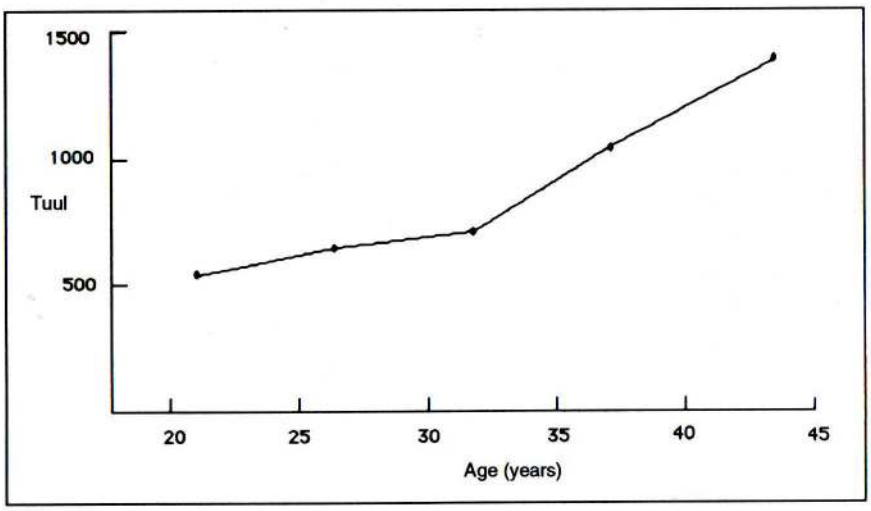


Figure 6.6. Changes in the absolute threshold for dark adapted vision as a function of age expressed as visual sensitivity (the reciprocal of the absolute threshold).

Table 6.9. Changes in morphometric norms of the optic nerve in male Wistar rats

	Young	Adult	Senescent	Aged
Cross section (μm 2 × 105)	1.65	1.78	1.97	2.04
Total fibres/1000	79.8	75.7	67.8	51.9
Small fibres/1000*	64.7	60.3	53.0	36.9
Medium fibres/1000**	9.9	9.8	9.5	9.5
Large fibres/1000***	5.2	5.5	5.4	5.6

Young = 3m; Adult = 12m; Senescent = 20m; Aged = 30m
* = diam less than 1 μm; ** = diam 1–1.5 μm; *** = diam above 1.5 μm.

1μm are significantly affected by age. Nearly half of them were lost by 30 months with most of this loss occurring after 20 months of age.

As with vision there is a loss of auditory acuity with age. This is measured as a fundamental loss of hearing and is termed presbycusis. Presbycusis is classified according to the predominance of particular kinds of tissue degeneration.

Sensory presbycusis is associated with atrophy in the epithelium at the basal end of the organ of Corti and produces a cut-off in hearing high frequency tones.

Neural presbycusis results from a loss in the population of neurons in auditory nerve tracts when there is a loss in ability to discriminate speech.

Metabolic presbycusis involves atrophy of the stria vascularis leading to a loss of the ability to hear pure tones.

Mechanical presbycusis results from a stiffening of the basilar membrane which is expressed as a progressive loss of hearing as the frequency of sound becomes higher.

Taste sensitivity is also affected by atrophic changes in the sensory cells. It has been reported that there is a fall in the number of taste buds which begins in males at 50–60 years of age and in females at 40–45 years. There is an estimated loss of up to two thirds of the papillae in old age. Atrophy is also a characteristic of the ageing olfactory system which involves the loss of nervous elements in the nasal mucosa.

At this level of analysis the problem of sensory processing in old age comes down to a study of cellular atrophy and tissue involution and the endogenous and extrinsic factors controlling lifespans of post-mitotic cells.

HEAT, COLD AND RESPIRATION

Heat production in mammals is a useful model to illustrate the interaction between the various levels of homeostasis. In human subjects heat stress has been used to examine age losses of responsiveness to change in environmental temperature. In general, there is an increased degree of discomfort at high temperatures and the physiological responses in the old become more variable. One relevant feature is that men over the age of 40 have a greatly increased vasodilation in response to heat. Since in a hot environment heat loss is almost entirely by evaporation, the greater skin blood flow in old subjects would not be expected to have a marked effect on their heat regulation. Age differences in rectal and skin temperatures are not significant in heat-exposed subjects, therefore the view may be taken that the faster skin circulation of older men is unnecessary for heat regulation and places a needless burden on the general circulation.

On average, heat production, oxygen consumption and carbon dioxide elimination decrease with ageing; but the percentage proportion of the two gases in the expired air changes differently: namely, the proportion of O_2 increases while that of $C0_2$ decreases. Apparently aged tissues cannot use inspired oxygen at the same rate at which it is used at young or adult ages; and at the same time the elderly produce and eliminate carbon dioxide at a lower rate. Accordingly, heat production gradually declines with age. In one experiment a group of 50–90 year olds, unclothed and motionless, were exposed for some time to room temperatures of 5–15°C. Their reaction was compared with that of an 'adult' group of persons in the age range 20–40 years, who were treated in exactly the same way. The aged were less able than the young and adults to maintain their normal body temperature; and they consumed more oxygen during the cooling period than the younger group.

Disturbance of heat production in old age is also apparent in rats. The rectal

temperature decreases with age. When kept at a low temperature the body temperature of old animals falls to a lower minimum compared with young ones; and when kept at the high room temperature of 38°C, young rats increase their body temperature less than the old animals.

When rats are exposed to a low atmospheric pressure, their body temperature falls considerably, but is easily restored to normal in young animals at the ordinary room temperature. Older rats (14–20 months) were completely unable to react in this way, i.e. they showed a lack of adaptation to low oxygen pressure.

Although average figures of basal metabolism with ageing form a comparatively smooth declining curve, the range of variations in each age group, in particular in old people, is usually wide, sometimes very wide indeed. Thus, the highest value of heat production in the range of variations of men 70–79 years old (42.1 cal) is about the same as that in men 40–49 years old (42.9 cal). The highest value in the ninth decade (37.6 cal) exceeds the average values for the third decade (36.57 cal) and for the fifth decade (35.75 cal). Somewhat similar variations are observed for 0_2 uptake and $C0_2$ elimination. An idea of these variations can be obtained from the following examples; in old men aged 72, 72 and 73 years the respective heat production was 28.1, 35.7 and 37.3 cal/m²/hr; in four men aged 80, 81, 82 and 82 years basal metabolic values were 28.3, 38.3, 27.6 and 38.2 cal/m²/hr.

Again, in three old men aged 83, 84 and 84 years the respective heat production was 39.0, 35.7 and 30.6 cal/ m²/hr; in another three aged 72, 71 and 70 years the respective values were 40.4, 29.6 and 39.6 cal/m²/hr. In a study of 10 persons aged 80–109 years, and two who claimed to be 125–142 years old, the average value of basal metabolism was 20.9 cal/m²/hr. While unusually low figures, namely 11.5 and 17.5 cal/m²/hr, were obtained in the case of two men supposed to be between 95 and 100, in the case of the third man, supposed to be 100–105 years old, the value was 31.7 cal/m²/hr.

Similar wide variations are observed in rats. Of two colonies studied by the same investigators, one had an increased basal metabolism with ageing, in the second colony, the average heat production decreased with advancing age. In some of these rats, in spite of the decline of the average curve of basal metabolism, longitudinal measurements showed a slight or sometimes considerable increase of heat production when metabolism was compared in the same rat at the oldest, less old and adult age. For example, in rat No. 15 at the age of 807 and 897 days the heat production was 777 and 840 cal/m²/24 h respectively; in rat No. 16 at the age of 804 and 1060 days, respective values of basal metabolism were 729 and 735 cal/m²/24 h; in rat No. 29 at the age of 454 and 867 days the respective heat production was 565 and 631 cal/m²/24 h; in rat No. 31 at the age of 580 and 913 days the respective values were 632 and 738 cal/m²/24 h. In a long-term study on dogs there was no detectable change in basal metabolism with ageing over a period of 12 years. In general it is thought

that low values in older age groups may indicate an abnormal pathological situation.

A failure of temperature adaptation is most clearly seen under laboratory conditions in response to cold stress. Old female mice have a high mortality rate following exposure to a temperature of 6–7° which is not seen in young mature females. The percentage surviving for 14 days falls off linearly with age (Figure 6.7). This experiment measures the ability to generate heat above the level required for maintenance of life. At each age there is some normal biological variation within the group in the ability to produce heat. With increasing age the average ability to produce heat declines and the fraction that cannot produce enough heat to maintain life increases.

The failure of integrative function which is responsible for the death of older animals appears early in the experimental period. Old mice which survive the first 2 days of cold exposure have a good chance of surviving for the next 12 days in the cold. Mortality of old mice is reduced when they are slowly adapted to the lower temperature, but the adaptive process is less effective than in the young.

A number of the individual physiological responses of rodents to cold, such as the increased oxygen uptake, rise in blood sugar, loss of body weight, and changes in blood cell count also become less marked with age. Studies on humans suggest that the mechanisms for minimizing heat loss also deteriorate with age.

Responses to temperature are intimately linked with the basal metabolic rate. There is a significant fall in basal heat production and carbon dioxide elimination as humans age. There is also a larger day-to-day variation in several aspects of respiratory function, which may be of significance with regard to the

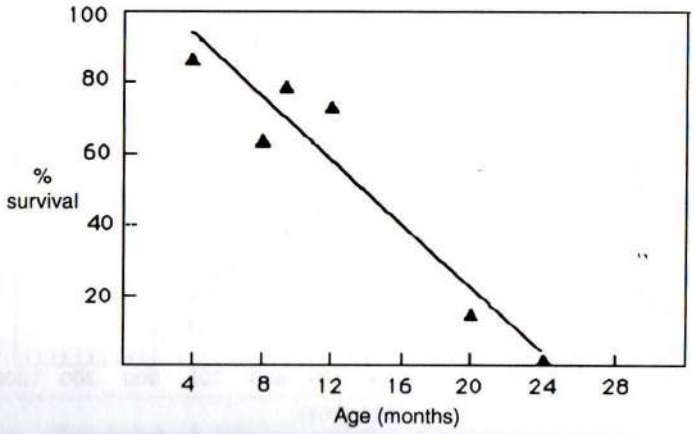


Figure 6.7. Survival of mice at a temperature of 7° over 14 days

efficiency of adaptation. With regard to experimental hypoxia there is a marked decrease in adaptation of old animals.

An overall measure of ability to react to heat stress is the maximal amount of gram calories that can be expended by an animal when maximally stressed. When mice are forced to do continuous work at elevated temperature until the onset of convulsions it was found that physiological capacitance increased during growth and then underwent a linear decline after maturation (Figure 6.8).

Losses of cells, which represent the heat-producing compartment of the body, would be expected to result in the decline in basal metabolic rate and this has been confirmed by direct measurements of oxygen uptake and heat production. The decline in oxygen uptake goes in parallel with the loss of body potassium, which may be taken to mean that the basal needs of the remaining cells at any age are adequately served by their energy-generating systems. However, it is also possible that there is an impairment in the capacity of atmospheric oxygen to enter the capillaries of the lungs due to a separate failure in this organ.

Elderly and old subjects show diminished vital lung capacity at the expense of all its component volumes, increased residual volume, decreased ventilation effectiveness, decreased bronchial patency, bronchospasm, decreased filling of the lung with blood, reduced elasticity, and increased rigidity of pulmonary vessels and disturbed uniformity of pulmonary ventilation.

There is a significant improvement in the pulmonary volumes of elderly

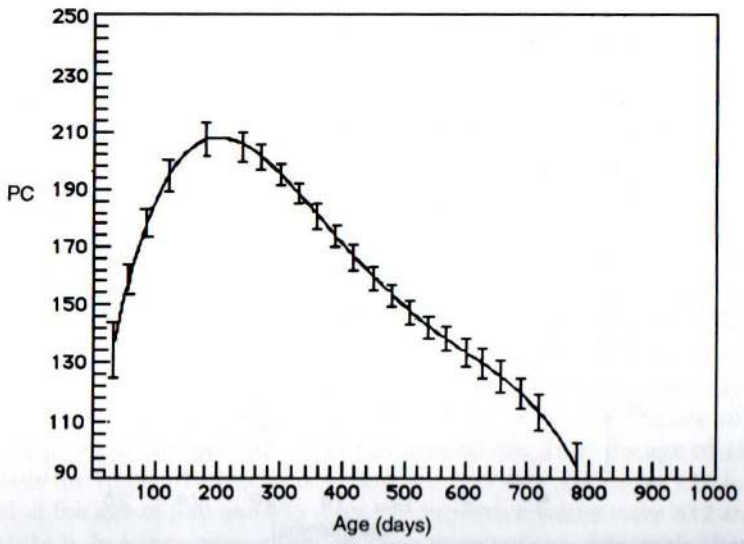


Figure 6.8. Physiological capacitance (CP = gram caloric output) or mice at different ages

people after 8 weeks of systematic physical training as expressed by an increase in vital capacity.

The formula for the diffusion of oxygen into lung capillaries is that D, the diffusion rate, is equal to V/p–pl, where V is the quantity of oxygen absorbed per minute, p is the tension of the oxygen in the alveoli, and pl is the tension of the oxygen in the vessels that come from the lung.

These are measurements that can be made with some accuracy and they show that there is a 60% decline in the capacity of oxygen to gain entrance to the lung capillaries from the early 20s to the 80s (Figure 6.9).

The amount of collagen that is present at autopsy in the lungs of men and women may be calculated from the amount of hydroxyproline, a characteristic amino acid of collagen. Both sexes show a gradual increase in lung collagen with age regardless of the cause of death. From this aspect, the lungs of the elderly would not be expected to be as efficient as those of younger people with a more highly organized cellular exchange surface.

Ageing of the lungs is but one example of many failures at the organ level which reduce physiological efficiency. These include the general decline in work performance and the lowered capacity of heart, kidneys and liver to deal with chemical and physical loads. An important principle of ageing is that the decreased physiological efficiency is expressed as a longer response time and a fall in the maximum response capacity. Also, an important generalization is that the performance of a wide range of physiological systems declines in a linear manner from the third decade at a rate of about 10% per decade.

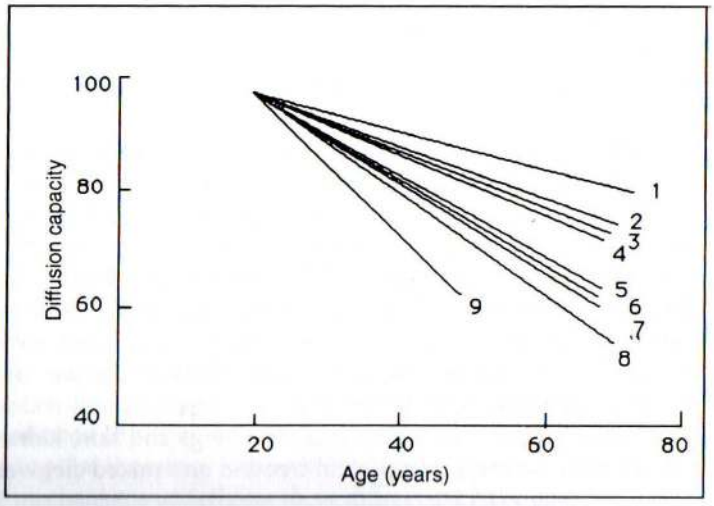


Figure 6.9. Regression of diffusion capacity of human lung against age by nine different methods of measurement

WATER METABOLISM

There are two kinds of water metabolism, endogenous and exogenous. Endogenous water is produced by cellular elements of the organism in the process of metabolism of tissue proteins, fats and carbohydrates. Ingested water is the source of exogenous water.

Water metabolism is closely connected with, and dependent upon, water absorption from the gastrointestinal canal, water excretion by the kidneys, the renal regulatory mechanism, water expiration by the lungs, and perspiration of the skin. Accordingly, water metabolism is also related to the function of the kidneys, intestinal canal, lungs and skin, and to the effects of ageing upon these organs. There is, however, a tendency to explain nearly the whole water metabolism by the regulative function of the kidneys, and consequently to underestimate the most important activity of the cells of all the organs and tissues. The cells possess their own digestive, excretory and regulatory systems. For example, it is apparently due to these cellular systems and their activity that water administered in excessive amounts is collected in the extra-cellular compartments of the organism, and not in the tissue cells.

The water content in the liver and muscles of dogs, before and after intravenous infusions of saline solution remained normal; i.e. the infused water was stored in the extra-cellular compartments. In human subjects remarkably large amounts of water from intravenously infused fluids can be retained in the extra-cellular compartments without any harmful effects to the organs and tissues.

Water metabolism can be studied in two ways: as water exchange only, and as water tolerance after administration of varying amounts of fluids, together with the investigation of the effects produced by water intake on organs and functions. There are only a few investigations on the effect of ageing on water metabolism; and therefore only tentative conclusions can be drawn from the data obtained.

Direct investigation of water metabolism has been carefully performed on rats aged 30, 80 and 540–720 days. During experimental periods the animals were kept in a closed metabolism apparatus, through which air, free of moisture and CO_2, was circulated. This arrangement made it possible to investigate not only water metabolism (with estimation of the water output through the lungs and skin, kidneys and intestines), but gaseous and nitrogenous metabolism as well. The experiments were performed on three diets (mixed food, meat and a protein-free diet). The results showed a considerable decline of water metabolism with ageing, both in the income of exogenous and endogenous water, and in the water expenditure through the lungs and skin, kidneys and intestines. The results obtained on a protein-free and on a mixed diet were very close.

When the total water income, the percentage of water supplied from exogenous sources, and the percentage of endogenous water are estimated,

these values tend to be constant for all age groups: thus, for rats aged 30, 90 and 540–720 days the respective values of the 'exogenous' percentage were 82.7%, 82.2% and 75.5%; of the endogenous—17.3%, 17.8% and 24.5%.

The balance of the water metabolism was highly positive in rats aged 30 days, namely about 40.2 g of water was retained in the body of these animals; in rats aged 90 days only 3.1 g of water was retained; and none in senescent rats. The high retention of water in young rats is explained by its use for building new tissues in the growing organism.

From the data of gaseous and nitrogenous exchange, changes in the bodyweight and the results of water metabolism, it has been concluded that the main source of endogenous water is cellular metabolism of carbohydrates.

Of all the homeostatic systems of the body, water balance has been signalled out as a suitable experimental model for gerontological studies. The whole body failure is clear-cut, the hormone mechanisms are well defined, and there are simple experimental procedures for the individual examination of both people and animals. Changes in water balance with age may be explored in human subjects by giving them standard drinks of between 0.5 and 2 l, after which serial blood samples are taken to measure the extent of dilution of the blood, and urine collected to measure the rate of water elimination by the kidneys. This reveals that there is a definite difference in water tolerance between different age groups. Most young and adult men drinking 1500 ml of water, or even more, show only a slight decrease in the protein concentration of the serum or no decrease at all. This means that there was only a slight blood dilution or none, and the water load is excreted rapidly. On the contrary, in old people, given smaller amounts of water, there is a definite blood dilution measured by a fall in the concentration of haemoglobin and solids, in most cases by 6–16 %.

Another way of investigating these changes is to give the water load by injection intracutaneously. This is absorbed in old people within 2–6 hours, while in young persons within one hour. This type of research also shows that the loss of electrolytes from the blood of old people, whose metabolism, and the mechanisms regulating it, are less stable than those in young persons. Prolonged, or more or less regularly repeated administration of large amounts of water produces similar, but more pronounced effects, than those in the experiments of short duration. Such loss of sodium chloride occurs in old people even after drinking comparatively small or moderate amounts of water. Another important finding is that in a few old people investigated, water tolerance is close to that in adults. These water tolerance experiments on man are confirmed by direct investigation of water metabolism on animals, suggesting that the rate of water metabolism declines with ageing in all mammals.

The mechanisms underlying these differences have been the subject of much detailed investigation. Clearance studies with diodrast and aminohippuric acid indicate that renal plasma flow is diminished in old subjects. There is also a fall in

the uptake of aminohippuric acid by isolated tubules. Similarly, the capacity to reabsorb glucose from the glomerular filtrate shows a linear decline with age. which amounts to about 19% from the third to the ninth decade (Table 6.10). The changes in filtration rate parallel the changes in reabsorbtive capacity which may be of significance in relation to the age-dependent depression of glucose tolerance in humans, and the failure to induce a reduction in the alkali reserve after treatment of old dogs with anaesthetics.

AGEING OF THE NERVOUS SYSTEM

It is probably true to say that ageing of every organ of the body is characterized by a loss of its innervating neurons, and in the brain itself by a loss of cells from particular regions (Figure 6.10). Also demyelination and axonal degeneration occur with increasing frequency after the fifth decade.

Apart from the well-established age-involution of nervous tissue and the appearance of 'age-pigment' in neurons of the central nervous system, there are few indications of there being a central cause of the failure in day-to-day co-ordination in old individuals. The failures seem to be compounded of several general changes, some of which may be connected with an inadequate nerve supply to particular effectors. For example, the excitation threshold for electrical stimulation of nerves is higher in old animals than in young ones. Also there is a decrease in the activity of choline acetylase and choline esterase, the key enzymes in transmission of the nerve impulse. It may well be that the decline in the number of neurons and motor end-plates with age also contributes to the loss of flexibility of nervous responses. These changes in the nervous system may well be sufficient to account for the slow acute responses. Loss of motor end plates is not the only aspect of ageing of the brain/effector interface. The motor end-plate is the expanded terminal of the motor nerve together with the specialized receptor zone of muscle membrane underlying it.

Table 6.10. Various measures of kidney function in middle-aged and old men

Age (yr)	Urine[1] specific gravity	Glucose[2] reabsorption	Clearances		
			Inulin	Diodrast[4]	Urea[5]
40–49	1.0293	315	121.0	574	95
50–59	1.0287	308	99.3	500	86
60–69	1.0277	260	96.0	442	82
70–79	1.0253	239	89.0	354	65
80–89	1.0238	219	65.3	289	61

[1]Addis–Shervky concentration test [2]mg/min [3]Glomerular filtration rate ml/min/1.73 sq.m. [4]Renal plasma flow ml/min./1.73 sq.m. [5]% of value for young men

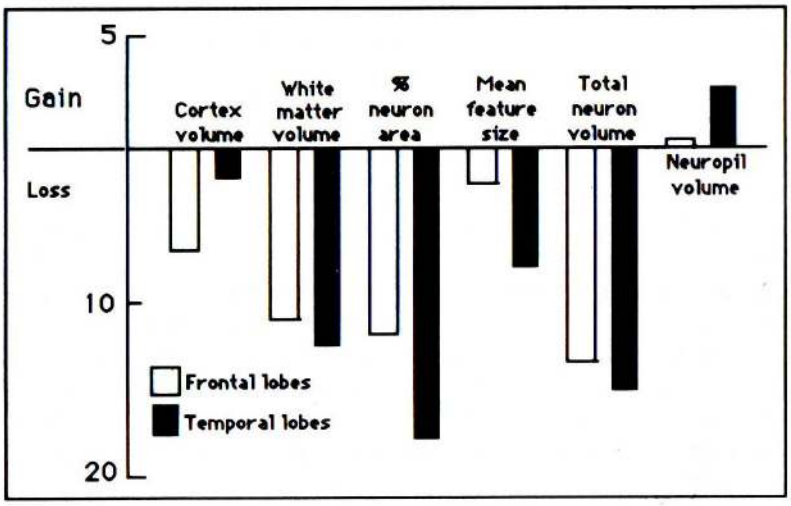


Figure 6.10. Mean loss or gain of tissue in the cerebral hemispheres by the eighth decade as measured by computer assisted imaging in brains of cadavers free of neuropathological disorders

Several age changes have been described at motor nerve terminals including terminal axonal sprouting so that on average each motor nerve may supply more muscle fibres than in young subjects. Multiple swellings may develop on terminal nerve branches particularly in those supplying the lower limbs, and there may be an increased complexity of the end-plate structure.

THE ENDOCRINE SYSTEM

There are many points at which complex physiological systems can fail; at the input of a need to the sensory detector; transmission of the need to the co-ordinating system; transmission of the response from central control to the peripheral effector; and activation of the effector. Where physiological responses involving neuroendocrine integration have been examined ageing has been found to produce independent failures at all of these points in the chain of command.

For example, at the effector end of the hypothalamic/pituitary/adrenal axis, isolated cells of the rat adrenal cortex reveal fundamental age-dependent failures with respect to baseline corticosterone release and synthesis in responses to the addition of trophic hormones. These cellular changes may then be 'dissected' at lower levels of complexity to explore underlying possibilities of changes in membrane-bound hormone receptors, the intracellular enzymes

responsible for steroidogenesis and the efficiency of the genetic system which brings about long-term differentiation of the cortical cells in response to chronic stress.

Some of the age changes seen *in vitro*, although they no doubt reflect some general loss of regulatory capacity, may not have the same significance for the body functioning as a whole.

Hormones are integrated into the pattern of development in two ways. They act as controllers, in that they release or trigger the developmental potential inherent in certain tissues. Actions of this type are sometimes irreversible, but usually the target tissue returns to its former state when the concentration of hormone falls to the initial level. Second, hormones invoke responses that offset undesirable changes in the external environment. From the latter point of view, the ageing of endocrine systems takes place in two stages. Initial development of endocrine responses makes the animal more adaptable and independent of its surroundings; later changes decrease the capacity for adaptation to the environment.

Several biological aspects of development are of importance to the endocrinologist dealing with the ageing of endocrine systems. These are related to the source of the initial stimulus that leads to the modification of an endocrine response. The stimulus may be internal or external.

The most obvious modifications of the endocrine system that fall into the first category are those linked with the attainment of sexual maturity. This applies particularly in the female, where there is a precise chemical control of the sequence of events in the reproductive cycle. In this context, the corresponding alterations in the endocrine system are brought about by intrinsic mechanisms, although the various responses may be synchronized by external factors.

The basis for alterations of the second type, where the stimulus lies outside the organism, underlies the development of many lower vertebrates. The chemical nature of the stimulus is most clearly seen in the environmental factors encountered by euryhaline fish, where a spawning migration from fresh water to the sea brings about profound changes in the endocrine system. Some of the endocrine changes appear to accelerate the deterioration of those organs not directly involved with reproduction; other changes, such as those connected with the regulation of salt balance, seem to be reversible. However, even though the immediate stimulus which activates the endocrine system comes from the environment, the impulse to undertake the migration may be intrinsic.

The above features of the developing individual determine the changing patterns of homeostasis with age, and, in order to discuss the ageing of mammalian endocrine systems, four aspects related to intrinsic stimuli may be considered.

1. Changes in endocrine controlled norms.
2. Changes in the responsiveness of target tissues to hormones.

3. Changes in the function of endocrine organs.
4. Changes in the physiological and biochemical action of hormones.

Changes in Norms

At each stage of life, the chemical composition of the mammalian body is maintained within narrowly defined limits, through the action of endocrine systems. However, new intracellular norms for enzymes and metabolites are established as animals move from one stage of development to another. In general, the most rapid changes of this type are found in the early postnatal period. Arguably the greatest changes in endocrine norms are expressed in the development and ageing of the human female reproductive system (Figure 6.11).

Few measurements have been made in the older age groups with regard to intracellular metabolites, but age variations have been noted in the concentration of several plasma constituents. Bearing in mind the fall in metabolic mass with

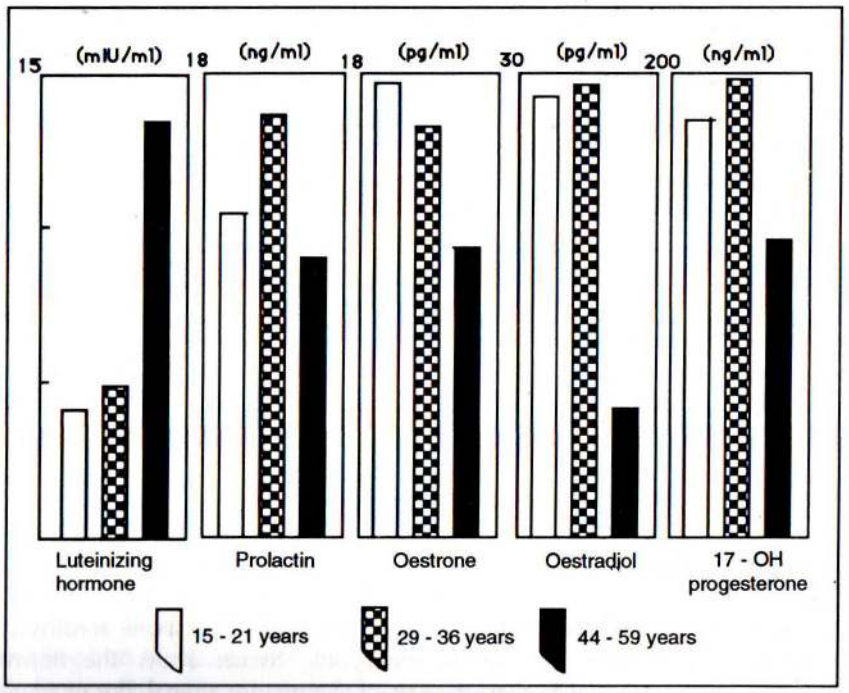


Figure 6.11. Changes in the daily amplitude of plasma hormones in women over the reproductive lifespan

age (age involution) it is likely that a decreased rate of metabolism would tend to produce a temporary increase in the concentration of most plasma metabolites which have a direct dietary origin. The fact that there is not a general rise in the concentration of these substances with age suggests that adaptive changes in the endocrine system occur to offset this tendency, but we are largely ignorant of the details.

Every endocrine response, even though the end-point is the restoration or maintenace of a constant concentration of some chemical in the body fluids, is always associated with changes in the expenditure of energy to maintain constancy. Thus, by its nature, a hormone brings about an alteration in metabolism and the temporary or permanent attainment of new norms. At this point it may help to define regulation of norms as distinct from control of norms. A regulator maintains a norm within narrow limits; a controller sets the norm at a new level. For example, an increased rate of synthesis of the hormone and also, particularly in long-term hormone action, a stimulation of the formation of a component of the effector system in the target tissue (a new norm) may accompany hormone secretion. In this way, there may be requirements for an increased supply of energy yielding substances and precursors of macromolecules. These changes are important in responses that result in enzyme synthesis. Therefore many aspects have to be considered in relation to ageing of such systems. There may be no age alterations in, say, the concentration of a particular metabolite, but several new norms may be established as the organism ages associated with a tendency for the steady state equilibrium of this metabolite to shift due to the ageing of other components of the system (for example organ or cellular hypertrophy).

In ageing mammals it is difficult to relate the observed changes in plasma metabolites to a variation in the endocrine organ rather than to alterations in the enzyme activity of the target tissue. If there was a fall in the efficiency of either, the appropriate endocrine organ or the enzymic process that previously maintained a particular metabolite at a constant level, the concentration in the body fluids would tend to change with age. However, in this complex regulation/control system, a change in one of the three potential variables, availability of metabolite, activity of endocrine gland, and rate of enzyme synthesis, could well lead to alterations in the other two.

Changes in Target Tissues

Little work has been done on the early development of hormone sensitivity. However, it is known that certain embryonic tissues show the normal qualitatitive response that is characteristic of the mature animal. For example, there is evidence that fetal liver and thymus are affected by adrenal steroids in the same way as in postnatal life. Other embryonic and early postembryonic

tissues are also sensitive to hormones. An adequate hormonal status is essential for several normal morphogenic events to occur, such as sex differentiation, and for ontogenesis and maturation of some physiological functions. Hormones may also impose new permanent and irreversible orientations on crucial structures. Unusual reactions may be elicited which can sometimes only be evoked during a short, well-defined period. Often, effects are obtained which result in the abnormal differentiation of target tissues, as in the case of androgens acting on the secondary sexual organs in the female fetus. Presumably these abnormal reactions are connected with the incomplete differentiation of the target tissue. An abnormal response to androgens is not confined to vertebrate sexual tissues. It is also found in the embryonic lymphoid tissue in birds, and may result from common mechanisms of differentiation within the vertebrates.

From work on the subsequent changes in the postembryonic response to hormones, it appears that target organs for hormones become sensitive to the appropriate hormones at stages of early development that coincide with the differentiation of the target tissues. A gradual rise in the degree of hormone sensitivity is implied from some experiments, but few investigators have been concerned with this aspect in detail. In the later stages of life it has been found that tissues become less sensitive to some, but not all, hormones.

The complicated nature of these changes is illustrated by the endocrine control of renal function. In the human kidney, electrolyte control is not precise until 2–3 weeks after birth. This situation is thought to be connected with the slow development of the loop of Henle. At the other end of the time scale, it is known that the kidney of old animals is not so versatile in its response to hormones as in early adult life. Experiments with the rat have shown that the antidiuretic response to exogenous vasopressin depends on the age of the animal. More hormone is required to produce a given response in very young and old animals compared with animals of intermediate age. With respect to other polypeptide hormones it has been shown that there is an age-dependent decrease in sensitivity to insulin, growth hormone and ACTH. A loss of sensitivity is not general for all hormones. For example, the characteristics of lipid mobilization and deposition in humans treated with norepinephrine suggest that age has little effect on the endocrine response. Also, despite the drop in sensitivity of tissues to ACTH the assessment of the hypothalamic pituitary adrenal axis indicates no decrease in functional integrity.

Some hormones are more effective in old animals. Thus, exogenous thyroid hormone may bring about a loss of body weight, which for a given dose is greater in old rats than in young ones. Also, the effect of thyroxine or thyrotropic hormone, particularly in multiple injections, on the basal oxygen consumption of rats, is more marked in old animals. However, when effects of a single injection are compared on oxygen consumption and heart rate it is apparent that old rats have to work harder to consume oxygen than young ones (Figure 6.12). The heart rate takes longer to return to normal in old rats.

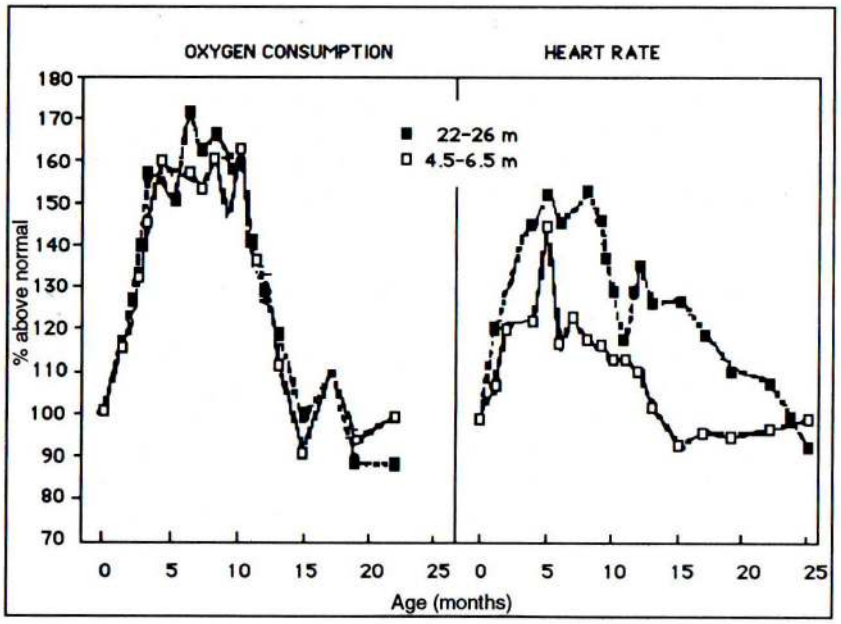


Figure 6.12. Effect of a single injection of 1 mg thyroxine on basal oxygen consumption and heart rate of young and old rats

In apparent contradiction to these findings, thryoidectomy has less effect on the metabolism of old rats compared with young ones. Possibly in addition to differences in tissue sensitivity to thyroid hormones there are changes in the role of the thyroid gland with age.

Tissue sensitivity to thyroid hormones is bound up with the state of the oxidative enzyme systems. In this respect, although whole body measurements of oxygen uptake suggest that there is no drop in the basal metabolic rate per cell, test-tube experiments using a wide range of tissues show an age decline in *in vitro* respiration.

Evidence for the impairment of the enzyme systems of aerobic metabolism may be obtained from incubated tissue slices or homogenates prepared from animals of different ages. Most experiments show a decrease in oxygen uptake expressed per unit weight of tissue. Variability between different experiments seems to arise from the age range over which the measurements were made. Part of the variation is best interpreted as representing a decline in aerobic metabolism during the first year of life, which coincides with the drop in growth rate. During the second and third years there is a further fall in tissue respiration. Expressing oxygen uptake per unit of DNA seems to produce the same

variability indicating that the effects are not due to a decrease in cellularity of the tissues. These large percentage changes probably reflect an *in vivo* loss in efficiency of aerobic metabolism. However, changes of the same magnitude do not show up at the level of the basal metabolic rate expressed per unit of body potassium, suggesting that the failure revealed *in vitro* is only limiting when there is a need for acute and chronic responses of oxidative metabolism to environmental stimuli, which require increased demands above the basic level.

Only a small number of results are available on the changes in sensitivity of human tissues to steroid hormones, and all of the following results come from work on laboratory rats and mice. It seems that there is a diminution in the responsiveness of the kidney to aldosterone and cortisol. As far as the metabolic action of corticosteroids is concerned, cortisol is more effective in inhibiting growth in 5-week-old rats that in 3-week-old animals. This difference may be connected with changes in the endocrine control of growth with age. Further it is not possible to obtain a loss of weight with cortisol treatment in 3-week-old rats whereas 5-week-old animals show this response at quite low doses of steroid.

During the early postnatal period there are large changes in the response of enzyme synthesis to glucocorticoids. The basic reaction in the process of enzyme induction, the formation of RNA polymerase, does not respond to corticosteroids until 3 weeks after birth. Also, the extent of enzyme induction after cortisol treatment depends on the age of the animal. Young rats show a large increase in liver glutamic pyruvic transaminase but the percentage change in enzymic activity gradually diminishes with age. With tryptophan pyrrolase, another enzyme that is induced by cortisol, the kinetics of enzyme synthesis vary with age.

With regard to thyroid control of glycerophosphate dehydrogenase in rat liver, all animals in all age groups are fully capable of responding to the presence and absence of thyroxine in the same manner, suggesting that age effects on enzyme induction are not general. Despite this evidence the age-independent modification in the ability of tissues to synthesize enzymes in response to a variety of environmental stimuli has been proposed as a common biochemical expression of ageing. In general, it appears that impairment of enzyme induction occurs widely and may take the form of a decreased level of enzyme, a drop in the magnitude of response, and a delayed response.

In assessing a declining sensitivity to injected hormones the view may be taken that this reflects a lower steady-state concentration resulting from an increased rate of metabolism or clearance. Another viewpoint is to consider the possibility of a loss or modification of hormone receptors. A third approach requires an examination of the biochemistry of the target cells in relation to possible variations in the enzymic potential of target tissue to response to the hormone. Little work has been done with regard to all three methods of investigation. There appears to be a fall in the rate of metabolism of steroid hormones with age, possibly related to decrease in metabolic mass. Incidental to

this it might be expected that a given dose of hormone would be more effective in an older animal, but this is not always so.

With regard to hormone uptake, many studies have involved the use of steroids where methods are available for the isolation and characterization of these compounds and their metabolites. However, few workers have been concerned with age variations. In the human red cell, which is probably the simplest model system for the study of transport processes, there is a rise in the uptake of triiodothryonine with age which reaches a plateau early in adult life. Here, as in other studies concerned with changes in the uptake of hormones from plasma it is difficult to assess the contribution due to changes in the uptake mechanism as opposed to alterations in the proportions of total hormone available to the cell, because the capacity of plasma proteins to bind the hormone and render it inactive may alter with age. In this context, the renal enzyme system which inactivates parathyroid hormone becomes less active later in life. Similarly it has been postulated that there is a diminished rate of excretion and/or inactivation of thryoxine in older rats.

With reference to possible age variations in receptor structure we are still largely ignorant of the chemical nature of the site of hormone action. For steroids it is known that cell proteins are important quantitatively but no information is available on age-related variations in protein structure and their quantitative influence on the endocrine response.

It is thought that cell proteins reflect the process of ageing in their tertiary structure. Such changes in the configuration of proteins might affect the binding of steroid hormones to cells as well as the biochemical properties of the steroid-protein complex. But in skeletal muscle, which is a target tissue for steroids, and undergoes considerable changes in function with age, the age-related alterations in protein structure as determined by indirect enzymatic methods are not very marked.

As to the biochemical potential of target tissues, it appears that the enzyme pattern in some tissues is not affected very much by age, but this is not general. Muscle undergoes marked changes during the lifespan. There is a decline in the work performance which occurs at a time when there is a large fall in relative cell mass. At the biochemical level there is a diminution in the capacity for aerobic energy production and a rise in the activity of glycolytic enzymes. It has also been suggested that an observed drop of 80% in the Mg-activated ATPase in the muscle of old rats is connected with the age-dependent loss of contractile efficiency. All of these biochemical changes could well be sufficient to alter the response of muscle to hormones.

It has already been pointed out that the ageing kidney becomes less sensitive to antidiuretic hormone. The kidney of old animals is also less responsive to stimuli that bring about compensatory growth, suggesting that there is a general decline in biochemical efficiency. This is reflected in the slow rate of turnover of nucleic acids after unilateral nephrectomy in old rats. An age-dependent deterioration in renal transport processes found in intact animals

is also found in *in vitro* preparations of kidney. The phenomenon coincides with a fall in the concentration of the ATPase thought to be a component of the active transport system. It is clear that the loss of hormone sensitivity is only one aspect of the ageing process in the kidney but it may well be linked with specific changes in the enzymes basic to ion transport.

Experiments on the lipolytic response of rats to epinephrine and theophylline lend support to the idea that the loss of responsiveness to catecholamines is not due to faulty receptors. On the other hand, mitochondria in the liver of old rats show aberrant growth responses to thyroxine treatment of the intact animal indicating that there is a loss of the capacity to make an integrated response. In summary, the generalization may be made that age variations in metabolic pattern of target tissues are important factors leading to a decrease in tissue sensitivity to hormones., However, these changes at the molecular level are not specifically confined to effectors of the endocrine system.

The Pancreatic/Hepatic Glucokinase Model

In the final analysis we return again to the difficulty in assessing a holistic response in terms of the relationships between stimulus, hormone release, and changed enzyme activity. It may be better to work in the other direction, from the biochemical change of known significance to homeostasis. For example, hepatic glucokinase is an adaptive enzyme playing a vital part in the regulation of blood glucose, which responds to the absorption of glucose from the intestine. The enzyme responds slowly in 2-year-old rats compared with first year cohorts. The response is delayed by up to 12 hours although the increased enzyme activity is the same.

Insulin is required for the glucokinase response, and the direct treatment of hepatocytes by intraperitoneal injection of insulin produces the same response in glucokinase in young and old animals. Binding of insulin to hepatocyte cell membrane receptors is not altered with age either in terms of the number of sites or their affinity for hormone. This indicates that the age impairment lies at the level of interactions of glucose with the pancreatic cells. A simplified version of these possibilities is presented in Figure 6.13.

Insulin secretion from the pancreas into the hepatic portal vein takes place in two phases. One is almost immediate and the second occurs after a delay. Ageing is associated with an enhancement of the first phase and a delay in the second with a reduced magnitude of response.

Glucagon, secreted by the alpha cells of the pancreas is the main antagonist to insulin and during the initial phase following glucose administration its levels are high relative to that of insulin. It is therefore likely that the second phase of insulin secretion is more effective in promoting the synthesis of glucokinase and uptake of glucose than the initial phase of secretion.

A further complication is that the pancreas contains two populations of the

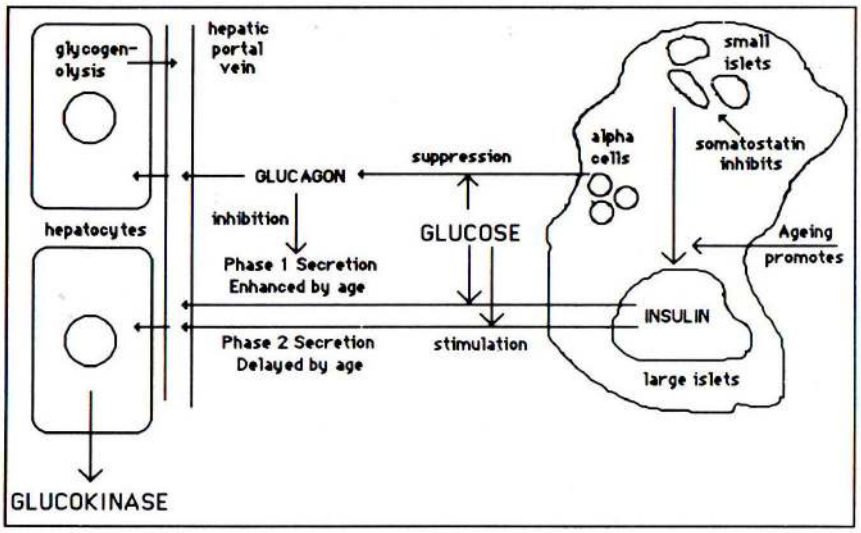


Figure 6.13. Possible interactions between glusoce absorption, pancreatic hormones, and ageing

insulin secreting islets which differ in size. Small islets predominate in young animals and there is a shift from small to large islets as animals get older. At the same time the small islets lose their ability to secrete insulin in response to a rise in plasma glucose. This appears to be due to an age-related increased local production of somatostatin by cells in the small islets: somatostatin is a potent inhibitor of glucose-stimulated insulin secretion.

Changes in Endocrine Organs

The most obvious starting point to discuss changes in endocrine organs is with the secretion of its hormone. Changes in secretion occur either through an ageing process intrinsic to the gland or through an alteration in the rate of metabolism or excretion of the hormone, which change the rate of secretion through feedback regulation. In the latter two situations it is necessary to postulate a feedback loop that operates to maintain a fixed concentration of plasma hormone. A fall in the rate of hormone metabolism is counteracted by a decease in the secretion rate. This appears to apply to the decline in the secretion of aldosterone and thyroxine in ageing humans. Here the change in the function of the endocrine organ may be compounded of several factors outside the gland, such as the quantity and concentration of metabolic enzymes, hepatic blood flow and the uptake of hormone by tissues other than the target

organ, and interactions with changes in the levels of other hormones. With regard to the latter possibility it is likely that the marked reduction in plasma aldosterone in women at about the time of the menopause is connected to changes in the levels of brain and ovarian hormones (Figure 6.14).

For the sex steroids, it may be inferred from the age-related fall in the excretion of urinary steroids that there is either a fall in the concentration of plasma hormones or a decrease in their synthesis and metabolism. Of these urinary hormone metabolites, the decrease in 17 ketosteroid excretion is due largely to a fall in androsterone. Urinary 17 ketogenic steroids also decline with age. Both changes are reversed by thyroid hormones. There is also a decline in the excretion of 17 hydroxycorticosteroids in human male urine which occurs at about middle age and correlates with a 50% fall in the excretion of pregnanediol. As this is not matched by age differences in the concentration of plasma corticoids it may be concluded that there is a lowered rate of secretion by the adrenal cortex.

Based on these changes in urinary steroids it has been proposed that the 17 ketosteroid to 17 hydroxycorticosteroid ratio of urine, which on average declines steadily with chronological age, is a useful measure of the physiological age of the human adrenal cortex. Much more well-defined changes in plasma levels, metabolic clearance and blood production rates are available for oestrogens in women, which have been connected with a postmenopausal decline in ovarian oestrogen production.

There is no change in the concentration of plasma-bound iodine with age, and from this it is inferred that the concentration of thyroid hormones is not

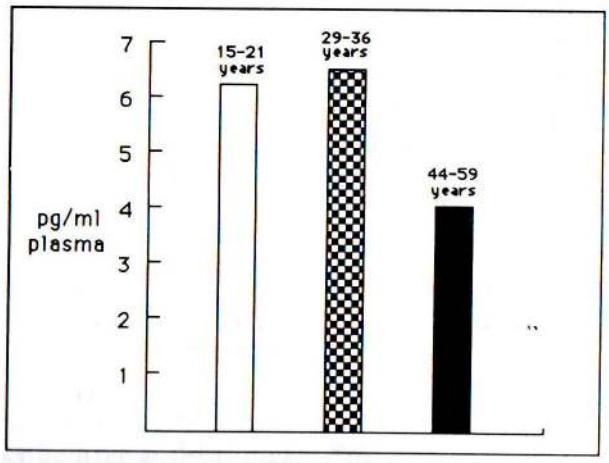


Figure 6.14. Changes in the diurnal rhythm adjusted mean value of plasma aldosterone in women

affected in old animals. This is borne out by measurements on triiodothyronine in rats where most of the increase occurs during the first year of life.

For corticosteroids, there is sound chemical evidence for age changes in the concentration of circulating corticosterone in rats. This occurs during the early phase of growth, peaking at about 1 year of age. Thereafter it falls back to a level characteristic of the third and fourth months of life.

In humans there does not appear to be a marked difference, comparing the concentration of plasma corticosteroids in young adults, with that in people over 65 years of age. On the other hand there is a phase shift in the circadian rhythm.

A complication in this kind of approach is that neither the total plasma hormone nor its secretion rate is a guide to the concentration of biologically active hormone when a large proportion of the hormone interacts with the plasma proteins. The specific binding proteins in the plasma may vary independently of endocrine secretion so that a variable proportion of the total hormone is in immediate diffusion equilibrium with the cells. In this connection it is established that large changes in the plasma protein pattern occur during the lifespan of a number of mammals.

However, the main problem concerns the lack of cheap, routine analytical methods to make the many measurements necessary to define the amplitude of diurnal rhythms and detect possible shifts. An indirect approach is to study the capacity of preparations of endocrine organs to synthesize radioactively labelled hormones *in vitro*. By this means it becomes easier to measure the relatively larger amounts of steroids produced in the experiment. This method has been more widely used to study differences in the rate of synthesis of androgens and growth hormone.

A third approach to the problem of hormone availability may be made by measuring the amount of hormone stored in the endocrine gland (Table 6.11). It is difficult for workers in this field to agree on a common interpretation of the changes in the quantities of stored hormones For example, a decrease in the concentration of hormone in the endocrine gland may be regarded by some to indicate a fall in the rate of synthesis, while others would see the same phenomenon resulting from an increased rate of secretion with a concomitant

Table 6.11. Changes in the content of adrenal catechol amines (μg) in male rats

	6 months old	30 months old
Epinephrine	15.0	16.8
Norepinephrine	7.2	55.0
Dopamine	0.09	1.7
Dihydroxymandelic acid	37.9	24.3
Left gland weight (mg)	31.8	102.0

high rate of synthesis. Where there is often a marked change in the size of the endocrine gland, as is the case for the adrenals, the difficult question arises as to whether the change in size, for example an increase, is a response to declining synthesis of a crucial hormone.

With respect to the anterior pituitary gland it appears that there are no age-related changes in the amount of stored growth hormone and TSH. In the case of growth hormone it has been shown that a constant quantity is maintained in the gland throughout life without an impairment of the synthesizing capacity of the tissue. The major factor that limits the amount of circulating growth hormone appears to be the concentration of releasing factor passing from the hypothalamus. Old rats have none of this releaser. Attention has also been paid to the blood levels of somatomedin-C/insulin-like growth factor. This is a growth-hormone dependent anabolic peptide highly correlated with increases in nitrogen metabolism and body weight. It falls with age. In contrast to growth hormone, the amounts of gonadotropins, FSH and LH in the pituitary increase considerably with age.

Comparative work on the insulin content of the pancreas shows the dangers of generalizing from the situation with respect to stored hormones in a single species. In ageing cattle there is a decrease in the insulin concentration, in rats an increase, and in humans no change with age. It is not known how these species differences are related to secretory activity.

The proportion of endocrine tissue in the body varies with age. In the case of the thyroid there is a decrease in the weight of the gland relative to the body. This change is most prominent during the early stages of postnatal life. For example in the dog during the first 8 weeks of life there an almost 50% fall in the relative weight of the thyroid. The relative weight of the adrenals and parathyroids also declines with age. Similar but opposite changes in the weight of the gonads also occur. These alterations in the relative weight of endocrine organs might have a bearing on the effective concentration of circulating hormones because of corresponding variations in the relative volume for distribution of the hormone. A large fall in relative cell mass of an endocrine organ also indicates a corresponding rise in the rate of hormone synthesis per cell.

Little is known of age-related changes in the sensitivity of endocrine organs to the primary stimulus that results in hormone secretion. The problems are similar to those encountered in the assessment of the mechanisms responsible for changes in target tissue sensitivity. It is established that homeostatic adjustments to the ingestion of water, sodium and potassium initially become more effective with age. But more fundamental information is needed on the relationship between stimulus and response. For example, from the point of view of changes in the endocrine organs it would be of interest to know the effect of age on both the secretory capacity and the time of response of the zona glomerulosa of the adrenal cortex to various concentrations of plasma sodium.

Changes in Function

Most of the evidence that points to changes in the nature of hormone function with age has come from experiments on the action of exogenous corticosteroids and neurohypophysial hormones. The underlying principle is that age-dependent changes in the characteristics of the response are related to intrinsic changes in the homeostatic mechanisms. This applies particularly to the action of neurohypophysial hormones and adrenocorticosteroids on electrolyte excretion in rats. In the early stages of life, vasopressin inhibits water loss in older animals. Similarly, cortisol increases the excretion of sodium and water in young rats but reduces water loss in older animals. The age difference here is most likely related to the fact that the younger, in contrast to the older, animals had not reached an age at which they were normally weaned. It is in this period that a difference is noted in the metabolic effects of adrenal hormones. For example, after adrenalectomy a 12-hour period of starvation proved fatal to the majority of animals in a group of very young rats 20 days old, but old and adult rats aged 60 days to 18 months survived longer than 24 hours. Again this difference if probably linked with the endocrine control of energy stores, and related to a change in feeding behaviour after weaning. Differences in hormone action are not confined to this early period. Aldosterone has little or no action on the excretion of potassium in 5-week-old weaned rats, but produces a marked inhibition of potassium output in rats aged 1 year or more.

It is not known how these different effects of hormones are related to the development of homeostatic systems. During the first weeks of life after birth there are many changes in the rat's physiology. In addition there is also the possibility that some of the alterations in responses to hormones are connected with age-linked variations in hormone sensitivity.

Ageing and Hormone Deficiency

If it is anticipated that a hormone deficiency is responsible for an ageing phenomenon the obvious test is to treat the animal with the hormone in an attempt to restore the youthful characters. From studies of this nature with sex steroids it appears that age differences in biochemistry, physiology and behaviour are not related solely to hormone deficiencies. Research which compared eunuchs with normal men, and the effects of castration on the lifespan of domestic cats, have indicated that endogous sex steroids have a life-shortening action, particularly if they are present during the growth period.

With reference to the anterior pituitary gland, although the maximum body weight gain attained during growth of rodents is increased by exogenous growth hormone, the normal termination of growth is not due to a lack of this hormone in the plasma. Also, it is well known that several features of old age are

similar to those encountered in hypothyroidism, but there is no evidence for a general decline in the concentration of thyroid hormones with age, and most symptoms of old age cannot be alleviated by thyroxine.

The characteristic age-related changes in the water and electrolyte content of cells are similar to those in diabetes insipidus and point to an impairment of neurohypophysial function. Support for this idea comes from experiments in which the pattern of salt and water distribution in old rats was partially restored to that characteristic of earlier stages of development by treatment with neurohypophysial hormones. Further, the combined treatment of old rats with posterior pituitary powder and cortisol resulted in marked prolongation of life. In the old rats used in the above experiments the neurohypophysis was depleted of neurosecretory material and failed to respond adequately to an osmotic stimulus. It is difficult to tell whether or not these phenomena are due to the onset of an age-dependent syndrome centred on the hypothalamus and pituitary. In this respect they may well be strain dependent. From similar work it is thought that changes in water metabolism in old rats are due mainly to an intrinsic regression in renal function and are not mediated solely through an alteration in the pituitary system.

When growth hormone was given daily in the long-term treatment of middle-aged rats although there was a significant augmentation of body weight gain and water consumption, no effects were observed on several other metabolic and physiological variables, and there was no change in lifespan. Further, growth hormone failed to prevent the final decline in body weight as rats approached death. It is also notable that, although hypophysectomy retards the ageing process in connective tissue and prevents changes in renal function that may be regarded as detrimental, the lifespan of hypophysectomized animals is shorter than normal.

Thyroidectomy is known to retard the growth rate and rate of general development, but a hypothyroid state does not increase longevity. The condition results in the unbalanced development of various organs, and in several respects the hypothyroid animal resembles one that has aged prematurely.

Treatment of rodents with glucocorticoids inhibits growth. From early work on these steroids it was thought that this was a toxic reaction related to the stress role of these hormones. However it is known that in some circumstances rats treated with corticosteroids can be maintained for a considerable time despite a marked inhibition of growth. It is also evident from comparative studies on other animals that corticosteroids have a more general effect on reducing mortality. Of 38 drugs tested on *Drosophila* cortisol and cortisone were the most effective, giving up to a 40% increase in lifespan. Cortisol is also particularly effective in increasing the lifespan of cells in tissue culture. It has been proposed that this may be related to their action in stabilizing intracellular membranes.

THE IMMUNE RESPONSE

The importance of the immune response in gerontology is that, as a cellular system, it offers a model of cell differentiation and function, and at the level of the whole animal it is a vital homeostatic system responding to environmental challenges. Also, the immune system has been implicated in a general immunological theory of ageing and disease.

The immune system is the body's defence mechanism against foreign cells and substances, which clinically and experimentally reacts against skin and organ grafts. The main agents of the immune system are special kinds of white cells which circulate in the blood and lymph. These cells are called lymphocytes and are the dominant cells in lymphoid organs such as the spleen and lymph nodes. These organs are composed of reticular epithelial cells, held on a connective tissue framework and the lymphocytes occur within the cavities of this sponge-like mesh. The function of the lymphoid organs is to filter foreign particles and cells from blood and lymph, enabling the lymphocytes to neutralize them. Local accumulations of lymphoid tissue are found at strategic positions such as beneath the linings of the respiratory and gastrointestinal tracts, the pharyngeal tonsils and the intestinal Peyer's patches and appendix.

Lymphocytes are derived from haematopoietic stem cells, which also produce other cells of the blood, such as granulocytes and red blood cells. During development haematopoietic stem cells in the bone marrow produce cells which colonize the thymus and peripheral lymphoid tissues. The micro-environment of the recipient tissue, such as spleen, lymph nodes or tonsils triggers further differentiation of these lymphocyte precursor cells. The end-point of these lineages are, in the main, two functionally different classes of immunocompetent cells. Under the influence of the thymus the cells differentiate into lymphocytes called T cells (thymus-derived cells). T-lymphocytes are responsible for what is termed cell-mediated immunity, which involves direct contact by lymphocytes with foreign cells, such as grafts, and may be incipient tumour cells, or particles, to destroy them. Immunity to these agents can only be transferred by T-lymphocytes and not serum antibodies.

Under the influence of a bursa of Fabricius in birds and possibly the bone marrow or a bursa equivalent in mammals, bone marrow precursor cells differentiate into another kind of lymphocyte called B cells (bursa-derived cells). B cells produce the cells that form the antibodies which circulate in the gamma globulin fraction of the blood. In the presence of an antigen they interact with T cells and other kinds of white cells to multiply and differentiate into plasma cells which synthesize and secrete specific antibody. T cells play a role in regulating the formation of plasma cells in a complex and as yet not fully explained feedback system.

Possible failures in immunocompetence can be assessed at the cellular level in

several ways. For example T cells are responsible for delayed hypersensitivity. Their functional competence may be measured by the redness and swelling produced by a test antigen injected into the skin. The T-lymphocytes of immune subjects respond by triggering a local inflammatory reaction which reaches a maximum 48–72 hours later.

Another test is to measure the rejection of skin grafts. This is effected by T-lymphocytes which have been sensitized to the graft's foreign antigens. A third method is to measure the mitogenic response of lymphocytes to substances such as phytohaemagglutinin and concanavalin. Cells from animals with low levels of T cells have a diminished response to these mitogens or to foreign cells. A variation of this method is to measure the amount of substances called lymphokines which are produced by T cells in contact with a mitogen or antigen. Fourthly, since an immune T cell will kill target cells bearing the antigen to which it has been immunized, the killer response can be used to measure the extent of T-cell immunity.

Assay for B-cell activity depends on the measurement of serum antibodies, determination of the numbers of antibody-forming cells in lymphoid tissue, and the response of lymphocytes to B-cell mitogens such as endotoxin and lipopolysaccharide.

Using these methods it has been found that immunity homeostasis declines with age. Age involution of the thymus which provides the micro-environment for differentiation of T-cell progenitors is evidence of a decrease in the body's potential to produce T cells. All of the other lymphoid tissues, like the thymus, generally reach their maximum relative size immediately after puberty, although the resultant loss of cellularity is not so dramatic. In line with these changes the number of T cells declines. Further, human and animal studies have revealed a decrease in humoral immunity. This is seen in the primary response of the immune system to an antigen. However, the secondary immune response does not usually show a decrease with age. The difference is that the former depends on the combined action of T cells and B cells whereas the latter is a function of the B cells only.

The mitogen response assay also indicates that T-cell function declines with age, and the suppressive effects of T-cell populations on B cells also decline. These data give rise to the hypothesis that a decline in a control mechanism regulating the antibody-forming system results in the appearance of cells which synthesize autoantibody with specificity against 'self' antigens.

There is also decline in cytotoxic T-cell function with age which can be measured directly *in vitro* or indirectly by foreign-tissue rejection, delayed hypersensitivity and graft-versus-host reactions.

Taking all of these results together there is now general agreement that ageing is associated with altered humoral and cellular immunity in rodents and man. Further, this is centred on an impairment of lymphocyte proliferative activity. T-cell proliferation is driven by complex interactions between cells and

monokines/lymphokines. In particular, interleukin-1 produced by cells of the monocyte/macrophage series is the first signal for lymphocyte proliferation following mitogen/antigen activation. Subsequently, interleukin-2, a glycoprotein produced by a special type of T-lymphocyte, binds to a specific lymphocyte receptor and is able to sustain continuous proliferation of T cells. Tests for the production of the two interleukins (IL-1 and IL-2) involve the culture of monocytes from young and old subjects and the use of the supernatant culture, containing secreted IL-1 and IL-2 as a stimulus for the growth of mouse thymic cells responding to mitogens, such as phytohaemagglutinin. The rate of DNA synthesis can be used as an assay for these interleukins (Figure 6.15). Current ideas surrounding the loss of human immunocompetence with age favour a limitation in nuclear responsiveness to cytoplasmic signals as a central mechanism.

Autoimmune Disease

Autoimmune antibodies are antibodies which react specifically against the body's own cells. They have been detected in the tissues of old subjects but

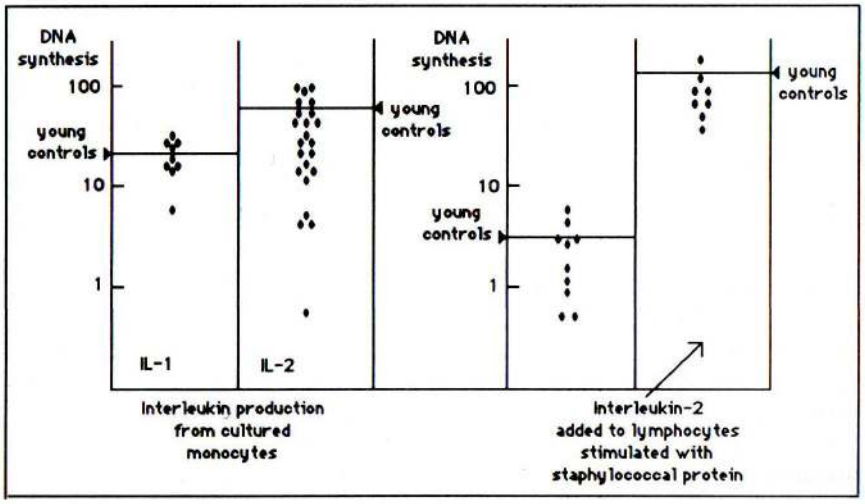


Figure 6.15. DNA synthesis in mouse thymic cells responding to interleukins produced by cultured human monocytes (● = individual results from old donors), and human lymphocytes from individual old donors (•) responding to pure interleukin-2. (Results from old individuals are compared with the means for a young group)

their significance is in dispute. At one extreme there is a school of thought that autoantibodies cause several age-related illnesses, such as vascular disease; at the other pole is the strong belief that autoantibodies are not associated with any disease. Another viewpoint is that the autoantibody response is a way for the organism to rid itself of damaged tissue. Therefore autoantibodies increase with age because of the rise in dead and dying cells. There are other hypotheses that autoantibody is antibody directed against viral-induced cell surface antigens on virus-infected cells. It also could be the result of a breakdown in control mechanisms which then allows an 'escape' and proliferation of a particular clone of cells which make autoantibody. There is other evidence of age-associated synthesis of abnormal antibody protein which could reflect changes in the proliferative capacity of individual B cells. Whether this results from a deficiency in a T-cell control effect on B cells is not certain.

Since cellular ageing appears to be a general feature of all kinds of cells regardless of their state of differentiation it is reasonable to suppose these principles apply to the cells of the immune system. In this respect, loss of the immune response is a result of ageing not its cause. The cellular deficiency may be due to a decrease in the number of precursor cells; an inability of precursor cells to divide and differentiate; and this can lead to fewer and/or defective immunocompetent effector cells.

Failure of immune homeostasis may be directly responsible for some causes of deaths which increase with age, such as mortality of infectious diseases. With respect to the incidence of cancer it may be that the immune system loses its protective effect, both in preventing the occurrence of malignant cells or in stopping the growth of tumours by killing them. On the other hand, antitumour antibody may in some way stimulate tumour growth. Therefore, it could be that T cells suppress tumour growth while B-cell products encourage tumour growth. So far there is a lack of precision in establishing a definitive demographic relationship between cancer incidence, the level of immune competence, and the ratio of T-cell activity to B-cell activity, partly because of technical and, with humans, logistical problems, but also because of the large variability in immune function between individuals.

VARIABILITY IN HOMEOSTASIS

It is frequently observed that as a population of animals ages the variability of physiological and biochemical parameters increases. There are three possible sources of variability. First, an increased error of measurement. Published data often give no indication of experimental error and this can only be ruled out as a source of increased variability where there is no change in the mean value with age. The second source of variation is the drift in norms within individuals as expressed by increased phenotypic and genotypic variation. In this sense there

are certainly shifts in population norms with age. Third there is a possibility of increased random variation within individuals due to a greater overshoot and undershoot among the various homeostatic systems at both the physiological and biochemical levels.

The only way to distinguish between an age-dependent variability in norms and a variability in individual homeostasis is to carry out longitudinal studies, but as yet few such investigations have been made. But there are many indications of increased population variability with age and it is unlikely that they are all simply related to measurement errors.

One of the areas of possible functional age variation is that of blood homeostasis, since the composition of the blood would be expected to mirror any general deterioration in regulatory systems. There is certainly a great variability in the blood constituents among humans, but an exhaustive analysis of 21 norms in 478 individuals and 15 in 284 subjects has led to the conclusion that the observed variability is independent of age. This does not rule out age-linked loss of accurate homeostasis because homeostatic oscillations could be masked in natural populations by virtue of great intrinsic genetic and phenotypic variation.

Within inbred laboratory stock, variability does increase with age. One of the first indications of this came from work on ageing in rotifers, where it was observed that homozygous populations derived from a single egg gradually become more variable with regard to reproductive function. It is difficult to avoid the conclusion that this is due in large part to some kind of random phenotypic drift in individuals. Inbred rodent populations also show increase variability at both the physiological and biochemical levels. However, from the point of view of the underlying mechanism, an analysis of variability in structure may offer the opportunity of defining ageing at the level of individual cells. Thus, in human subjects, within the age group 65 to 69 years, there is an increased coefficient of variation for the numbers of mitochondria per human hepatic cell, and the circumference and total size of these organelles. Further, the inability to maintain the norm for mitochondrial structure is manifest in the injurious effects of thyroxine which appear with increasing frequency in the cells of senile rats. There are also similar age differences in the response to chronic hypoxia in the fine structure of cardiac muscle and the cells of the autonomic ganglia.

Increased variability within a population as a concomitant of age is important because it may increase the chances of death of individuals showing extreme variations. This is particularly so if the variation is associated with a decline in the mean resistance to death.

It is difficult to make similar generalizations at the molecular level. The main difficulties are connected with the lack of standard techniques, age ranges over which the measurements were made, the mode of expressing the results and the species and strains of animals used for the work. Attempts to trace whole body

physiological failures to the molecular level have so far failed. No doubt there are underlying changes in enzyme activity, but the relatively crude methodology of measuring their activity in cell free extracts has not shown any consistent pattern, either between tissues, or between enzymes in a particular tissue.

Taking a selection of 95 sets of data on rodent enzymes indicates that most enzymes do not change their activity, expressed either per unit protein or DNA. Those that show an increase are just about balanced by those showing a decreased activity. These shifts in the specification of enzyme patterns, although not indicative of a general loss of the capacity to synthesize enzymes, are consistent with the view that the youthful genetic specification of tissue biochemistry cannot be maintained. Despite the difficulties in making suitable comparisons because of differences in animals and techniques, this number of experiments probably reflects the best possible random sample available across age and strains (Figure 9.4).

This points the direction that the search must take. The ultimate explanation of failures to maintain a youthful pattern of protein catalysts must, on the one hand, involve the flow of information from DNA which specifies the type and quantity of enzymes, and, on the other, the postsynthetic inactivation of their catalytic centres. Enzyme activity measured in isolation tell us nothing unless it can be linked with the alterations in tissue and cellular structure defined at the cellular level.

BIOLOGICAL RHYTHMS

Organisms at all levels of biological organization have rhythmical activities with periodicities approximating to calendar intervals such as the solar day, the lunar month, and the planetary year. These rhythms:

1. Have frequencies that are resonant with environmental uncertainties and ecological opportunities.
2. Are modulated and influenced by internal and environmental inputs.
3. Persist when isolated from environmental modulators.
4. Change their characteristics such as periodicity and amplitude during development, ageing and disease.

A range of physiological systems take on an approximate 24-hour cycle when individuals are isolated from time clues. These are called circadian rhythms. For example when isolated in the dark, circadian rhythms emerge in body temperature, pulse rate, and the blood levels of hormones. Many of these rhythms can be detected in normal life where the circadian periodicity is synchronized to the individual's 24-hour activity pattern (Figure 6.16). The existence of such variations makes it imperative that they should be used in the

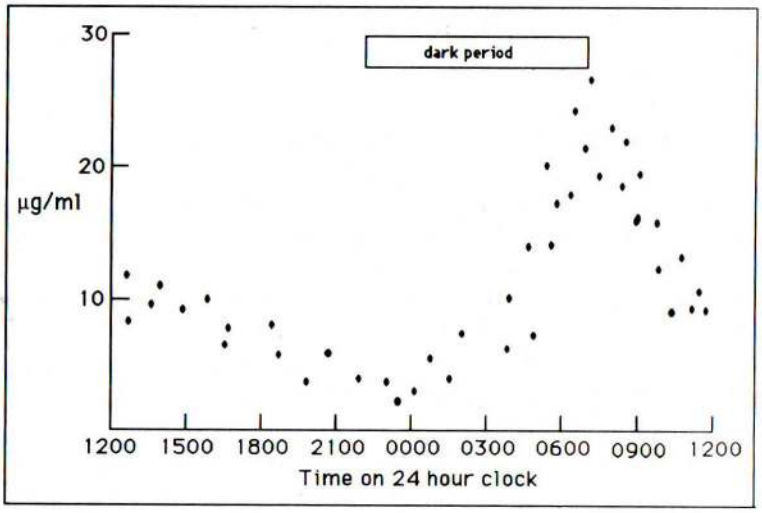


Figure 6.16. Diurnal rhythm in human plasma cortisol

study of physiological functions since they express the underlying fundamental homeostatic mechanisms. Their importance emerges when examples are found where an increased risk of disease or malfunction is found to correlate with a particular phase of a diurnal rhythm. For example, it has been reported that the rhythm-adjusted mean blood pressure, and risk of developing diseases associated with high blood pressure have a negative correlation with the amplitude of plasma aldosterone. Clearly, an experiment with multiple sample points within a given time span will provide a better interpretation of the function being measured compared with a single sample protocol.

However, there are major conceptual difficulties with this field of endeavour. Biological rhythms exist without doubt but so far their function is obscure. Their ubiquity points to important selective advantages in the evolution of life. They may simply reflect the universal importance of feedback loops at all levels of biological complexity as stabilizers of cellular, organismal and organism function. This overview was expressed diagrammatically by Franz Halberg as life's 'rhythm web'. A tireless promoter of the study of biological rhythms, particularly in the field of endocrinology, Halberg also presents a grand scheme for research in chronobiology where the various time scales of life, including ageing, are woven into a new multidimensional statistical physiology (Figure 6.17). This vision has yet to bring the study of ageing into the mainstream of biology.

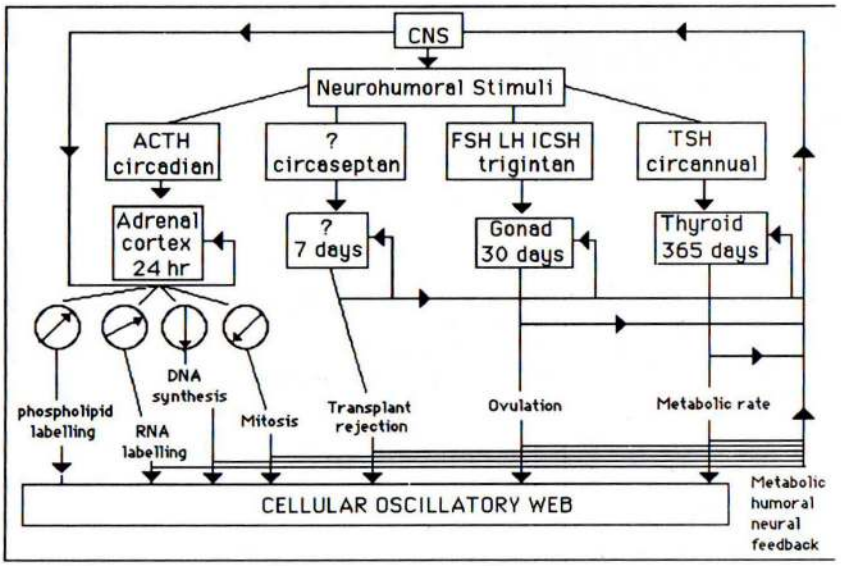
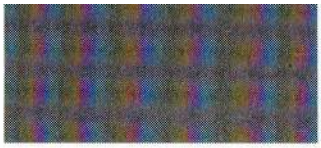


Figure 6.17. Halberg's theoretical rhythm-webs involving interactions between neuronal, endocrine and cellular systems



CHAPTER 7 Ageing as a loss of cellular homeostasis—1

At a time when knowledge of gene anatomy and function is increasing explosively, there are remarkably few ideas about how genes cause cells to construct tissues, organs and organisms. Despite the overwhelming dependence on form in biology and medicine, it is not yet possible to describe quantitatively any of the morphogenetic phenomena shown by higher organisms. (Roth 1983)

DEFINITIONS

Cellular homeostasis is usually taken to mean the sum total of all processes that govern the utilization of resources to promote cell division, cell enlargement, the production of organelles, and the maintenance of turnover of all intracellular components. However, the study of cellular homeostasis cannot be separated from the wider issue of maintenance of biological form. At the cellular level, 'form' is an expression of the patterning of cells in relation to the extracellular structures in which cells are embedded.

Early development is due to the progressive restriction of the developmental repertoire of embryonic cells so that cell lineages progress toward a form which, at maturity, is the stable expression of a particular phenotype. The microanatomy of a phenotype is defined at the cellular level in terms of sets of particular tissues. Ageing, as a loss of this anatomical stability, is therefore bound up with questions as to how genetic determinants are selected to be regulated in a specific differentiating cell population. In this broad context, the goal of cellular homeostasis is to generate precisely localized, uniquely differentiated cell populations and maintain them within a complex, spatially organized tissue structure. Histological examination of many kinds of tissues in a wide range of species shows that this precision declines after maturity.

The explanation of gradual failure to control and regulate tissue patterns as a phenotype ages resides in the history of particular cells, and if they are dividing, their lineages and the experiences of their ancestors. It is also important to take into account a cell's own environment, be it other cells or highly organized, noncellular components of the extracellular matrix. Changes in this environment could affect its ability to detect homeostatic signals in its neighbourhood which

would affect its ability to use some genetic determinants and ignore the existence of others.

To analyse ageing of organisms from the aspect of what might be termed their cellular ecology, a range of systems should be selected to model all of the above features. Whilst this chapter will concentrate on models of differentiated tissues it should be borne in mind that models of control and regulation of cells in the developing embryo provide points of reference for ageing, post maturation. From research into cell interactions in development the following events and processes should command the attention of gerontologists:

1. The regulation of morphogenetic cell movement.
2. Cell surface properties.
3. Cell contact and adhesive interactions.
4. Structure and function of extracellular matrix.
5. Association of the extracellular matrix and cell surfaces.
6. Progressive restriction of capabilities in cell lineages.
7. Regulation of specialised phenotypic characters.

Some of the major processes that are relevant to deleterious effects of ageing on the cellular integrity of organisms are presented in Figure 7.1. All of these processes have been taken up by research as gerontological models in efforts to understand how it is that ageing results in a loss of division capacity and failures in cytological specifications.

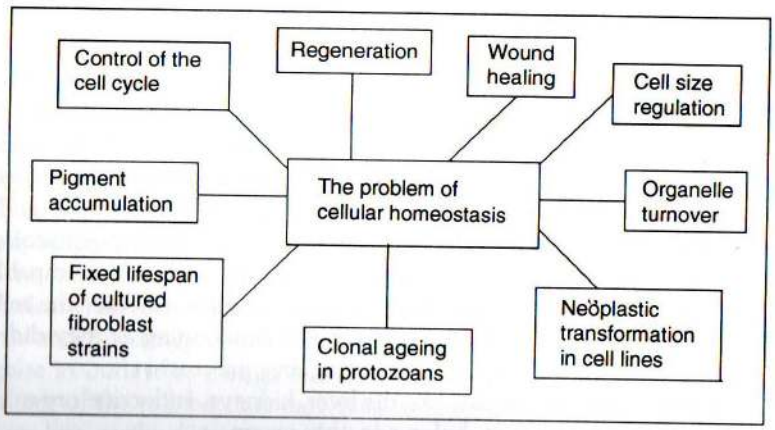


Figure 7.1. Major processes that have a bearing on cellular homeostasis

CLASSIFICATION OF CELLS

As a result of studies on the cellular changes in ageing mammals, chiefly mice, rats, rabbits, guinea-pigs, dogs and man, the cytological changes were first classified by Cowdry into four groups:

1. Vegetative intermitotics are relatively undifferentiated cells, the lifespan of which extends from one mitosis to the next. They reproduce themselves regularly and freely. To this group, for example, belong the basal cells of the skin epithelium, spermatogonia, bone marrow cells, etc.
2. Differentiating intermitotics are the cells which are in the process of differentiation but, like the cells of the first group, they reproduce themselves readily and have their lifespan limited to the period between two mitoses. This group includes the cells which are intermediate, and on the developmental pathway, between spermatogonia and spermatozoa, or between bone marrow cells and blood cells.

In some cases it is difficult to decide to which of these two groups a given cell belongs. Both groups reproduce freely, live only from one mitosis to the next and have the same final functional destination, namely to produce the highly specialized 'end' cells. Because of all those features Korenchevsky suggested they be called 'intermitotics'. Research is mostly directed to answer the question: despite their being in constant process of reproduction, do they age?

3. Reverting, postmitotics
4. Fixed postmitotics

To bring out the differences between the relatively undifferentiated intermitotics and the highly specialized and differentiated 'end' cells, Cowdry defined the latter as 'postmitotics'. For the majority of cells in this class, their individual life begins after the last mitosis and extends through youth, maturity and old age to death of the individual, i.e. they undergo the process of cellular ageing. They may be subdivided into two subgroups, reverting, and fixed postmitotics.

Reverting postmitotics, though they generally age and die, are capable of reversion, i.e. they are able under physiological stimulus, to enter the mitotic cycle, and give rise to two other cells capable of functioning as they did. The major question is: does reversion halt the ageing process?

Cells of most glandular organs like the liver, kidneys, endocrine organs, and probably skeletal muscle cells, belong to this group.

Fixed postmitotics constitute the highest level of differentiation and specialization. They do not multiply after the last mitosis, but like the whole organism they age and die. Nerve cells, rod and cone cells of the retina, cardiac

muscle cells, red blood cells and granular leucocytes are examples of this group.

In the cellular group of 'inter-mitotics' the mitotic division of cells continues up to the death of the organism. In the group of 'reverting postmitotics' of old animals, in normal conditions, mitotic divisions of cells are comparatively few or even absent. Reverting postmitotic cells potentially may live as long as the whole organism. Probably their limited multiplication occurs in order to replace cells lost accidentally. This is confirmed by the fact that, when the regeneration of a large part of the organ is necessary, the reverting postmitotics revert to the prolific mitotic activity, and the rate of multiplication of their youth. Some idea of the complexity of their ageing as a system is seen in the apparently contradictory phenomena, that explants of the normal liver from older animals grow much less satisfactorily than those from young animals, but the growth of explants from regenerating liver is independent of the animal's age.

The length of life of different types of cells is very different. In general, the life of those postmitotics, which can be replaced through the reproductive activity of their mother stem cells, (e.g. blood cells, epidermal cells, etc.) is shorter than the life of cells which cannot be so replaced (e.g. cells of the nervous system, retina, etc.). For technical reasons it is difficult to ascertain accurately the length of life in cells of different tissues and organs of higher organisms. In order, however, to show the magnitude of the variation range, the length of life of the majority of human nerve cells is about equal to the length of human life minus one year (since mitoses do not occur in these cells after the first year of age), while the length of life of neutrophilic leucocytes on an average is about 10 days. Therefore, the latter age and die about two or three thousand times more quickly than the former.

INVOLUTION AND HYPERTROPHY

Tissue involution is difficult to define in terms of individual organs. This is particularly true with regard to specific cell loss and changes in the morphology and function of cells that survive. Also, there are important questions regarding what happens to the space previously occupied by cells.

There are also serious methodological problems in separating changes in cellular volume from the surrounding extracellular compartment. They are to do with the difficulties in counting all cells cytologically, and measuring their volumes.

Nuclear changes vary in old age just as cytoplasmic changes do, both in the degree and frequency of their occurrence. The shape of nuclei may become irregular in outline or elongated. They are sometimes displaced towards the periphery of the cell, become dark-stained (pycnotic) or, on the contrary, pale, and are less easily distinguishable in the cytoplasm. The size of nuclei also varies, the mean size for a tissue being decreased or increased, or without change. Binucleated cells arise from mononucleated cells by mitotic or amitotic division of the nucleus with failure of the cytoplasm to divide. In fixed

postmitotic cells only their amitotic development is observed. This has been taken to mean that this exemplifies a mechanism by which chromatin of fixed postmitotics can compensate at the organ level for the loss of cells: there is a larger mass of nuclear and cytoplasmic material in normal binucleated cells. Binucleated cells, however, have been rarely observed in nerve cells of old individuals They are observed in reverting postmitotics at all ages, and in greater numbers in old subjects.

In human liver, there is no difference in the size of hepatocytes, but the size of the nuclei and the nucleocytoplasmic ratio increases with age. There are also increases in the variety of cellular and nuclear sizes, such that the maximum size increases and the minimum size decreases, both within and between individuals with age. The proportion of binucleate cells at first increases then decreases with age (Table 7.1).

A common finding is that cells in old tissues show extremes in size, larger and smaller than the maximum and minimum in adults. Korenchevsky summarized the main principles of this age-dependent cytological atrophy and hypertrophy in rats, as follows:

1. There is a five-fold increase in the percentage of atrophic cells throughout the maximum laboratory lifespan.
2. Both atrophic and compensatory hypertrophic processes in the cytoplasm exist together in the same organ.
3. The compensatory hypertrophy of the cytoplasm with advancing age in some organs reaches its highest level in adult age, and then remains stationary; in other organs it continues to increase until death.
4. Hypertrophic processes also prevail in the specialized cellular unit structures of skeletal myofibres, cardiac myofibres, renal glomeruli.
5. In contrast to the cytoplasmic hypertrophy, the average size of nuclei either remains stationary, or actually decreases in spite of possible occasional occurrence of large nuclei.

Table 7.1. Cytological changes human hepatocytes

Decade	5	6	7	8	9	10
Liver weight (kg)	1.48	1.17	0.98	0.80	0.62	0.65
Area of cells (u^2)	177.0	161.0	161.0	154.0	157.0	177.0
Variability coefficient*	18.7	20.6	20.0	20.8	22.6	21.4
Area of nuclei (u^2)	34.3	33.6	32.5	37.2	40.5	42.6
Variability coefficient*	18.1	23.1	24.3	28.4	28.2	32.7
Nucleocytoplasmic ratio (%)	20.0	21.0	20.5	25.0	25.7	24.8
Binucleate cell index**	521.0	709.0	765.0	704.0	543.0	526.0

*% variation in size: **numbers of binucleate cells/10 000 hepatocytes

6. The nuclear/cytoplasmic ratio, i.e. the relative size of the nucleus per unit of cytoplasmic mass, may be considerably different in adult and older ages as compared with that in young animals.

The functional significance of changes in the nuclear/cytoplasmic ratio is impossible to assess now, as it was in the 1920s, when it was the centre of a long-running debate amongst histologists, led by Minot. In a modern context, without any functional explanation, it is simply evidence for the generalization that old cells are less able to maintain the structural unity they once possessed.

It has long been the feeling of histologists that the tendency for cells to become larger in old tissues may be of significance in relation to the increased incidence of cancer with ageing. Their idea is that an increased activation of normal compensatory hypertrophic, and hyperplastic processes, may develop into pathological hyperplasias and metaplasias both in animals and human beings. The cellular populations so formed may actually represent possible latent malignancy, and may in their turn, be further transformed into adenomas or malignant tumours. Although it is clear that population growth of cells is regulated chemically at many levels involving serum growth factors, intracellular cyclic nucleotides and oxygen gradients, there are many situations where the geometry of an individual cell or the geometrical configuration of a population of cells seems to exert a profound influence on growth and differentiation. Thus, changes in the geometry of organs due to age involution may trigger new patterns of cellular growth, which may become cancerous.

In rats it is relatively easy to follow all the transitional forms of spontaneously developing tumours, which start as small groups of hypermetaplastic cells and gradually develop into tumours of various kinds. These structures begin to appear in most laboratory colonies after the 19th and 20th months of age. Causal factors, which stimulate the transformation of minute groups of hyperplastic cells into tumours, are unknown. There is evidence that a disbalance of endocrine factors might be one of the oncogenic causes, or at least a predisposing or co-operating factor, in the development of tumours.

Tissue culture experiments appear to be relevant to carcinogenesis in that normal cells taken from normal rats and mice may become transformed into 'immortal' cell lines when cultured. When these transformed cells are inoculated into the respective animals, they grow into malignant tumours. Both in tissue cultures, and in transplantations in animals, the malignancy remains unchanged over a number of years. Malignant metaplasia of cells in tissue cultures does not start to develop for at least a few months, or even a few years. This may indicate the operation of similar age changes *in vitro* to those involved with the development of neoplasia *in vitro*.

The failure of intracellular homeostasis is seen in the loss of structural integrity of organelles that have relatively short turnover times. For example, mitochondria usually change with ageing from short thick rods (their usual

shape at younger ages) into vesicles, or tenuous filaments, or, as a result of their fragmentation, they form into smaller proteinaceous granules, and apparently, pigment granules. Intramitochondrial vesicles, derived from 'swollen' cristae, may gradually increase in size and fuse to form larger amorphous vesicles.

The intracellular cytoplasmic membrane systems, exemplified by the Golgi apparatus, and the fibrillar structures which are a particular feature of mammalian neurons, also change with age. The normal delicate reticular Golgi network, as shown by special histological techniques, disintegrates into groups of argentophilic and osmophilic minute granules, or into irregular formations of stacked membranes of varying thickness.

AGE PIGMENT

Muhlmann in 1914 was the first to ascribe importance to the formation and accumulation of pigment granules in ageing cells. Using histochemical methods at least two varieties of pigments can be identified in old cells, as melanin and lipofuscin. The latter material is found in membrane-limited granules, and is composed of complex oxidized lipids, cross-linked and polymerized with protein and unsaturated peptides. The granules also contain iron and zinc in somewhat more than trace amounts, and significant quantities of hydrolytic enzymes, such as acid phosphatase. Lipofuscin occurs together with amyloid and both are a reflection of age (Table 7.2).

It is thought that lipofuscin is a largely undegradable end-product, derived from chemically-aged cell membranes and other cell components. The inert dead mass, which may more or less completely replace the active cytoplasm, has been thought of as a particularly exaggerated expression of the way the normal morphological and chemical composition of cells deteriorates. It is assumed that in cells accumulating pigment their function becomes greatly impaired, and death of cells may result. This pathological process should, indeed, be called pigmentary degeneration. This is particularly obvious in nerve cells which in certain parts of the brain become so full of the lipoid granule deposition that no cytoplasm free of pigment can be seen at all. Although

Table 7.2. Lipofuscin and amyloid in ageing mammals

Species	Age-related changes
Dog, cat	Lipofuscin deposits in cardiac and skeletal muscles
Monkey, rat, mouse	Lipofuscin deposits in neurons
Guinea-pig, mouse	Lipofuscin in Sertoli and Leydig cells of testes
Mouse	Amyloid in blood vessels, heart, gut and tongue
Insects	Lipofuscin in malpighian tubules

dominant in the tissues of old people and animals pigment accumulation in cells occurs in young tissues as well, and also in various infectious diseases, i.e. like many other kinds of degeneration, pigmentary degeneration is not specifically senile.

Lipofuscin and melanin pigments are insoluble in fat-solvents but are sudanophilic, i.e. are readily stained by the fat-staining dye Sudan black. When previously bleached, melanin loses its sudanophilic property while lipofuscin retains it. Lipofuscin appears as well-defined granules which can be easily counted, and from this point of view there has been much quantitative analysis of its incidence and progression. Also, current biochemical theories of ageing involve lipofuscin as a visible expression of deleterious effects of oxidative damage to lipids. In this respect, age-pigment will be considered in more detail in a later chapter.

Lipofuscin is particularly evident in old neurons although not all neurons are equally susceptible; certain areas do not accumulate lipofuscin at all. Because the lipofuscin is ultimately quite prominent in some neurons, and occupies so much of the cytoplasmic volume, it can be assumed that it displaces significant quantities of cellular organelles. Indeed, a decreased amount of endoplasmic reticulum, which is the protein-synthesizing apparatus, has been measured in neurons parallel with their increased content of lipofuscin. Although this has led many to assume that lipofuscin is a major element in causing cellular malfunction and death, this has never been directly demonstrated. The inferior olive, a purse-shaped aggregate of neurons in the brain stem, begins to accumulate pigment granules early in the first decade. Despite this concentration of lipofuscin there is no loss of cells in this region throughout the entire lifespan. It is evidence such as this which has given rise to the idea that age pigment is not toxic to cells although the possibility remains that cells containing large amounts of it are not so efficient as those which have none.

CELLULAR ECOLOGY

Age involution is not simply the loss of cells. It was established in the very early days of gerontology that atrophy is a very typical change in old tissues, and is characterized by changes in the relative proportions of pericellular components and fibroblasts. It is assumed that tissue involution of old organisms begins in the cells. The degree of cellular atrophy, however, varies greatly in different tissues and organs, from slight to complete atrophy. Very little attention has been paid to the 'filling-in' process that occurs after cells die. Degenerative changes in tissue as a whole vary both in degree and in the type of degeneration, but fatty, fibrotic and pigment degenerations are more common, while vacuolar and hyaline changes are less frequent. Atherosclerotic degeneration is a combination of fatty, pigmented depositions into the cells and interstitial

spaces, with infilling of calcium salts. While degenerative changes may be slight in some old individuals, in others they are very pronounced.

The latter may result in necrosis and complete disappearance of cells, usually classified by histologists as cytolysis. Cell death in this context differs from that of programmed cell death which is a commonplace feature of embryonic development, where its role is to establish the morphogenetic patterns of growth which determine shape and size of organs. A common secondary feature of cell loss in ageing is fibrotic degeneration, which constitutes a replacement by fibrous tissue of cells which were destroyed by atrophic or degenerative processes of ageing. Probably this occurs due to the normal interactions between differentiated cells and the surrounding fibroblasts similar to that which underlies the initial response to cell loss in wound healing, but little is known about it.

The acquisition of form and function during embryonic development involves a range of cellular behaviours, such as proliferation, movement, shape change and adhesion. These events take place within a pericellular matrix composed of characteristic macromolecules of three major classes—collagens, proteoglycans and glycoproteins. These components are highly interactive, both with one another and with the surfaces of the cells which they surround or abut. Their composition varies greatly in relation to their source, their locality, and the stage of tissue development, and it has emerged that these extracellular macromolecules play an important role, not only in maintaining the physical integrity and structural properties of tissues, but also in regulating the behaviour of their constituent cells. Because of the important role of the extracellular matrix in early development it is likely that macromolelcular interactions continue to be responsible for the integrity and biological properties of cells throughout life.

With regard to collagen, not only does it become more important quantitatively and qualitatively in terms of changes in molecular structure, but it also plays a dominant role in the modification of the elastic fibre network. Computerized image analysis of human dermal elastic fibres reveals a continuous increase with age in the relative surface area and the length of the elastic fibre system (Figure 7.2). These changes may explain the physical changes in skin such as the decrease in elasticity and the continuous increase in stainable fibres. If expressed throughout the body they may produce changes in cellular homeostasis at the level of maintenance of functional unit structures which depend upon the elastic fibre system to define their cellular arrays.

It is from such holistic considerations that we perhaps should be thinking of ageing as an expression of 'cellular ecology', and relating changes in cells to their total tissue environment. This requires a quantitative approach to the histology of the entire tissue. An example of this kind of approach to capillary wall tissue is given in Table 7.3. This study, on monkeys, revealed an attenuation of the capillary walls of the cerebral cortex and a decline in the

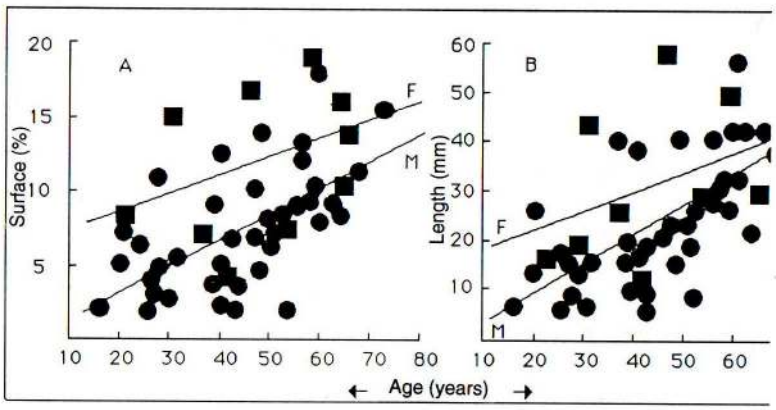


Figure 7.2. Changes in the elastic fibre network of human skin. Squares = female: circles = male

number of endothelial mitochondria per capillary profile. Capillary lumen size was not significantly altered. The thickness of the outer basal lamina increased between 4 and 10 years but no further change was observed between 10 and 20 years. Declining numbers of mitochondria are also a feature of other ageing tissues.

Although dominant in old organisms, none of these types of degeneration can be considered exclusively typical of, and occurring only in, old age. They are also, to a lesser degree, observed at younger ages and in various diseases. However, there is no doubt that pigment accumulation, fibrotic and atherosclerotic degenerations occur more often, or in a more severe form, or both, in elderly people. Atherosclerotic degeneration is probably due to the protracted action

Table 7.3. Cellular ecology of the capillaries in the occipital pole of the cerebral cortex in Macaca nemestrina

Cross section areas[1]	Age group years		
	4	10	25
Entire capillary*	36.6	28.0	20.7
Capillary lumen	10.4	9.4	6.1
Capillary wall*	26.3	19.4	14.6
Pericytes	1.3	0.6	0.4
Endothelial cells and basal lamina*	25.0	18.8	14.2
Inner basal lamina	0.12	0.09	0.08
Thickness of outer basal lamina[2]	0.11	0.14	0.14
Endothelial mitochondria[3]	3.8	2.5	2.8

[1] $\frac{1}{8}$[2] in u[2]; [3] number per capillary profile
*significant based on linear regression

of a range of damaging factors, with many different kinds of response which may produce lipid depositions, fatty degenerations and crude repair processes, all of which affect the form of fibrous tissue development.

THE NERVE CELL MODEL

Problems in quantifying cytological atrophy are particularly exemplified by studies of the loss of human brain cells. As far as cell loss is concerned, sections through human brains at autopsy indicate that the cortical mantle or grey matter of the cerebrum is thinner than normal, and the white matter, both in the convolutions and in the deeper centrum, occupies a smaller area. The grey matter, representing mostly cell bodies, diminishes faster than the underlying white (the fibre tracts) from age 20–50, changing from a grey/white ratio of 1.28 at 20 to a minimum of 1.13 at 50; thereafter, more white matter is lost than grey, so that the ratio rises to 1.55 by age 100. The cerebellum also shrinks slightly, again losing both grey and white matter, as does the spinal cord.

The human brain loses about 10% of its weight from maturity to old age which appears to be due mainly to a progressive loss of neurons, resulting in an enlarged fluid-filled space between the brain and its coverings. Other species do not show any change in brain weight. Within the human brain, some local functional groups of neurons are more affected by involution than others. The cortex of the cerebral hemispheres has been measured extensively by post-mortem cell counts, and it has been reported that only 50% of neurons remained in some areas by the ninth decade, with the greatest losses occurring in the frontal and superior temporal regions, which are also the areas of greatest gross atrophy For example, in the human brainstem the area responsible for head posture maintains a constant number of neurons. In contrast, in an area in the base of the fourth ventricle, with many connections with other parts of the brain, neurons are lost, from about 18 000 in youth to about 12 000 in old age.

The technical difficulties in assessing cell loss are due to the problems of staining cytoplasm distinctly for observation under the light microscope. It was only with the advent of electron microscopy that it was found that the extracellular space of human brain was much smaller than the 30–70% of total brain volume that had emerged from the use of light microscopy. Current estimates of the size of the cerebral extracellular space, by different methods, vary widely, from a low of 5% by ordinary electron microscopy, to 18% by chemical methods using markers of extracellular fluid, to about 20% by freeze-substitution electron microscopy. Very little reliable work has been carried out on experimental animals from the point of view of assessing terminal losses of brain cells. One of the better pieces of research provided evidence that extracellular space of the cerebral cortex in the 3-month-old rat is about 20%, and declines to about 10% in the 26-month-old animal.

Cell death leading to tissue involution is the dramatic end-point to a loss of cellular homeostasis. However, from changes in cellular morphology it appears that less fatal changes are a common feature of the cells that survive. For instance, alongside the losses of entire neurons in human brain, the remaining ones seem unable to maintain their dendrites and synapses. The dendrites not only decrease in number but the individual projections on each dendrite also decrease in number. These projecting 'spines' receive the incoming signals from closely apposed axonal terminals through the axodendritic synapse. The changes in cell morphology are probably related to the decrease observed in the enzymes involved with cholinergic transmission.

Changes in the neurofibril structure of human neurons are particularly significant in that they appear to be related to loss of mental function in the elderly. These microscopic abnormalities were classified in the early part of this century as the neurofibrillary tangle and the senile or neuritic plaque.

A tangle is a mass of intracellular fibrillar material, occupying much of the neuronal cell body. They are found in an increasing proportion of normal people after 60, and in almost everybody over 80, and appear to be a special feature of human ageing. There are especially numerous in the hippocampal cortex, which is concerned with memory. Plaques are extracellular structures situated between, and among, the neurons and measure 60–150 μm in diameter. They are also found mostly in the cortex and hippocampus. Unlike the neurofibrillary tangles, which are confined to humans, plaques are found in aged monkeys and dogs, although the detailed macromolecular structures are somewhat different to those in human nervous tissue.

Changes in the submicroscopic structure of the hippocampus are important for many reasons. In the rat hippocampus lipofuscin begins to accumulate at an early age and it accumulates more pigment per section volume of the cerebral cortex than other parts of the brain. The striking vulnerability of the hippocampus to such changes as hypoxaemia, and its involvement in human presenile and senile dementias also bring it to the fore as a model of practical importance.

Although present in all human subjects it has become increasingly evident that plaques and tangles are important features of cellular ageing that could well account for the functional changes which are common to some degree in the normal elderly, and to a much greater extent in people with greater mental disturbances. In particular, plaques and tangles are the hallmarks of Alzheimer's disease where they correlate with the degree of mental disability.

Under the electron microscope a neurofibrillary tangle is defined by large numbers of abnormal neurofibres in the cell body. Normal fibres in the adult neuron are of two types: hollow neurotubules composed principally of a protein called tubulin; and narrower, solid neurofilaments, predominantly composed of different protein, named filarin. Both of these intracellular structures have short side branches.

The neurofibrillary tangles appear to be derived from these normal structures, each fibril being a precisely organized double helix of two smaller filaments twisting around each other, with a periodicity of about 800 nm. These 'paired helical filaments' resemble normal neurofilaments without the side branches.

Tangles are also found in neurons affected by Parkinson's disease, which is thought to be the long-term result of a particular viral infection. They are present in adult brains from subjects with Down's syndrome. Both of these associations point to a DNA malfunction underlying the tangle. Similarly, experimental plaques identical to those of the dog and monkey have been induced in certain strains of laboratory mice by treating them with particular varieties of the scrapie agent, which is a type of slow virus.

Tangles are found in the brain of boxers, presumably as the result of repeated trauma. They have also been linked with aluminium accumulation, in that brain tissue of patients with Alzheimer's disease have increased levels of this element. A possible link between aluminium and abnormal neurofibrils, is that aluminium injection into the spinal fluid induces the formation of filamentous aggregates in rabbit neurons.

Senile or neuritic plaques have a central core of an abnormal, extracellular, fibrillar protein called amyloid, a complex proteinaceous substance which has affinities with gamma globulin. In this respect it has been suggested that it may be produced as an abnormal self-directed immune response. The core is surrounded with numerous distorted synapses, and axons dominated by clusters of paired helical filaments identical to those of the neurofibrillary tangle. Plaque contains fragments of mitochondria, and laminated granules termed residual bodies with hydrolytic enzymes. These granules are thought to be derived from mitochondria residual bodies and have considerable enzyme activity of the hydrolytic type.

BONE HOMEOSTASIS

Cortical bone is maintained as a well-defined tissue as a cellular homeostic system involving the formation of new bone by osteoblasts and its resorption by osteoclasts. New bone is laid down in concentric rings around the Haversian canal which feeds the cells through a network of blood capillaries. Bone resorption takes place within cavities containing osteoclasts, a 'cutting cone' of osteoclasts which makes a hole through bone to be filled with a new haversian system. Bone density rises from a level of 1.4 g/cm^3 in newly formed rings and matures over a period of about 6 months at a density of about 2.0 g/cm^3.

Cortical thickness is measured in human subjects from an X-ray of the hand and is measured at the mid-point of the shaft, its total diameter (D) and the diameter between the two inner tables of the cortex (d). Usually it is the second metacarpal of the left hand that is chosen because an X-ray of the hand is easily

standardized, and the relatively small dimensions of the metacarpal make measurement less cumbersome

Bone loss in old age may be regarded as a universal feature of human populations (Figure 7.3). Age brings about changes in the structure and mechanical properties of bone largely due to the continuing imbalanced activity of sub-periosteal osteoblasts. Excessive activity of osteoclasts results in a decline in the dry fat-free density of bones with increasing age, at rates which on average vary between sexes and races. Women lose about 8% of bone per decade, compared with 5% in men. A woman of 65 has a 6% chance of sustaining a fractured proximal femur if she lives to the age of 80. If she lives to 90 the risk increases to 22%. In the case of a man, the corresponding risks are 2% and 10%.

In women the most rapid decline in bone mass occurs after the menopause and probably results from lowered oestrogen levels, since bone loss is accelerated in oophorectomized women and can be reduced by oestrogen replacement. Resorption cavities become dominant particularly in, and near, the endosteal surface. There is also an imbalance between resorption and replacement of bone, particularly in the femur and radius, whereby new haversian systems

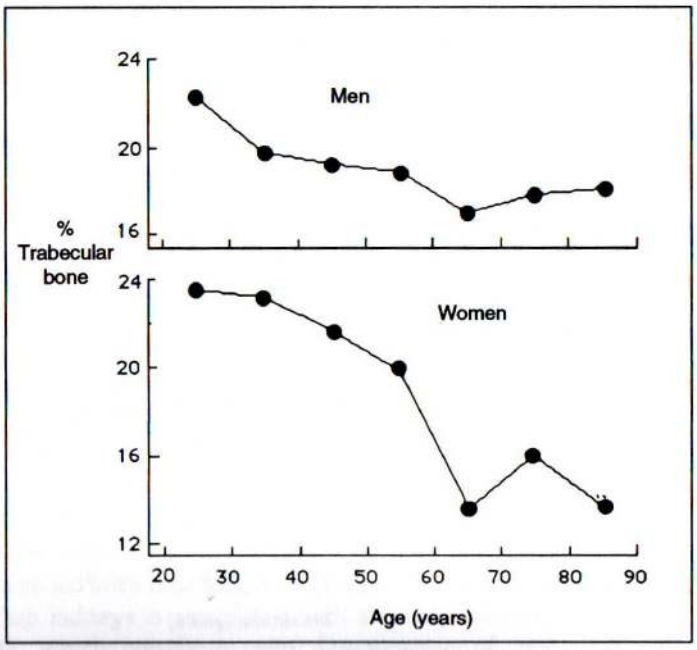


Figure 7.3. Loss of human trabecula bone expressed as a percentage of total bone in the iliac crest

are not completed. The central canals are maintained wider than normal. Cortical bone becomes progressively thinner and some of the trabeculae may be removed. Since this trabecular loss varies from bone to bone it probably reflects the response of surviving bone to mechanical stress.

The loss of cellular homeostasis in bone is expressed in osteoporosis, the most common bone disorder found in old people. There is a reduction in bone mass, the trabeculae are reduced in number and width and the cortex is thin. The numbers of both osteoblasts and osteoclasts are reduced. About 10% of women have symptomatic osteoporosis by the age of 55, and 20% by the age of 70.

The bone thinning causes increased susceptiblity to crush fractures of the vertebrae which, in turn, produce deformity of the trunk and thorax. The most common site for a fracture in a long bone is the proximal femur. One important cause is the high incidence of falls in old age due to poor vision, cerebrovascular disease, or dementia. Patients with fractured proximal femora have a lower bone mass than age- and sex-matched controls.

The mechanism behind the change in norms for matching bone loss with bone deposition is unknown. Numerous studies have demonstrated that oestrogen therapy prevents bone loss due to loss of ovarian hormones, and that relatively small doses of oestrogen are effective (Figure 7.4). However, this does not reveal what is behind the underlying trend in all members of an ageing population.

Another feature of calcium homeostasis is, paradoxically, the bone enlargement.

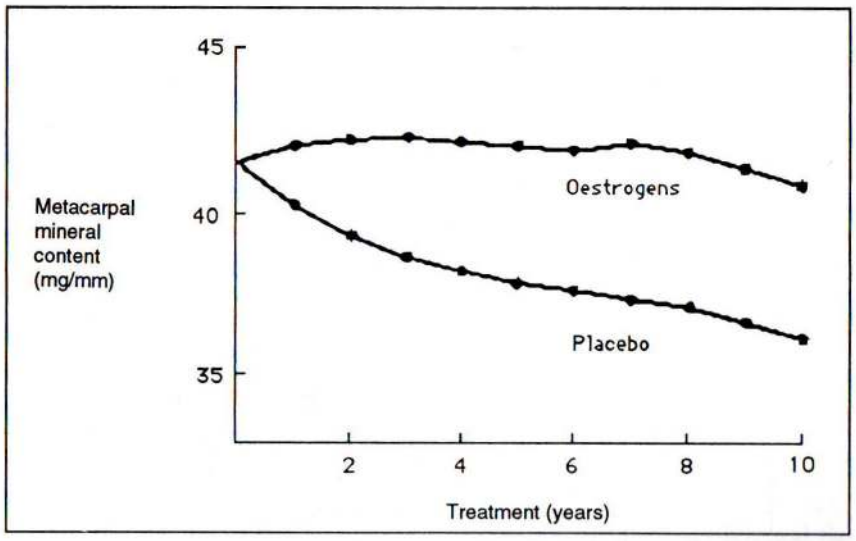


Figure 7.4. Effects of oestrogen (23.3μg/day) on metacarpal bone in oopherectomised women

There is a gradual increase in the diameter of the ribs, metacarpals and femur. Bones in the skull are also affected and this accounts, in part, for the changes in facial appearance which often take place with advancing years. These changes in relative proportions appear to be an extension of remodelling activities which in youth are responsible for changes in the strength of bones in relation to anticipated stresses and strains. Such remodelling is also found in experimental animals. The pig jaw has been used as a dental model to follow changes in jaw bones in relation to tooth placement. In wild rodents it has been found that there is a non-uniformity in the rate of growth of the skull which in the course of extended life in the laboratory causes a change in its appearance and proportions. In the case of the vole, *Microtus agrestis*, kept in the laboratory there is pronounced flattening of the skull and thickening of the zygomatic arches in the old-age groups. This is related to the fact that throughout life, the skull base grows steadily with age whereas the depth of the brain-case augments more slowly. This is a species characteristic which is not seen in the wild because all animals die before this allometric growth relationship produced a marked alteration in the angularity of the skull (Table 7.4).

TISSUE AGEING AND ORGANISM MORTALITY

In order for age-changes at the tissue level to be related to the survival curve for the whole organism they have to be somehow connected with the Gompertz curve which describes the exponential rise in mortality. This poses a problem at the organ level because the general feature of the ageing of organs is that they deteriorate at a more or less uniform rate which does not increase in line with the rise in mortality of the population. In contrast, the 'mortality' of isolated clones of fibroblasts does in fact follow a Gompertzian pattern and this is one of the attractions of using cell cultures as models for cellular ageing of the whole organism. Indeed, it has been postulated that the exponential rise in fibroblast 'deaths' is due to a cascade accumulation of errors in DNA template activity.

Another standpoint is to take the view that the shape of the survival curve of organisms is connected with the increasing risk of death occurring when the organ structural/functional specification falls below a certain level. This risk may be calculated for the population, at any particular specification, using the normal distribution curve of the measured function in the population and its variability. For any parameter that shows a constant mean rate of change, and an increased variability, the chances to an individual of any particular value being exceeded with time follows a gompertzian curve. For example, ageing of mammalian kidneys is associated with a steady increase in the radius of the glomeruli, which correlates with degeneration of individual tubules. The variability of this morphological characteristic also increases with age. The mortality of rats rises at about the time when the glomerular radius begins to

Table 7.4. Comparison of some measurements of skulls of *M. agrestis* from the laboratory and from the field.

	Percentage of skulls with different degrees of angularity							
	Field				Laboratory			
	None	Poor	Marked	Pronounced	None	Poor	Marked	Pronounced
Age (months)								
1–6	91.5	8.5			93.4	6.6		
7–12	41.5	43.4	15.1		9.8	27.9	52.5	9.8
13–18		17.2	55.2	27.6		15.3	49.1	35.6
19–24					3.4	3.5	43.1	50.0
25–39					7.1	28.6	64.3	

exceed 18μ. The percentage of the population that would be expected to have glomerular radii above 18μ may be calculated from the mean and standard deviation of a cross-section of the population and this percentage increases with age in a gompertzian manner (Figure 7.5).

When the population has encountered 40% mortality, about 60% of the population have glomeruli with radii of 18μ or more. A large proportion of animals in this particular strain appear to die of renal disease.

Another approach to this question has been made with brain cells. A cell suspension of the entire mouse brain was made by ultrasonic rupture of the organ and the neurons counted after fixing. This showed that a young mouse brain contained 5–6 million cells and, in extreme old age, where the survival of mice is less than 1% of the starting population, it decreased by about two thirds. The rate of loss of neurons increased throughout the life of the colony following a Gompertz function. Comparing the whole animal survival curve with that for neurons, it appears that mice and their neurons age at the same rate. The researchers put forward two possible interpretations of the data, depending on either the loss of critical cells, or critical networks. Taking the cellular viewpoint it is possible that the survival of a mouse depends upon certain vital functions, which in turn require the survival of 10–30 critical neurons. On the network idea the vital functions are compromised by the time 10 to 30 critical networks have lost 5% of their neurons, giving a mouse's chance of survival between 20% and 60%. This study is actually at variance with total analysis of brain DNA which in other work has failed to reveal any major change total brain cells in ageing mice. However, because of the different techniques used, it may be that the difference lies in the interpretation of the results from the isolated cell approach. For instance, it could be that brain cells become more fragile with age,

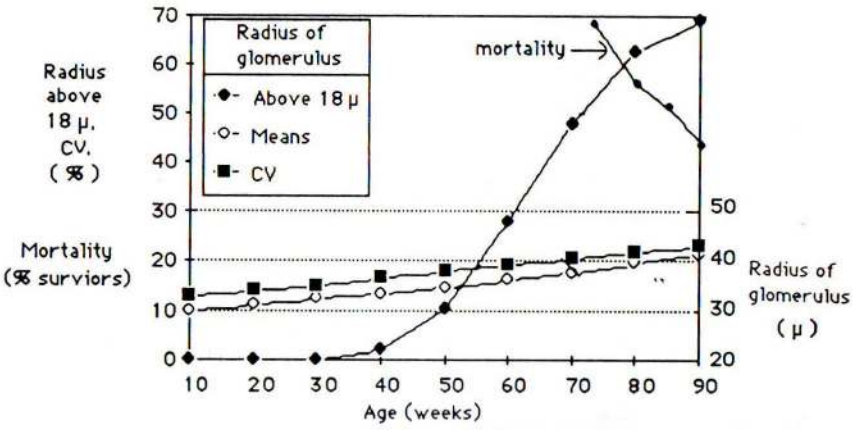


Figure 7.5. Age-related increases in the mean radius of rat kidney glomeruli, their variation and calculated percentages of radii above 18 microns

and an increasing proportion fail to survive the isolation procedure. Therefore gompertzian mortality could be a function of other events which also increase the fragility of neurons to ultrasonic energy.

It is important to remember that the exponential rise in mortality of fibroblasts is also a population phenomenon and that an exponential rise in a limiting factor is not necessary to explain it.

TURNOVER AND REGENERATION

The effect of age on cellular homeostasis expressed as turnover in renewing epithelia has been well researched. Much work has been carried out with models of the gut epithelium, particularly in mice, and skin epidermis, kidney cortical tubules, and tongue epithelium.

The general conclusions are that there is an age-dependent decline in the rate of cell proliferation in all cell populations, and the rate of rebirth in germinal layers slows down. The cell cycle lengthens with age in the oesophageal, gastric, duodenal and colonic epithelia. Changes in cellular proliferation are reflected in a fall in the number of proliferating cells in the stem cell compartment as well as in a slower rate of turnover of the entire epithelium (Table 7.5).

It was established in the early days of developmental biology that there is a fall in regenerative capacity with age. Not only the rate and extent of regeneration decline, but the quality of a regenerate may also change with age, usually becoming more variable in both form and function. In the invertebrate models, where this was first studied, the changes were connected with a decrease in the specific growth rate. In *Asellus* the relative rate of decline of regeneration with increasing body size (age) is at first greater for growth rate

Table 7.5. Turnover of basal cells in mouse oesophageal epithelium labelled with ^{3}H thymidine (individual animals and group means)

	Percentage of labelled cells			
	Infant	Young adult	Adult	Senescent
	12.7	8.5	5.7	2.5
	16.2	7.5	8.9	6.0
	10.7	6.7	5.5	7.6
	11.6	10.3	8.3	11.1
	14.9		8.2	6.4
	9.7		5.3	6.9
				6.9
Mean	12.6	8.3	7.0	6.6
Cell cycle (hr)	64	95	113	120

than for regeneration rate, and ultimately it is less. The specific regeneration rate, throughout, is about 10 times the normal growth rate (Figure 7.6). Abnormal regenerates in planarians are also associated with slow regeneration.

Effects of age on cellular activity are also apparent in the processes of wound healing. It is well known that even in great old age wounds heal in quite a satisfactory way; but the process of healing differs in some respects from that in young persons. Du Nouy, in 1936, on the basis of his experiments on animals, and observations on men, measured this process by a 'coefficient of the physiological repair activity'. He found the following values for this coefficient for man:

Age (years)	20	25	30	40	50	60
Coefficient	0.260	0.225	0.198	0.144	0.103	0.08

According to Du Nouy, the above-mentioned processes of wound healing, including presumably cell proliferation, decrease with ageing, being at 20 years of age about twice as great as at the age of 40; they were still weaker at the age of 50 and 60. However, the data relating to these two ages were only based on two and one patients respectively. Experimental confirmation of the relatively early decline in the speed of wound healing has since been obtained using rats; healing is significantly greater in young rats than in adults. The latent period between the time of infliction of the wound and that at which the healing growth of cells begins, increases with ageing, i.e. from 2 days in young rats, to 6 days in adults, and to 11 days in the oldest animals. The speed of retraction of experimental wounds also decreases with ageing. Other experimental

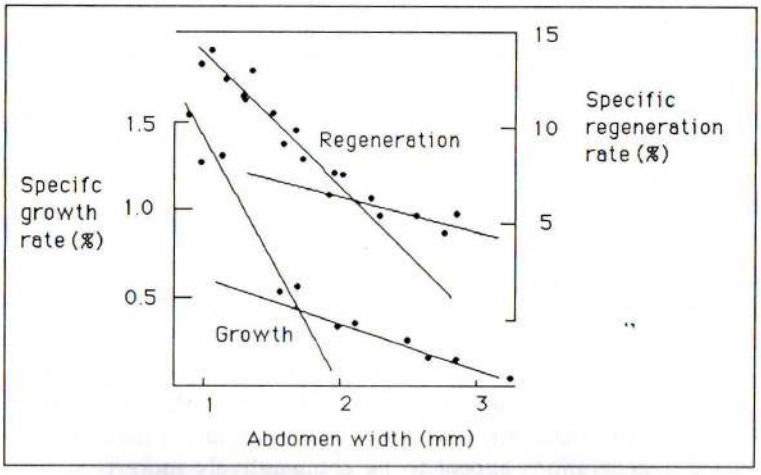


Figure 7.6. Relationship between growth rate and regeneration rate and body size in *Asellus aquaticus*

figures for humans indicate that on average a wound of 40 cm^2 heals in 76 days in a man of 40 years; 56 days in a man of 30 years and 40 days in a man of 20 years.

Attempts have been made to set up a simplified experimental healing system with fibroblasts, the dominant proliferating cells in whole body healing. This involved measuring the rate of regeneration in 'wounds' made in sheets of cells established on glass plates cultures. Cell division stops when all of the surface is covered by a monolayer of cells, a phenomenon termed contact inhibition. Healing of 'wounds' in the film was completed in 3 days in 8-day-old cultures; in 6 days in 19–25-day-old cultures, and in 8 days in 63-day-old cultures. Thus, the rate of regeneration of cells in wounds was inversely proportionate to age of tissue cultures.

Other work has indicated similar age changes in proliferative response in compensatory hypertrophy of organs. For example, after unilateral nephrectomy performed in rats aged 30, 60, 270, 360 and 540 days, the hypertrophy of the remaining kidney was 43.8, 34.6, 32.9, 23.0 and 22.8% respectively. After unilateral adrenalectomy, the compensatory hypertrophy of the remaining adrenal (as expressed by a hypertrophy quotient) was 35, 48, 28 and 20% in four groups of rats aged 1, 6.5, 11–17 and 20–22 months respectively. It is important to realize that these processes are not just the reflection of an increased mitotic activity.

Liver regeneration after experimental partial hepatectomy may be taken as an example of compensatory hypertrophy because it is the remaining liver mass which increases in size; the missing part is not rebuilt. The response, whether measured as the restoration of liver weight or DNA turnover (Figure 7.7), is slower in old rats. Furthermore, DNA metabolism triggered by the operation is not so intense at any stage during hypertrophy.

Both regeneration and wound healing are complex developmental processes, which take place in a well-defined sequence of stages. Also, in mammalian wound healing, the inflammatory response is involved, which calls into play very complicated cascades of cellular proliferation with many points of feedback control. In all of these respects the experimenter is dealing to a large extent with possible failures in homeostasis within the variety of tissues that take part. The results cannot be taken as a basis for generalizations about ageing of proliferative capacity.

From these cytological perspectives, atrophic and degenerative cellular changes are a major characteristic of ageing. In general it is possible to conclude that none of these regressive cellular changes are specific for old age only: they occur at all ages, and in various diseases. They vary greatly in the degree of their development between old individuals. Fibrous, atherosclerotic and pigmentary degenerations appear to be comparatively more typical for the processes of ageing. There are also degenerative changes in cellular organelles, namely in mitochondria and Golgi apparatus, and in the nuclei as well. There are alterations in the structural specifications of cell size which tends to increase.

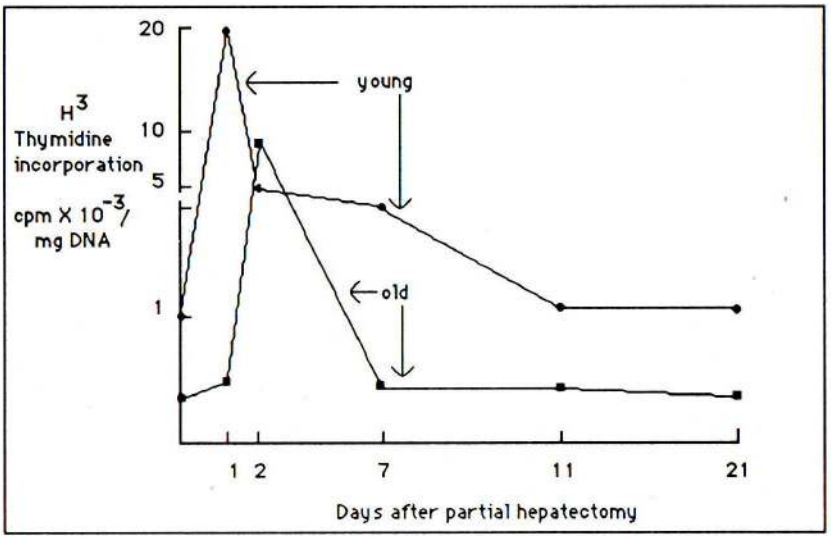


Figure 7.7. Turnover of rat liver DNA after partially hepatectomy

Cellular responsiveness decreases or slows down in old age as shown histologically, and in experiments on wound healing, regeneration and hypertrophic processes. Besides these regressive cellular changes which are present in old organisms, there are also 'progressive' processes, which involve the increased mobilization of resources for cell growth and division. These vary from normally occuring hypertrophy and hyperplasia of old cells to pathological metahyperplasias and finally tumours.

The mechanism by which renewing and renewable tissues respond to the ageing process is not known. In addition to possible fundamental intrinsic changes in stem cells, hormonal, nutritional, and vascular changes may be involved. Age dependency in the acceptance and growth of transplantable tumour cells (Table 7.6) has been ascribed to a possible wide range of physiological limitations in the host, such as failure to supply adequate capillaries and changes in immunocompetence.

Several of these different lines of investigation have come together through experiments on the development of the histolological picture of wound repair in rats. The microvasculature of the skin in old rats is characterized by staining positive to the periodic acid Schiff (PAS) procedure. The microvasculature of young rats is not PAS positive. After experimental skin wounding, histochemical ageing of the components of the microvasculature occurs rapidly in old rats. The freshly developed tissues are PAS negative, but during the next 8 weeks the microvasculature becomes increasingly PAS positive and eventually takes on similar staining characteristics to that of uninjured rat skin. In the main, PAS identifies glycoproteins and glycolipids, which in the old rat appear to be

Table 7.6. Growth rate of Walker 256 tumour cells injected into rats

Age at injection (days)	Number of animals	Mean tumour wt (g)
35	76	2.91
63	51	2.37
144	66	1.54
297	56	1.27
503	58	1.57

associated with arterioles, capillaries and venules. The reaction occurs in the arteriolar walls, and connective tissue coating the adventitia of the non-muscular venules. These structures develop in actutely wounded young rats but remain PAS negative. Although this model raises questions about it being a demonstration of accelerated ageing, it simply indicates that cellular homeostasis in old rats has become set to produce a new histological norm.

THE RED CELL MODEL

The human red cell has a finite lifespan and is normally removed from circulation after it has been in the blood for about 120 days. During any given period of time a certain number of cells are destroyed. The evidence indicates that the ones destroyed are those that have reached a certain level of biochemical deterioration. It is reasonable to suppose that the biochemical and biophysical changes concerned with ageing ultimately determine the removal of the cell from circulation and its destruction. There are three areas of investigation relating to ageing of red cells:

1. The structural components.
2. The chemical composition.
3. The energy forces.
4. The functional components.

Red Cell Ageing in vivo

Experiments on mice indicate that red-cell turnover increases with age with a rise in the number of circulating cells. Middle-aged rats have a higher mass of red blood cells than animals younger or older. Area measurements show an age-related decrease in cell size. Small but significant differences in red-cell homeostasis also occur in human subjects which affect the norms for morphology, mean corpuscular volume, and haemoglobin content (Table 7.7).

Table 7.7. Changes in human blood with age

Age	Cell volume[1]	Diameter[2]	Haemoglobin[3]	Haematocrit[4]	Viscosity[5]	Count[6]
80	88	7.8	13.7	38.5	12.0	4.6
21	87	7.7	14.2	37.8	9.9	4.8

[1]Corpuscular vol; [2]microns; [3]g/100ml; [4]%; [5]cp; [6]mill/mm[3]

These differences are related to changes in blood viscosity. In particular as age increases the deformability of the red cells decreases.

Any peripheral blood sample contains a mixture of cells of all ages and a technique is necessary for identifying and separating the cells into appropriate age groups Most of the published work uses a technique for ageing cells which depends upon the fact that when blood is sedimented the reticulocytes are preferentially distributed at the top of the cell column. As the reticulocyte is a young cell its position is indicative of cells newly formed and released into the circulation. The lower sedimentation rate of reticulocytes is due to their greater water content and consequent lower density. The remaining cells sediment in the column roughly according to age with the oldest found at the base. These are the most dense cells. One of the ways of separating these cells according to age is to rely on their differences in density. Centrifugation of a mixture of cells through non-water miscible mixtures of different specific gravities has been used in order to provide samples for analysis. By means of injection of radioactive iron into donor animals a limited population of new cells can be labelled and followed as an identifiable cohort through the cohort's entire lifespan. When this approach is combined with sedimentation of samples taken at various times after administration of the isotope, radioactivity first appears in the top layer of cells and then progressively descends the column, as the labelled cohort ages.

Young cells from the top of the sedimented column are more resistant to osmotic lysis than the older cells at the bottom, and if cells labelled with radioactive iron are subjected to different hypotonic solutions, the amount of radioactive haemoglobin liberated by cell lysis reflects the age of the cells destroyed. Serial studies by this technique indicate that red cells become progressively more susceptible to osmotic lysis during their lifespan. Age changes in the structure of the red cell are directly related to a decrease in surface area. Chemical analysis indicates less lipid in old cells and structural changes may also be inferred from the different ionic compositions and the behaviour in mechanical fragility tests. There are well-defined alterations in cell energetics, the rate of glycolysis decreasing progressively with increasing age. There also appears to be a lower level of active transport in older cells with a fall

in the steady state ratio of potassium to sodium. There is a decrease in the ratio of ATP to ADP, which correlates with a decreased survival of ATP in deficient cells as they age, both *in vivo* and *in vitro*. This relationship between age and enzymic activity has been sufficiently developed to enable investigators to estimate the mean age of a red-cell population on the basis of certain enzyme activities. There also appears to be an increased concentration of methaemoglobin in older cells. The increase being appreciable at about 50 days of age and steadily increasing afterwards. Alongside this a number of studies have demonstrated that the ability to reduce methaemoglobin to haemoglobin is a function of cell age. The capacity of old erythrocytes is less than in young or mature cells. The same decay curve is found for methaemoglobin reduction as for red-cell survival. The fall off in the reductive capacity of the cell may be explained by diminished glucose utilization with a resultant decrease in the generation of reduced hydrogen donors. The higher oxygen saturation in older cells (increased affinity) has been described to macromolecular changes within the cell and possibly to alterations in the structure of the haemoglobin molecule that progressed with age. Increased amounts of haemoglobin A_3 have been described in older cells. One of the component parts of this haemoglobin contains haemoglobin A with one of two sulphydryl groups of each beta chain blocked by a glutathione residue. It is also possible that the the increased affinity of haemoglobin for oxygen in older cells is dependent on the intracellular concentration of triosephosphate which decreases as red cells age.

In summary, it appears that largely through chemical ageing and consequent loss of metabolic capabilities the human red cell is unable to maintain the necessary cofactors to protect haemoglobin from changes leading to diminished function. These, or similar changes, may allow alteration in the cell membrane that eventually lead to destruction of the cell. Old red cells are more susceptible to haemolysis by immune antibodies. In addition there may be an accumulation of metabolites inhibitory to the metabolic activity of the cell. Although the red cell undergoes a process of ageing, and it seems probable that its final destruction is the result of this ageing process, there is no direct evidence to establish this as fact. It may well be that the changes noted so far are mechanisms designed to hold off final destruction as long as possible.

In addition to the segregation of cells in terms of their density, osmotic fragility may be used in order to destroy the older cells preferentially and the products of lysis from the younger cohorts may be analysed. Another method that has been used in experimental animals is to produce a population of very young cells by repeated drastic bleedings or *in vivo* haemolysis, however, the results from such methods are likely to differ from those derived from *in vitro* methods because the youngest cells are dominated by reticulocytes which differ considerably in total organization from the mature cells.

Young reticulocytes migrate more slowly under the influence of an electric field than the red cells. There is a reduced mobility of old cells taken from the

bottom of a centrifuged blood sample which is confirmed by separating out 10–15% of the youngest and oldest cells by differential flotation. Older cells loose their negative charge and the rate of agglutination by polyglycine also increases with age, which correlates with a lower electrophoretic mobility. This indicates that there is a correlation between the rate of agglutination and an age-dependent loss of surface charge from the cells. The surface charge serves to maintain red cells in dispersion, and any reduction of the net negative charge will favour red-cell aggregation. The bulk of the surface charge in human red cells is due to ionized sialic acid, which appears to be located on exterior glycoproteins and contains virus receptor sites and several antigens. When the surface charge of human, rat and rabbit erythrocytes is reduced by removing sialic acid enzymatically, reduction in sialic acid correlates with decreases in electrophoretic mobility and loss of PAS staining of membrane proteins. No changes in ATP levels or deformability result from this treatment. The survival rates of these cells in rats and rabbits is very much reduced indicating that there is a mechanism of red cell destruction which operates through a fall in the sialic acid content of the membrane in old cells.

Red Cell Ageing in vitro

Blood transfusions are given either to restore blood volume or to supply viable red cells, and for both purposes stored blood can as a rule effectively replace fresh blood. Cells stored under the best conditions in an acid citrate, dextrose preservative at between 4–7° C undergo morphological and biochemical alterations. They become more spherical with an associated increase in osmotic and mechanical fragilities. The glycolytic rate drops and organic phosphate compounds decline with the release of inorganic phosphate. There is also a fall in cell potassium and a rise in intracellular sodium. These altered cells when transfused to a recipient are destroyed in an amount dependent upon the duration of storage of the blood. Storage for three weeks results in a fall of 15% in the viability, and at four weeks there is a 40% drop in viability. Since the normal human red cell population is destroyed in the body at a rate of about l% per day the lowered survival of blood cells after 3–4 weeks storage is consistent with the expected *in vivo* losses from ageing. This indicates that the ageing of red cells that occurs in the body continues when the blood is stored. However, many of the changes in stored red cells are different from those in the body. For example, in stored red cells, ATP diminishes rapidly with time whereas in the body this change occurs very slowly. There is also evidence that during storage, young red cells deteriorate more rapidly than old red cells. Dog red cells with an average age of five days were stored for 20 days and transfused. Twenty-four hours later only 10% were left in the recipient's circulation, whereas cells with an average age of 60 days were found to have a 90% survival

24 hours after transfusion. Substances which retard the rate of deterioration of stored blood do not necessarily prolong the viability of red cells; also the osmotic fragility which increases in stored cells is affected by many factors which do not have any influence on viability after transfusion. In addition cells stored under conditions where they would become swollen are very fragile but as judged by transfusion criteria are very well preserved. This discrepancy between the changes occurring in cells on storage and those ageing in the body is not surprising when it is considered that the metabolic conditions on storage are greatly different from those in the body. The low temperature ensures that much less metabolic energy is available so that those processes that depend upon a steady supply of ATP may degenerate irreversibly. So far the techniques of blood preservation have been designed simply from the point of view of obtaining the largest number of surviving cells in relation to the longest possible storage time. In order to study red-cell ageing *in vitro* as a continuation of the process in the body it is necessary to duplicate as far as possible the conditions obtained during circulation, that is to have a high temperature, supply a good oxygenation and a steady supply of blood glucose.

INSECT MODELS OF CELLULAR AGEING

The fact that the cellular organization of all organisms is similar provides the logic for the view that the study of simpler animals may provide an understanding of how the human body functions. Many invertebrates, such as adult rotifers, nematodes and insects are examples of organisms composed almost entirely of fixed postmitotic cells. This had led to their use as models for the ageing of postmitotic cells. A particular advantage in using invertebrate animals as gerontological models is exemplified by the relatively lower costs in space, labour and time. For example, keeping 1000 fruit flies until they reach old age in about 2 months, is much cheaper than maintaining the same number of mice until the last one dies of old age after 3 years, particularly if the questions about ageing can be answered just as well using fruit flies. Generally invertebrates have been particularly useful models in exploring the relationship between genetics and lifespan, parental age and lifespan, and temperature and lifespan.

Insects provide a great, but as yet unrealized potential as models for the study of the ageing of cells in organs as opposed to systems. One of the advantages is the experimental freedom in terms of organ transplantation for separating intrinsic and extrinsic components of ageing. Also, organ atrophy is a feature of insect ageing and it closely resembles that in vertebrates, being associated with identical cellular and subcellular changes. Throughout life, insects lose cells from all postmitotic tissues because of random or stochastic damage.

There are no large-scale, age-related decreases in dry weight and protein

content of the brain of the blowfly, *Calliphora*, during the whole lifespan. However, intercellular changes involve an increase in autophagic vacuoles, loss of ribosomes, the appearance of 'giant mitochondria' and lipofuscin-like material, as well as condensed nuclear chromatin. There is possibly a causal relationship between some of these changes and metabolic rate.

The study of nuclear changes with age has been concerned with the structural and functional properties of chromatin and to a lesser extent with total DNA content. The various studies concerned with DNA content have failed to show significant change with age. However, many studies at the ultrastuctural, histochemical and biochemical levels have indicated significant age-related changes in chromatin structure and function. These changes are similar to those known to be associated with the conversion of active chromatin to an inactive state. Analysis of interphase chromatin condensation patterns has shown a significant increase in the amount and distribution of condensed chromatin in a variety of housefly cell types and has indicated a non-uniformity in the rate at which these cell types age.

The gradual physiological decline of those insects that feed as adults is comparable to that seen in mammals. Therefore, even though insects do not die from the specific age-related degenerative diseases of mammals, it is reasonable to use insects as human models. For example, ageing of *Drosophila* is accompanied by organ atrophy and a number of general cellular and subcellular changes, which include the following; glycogen loss, accumulation of neutral fat and agepigment, and a decrease in the number of ribosomes and mitochondria. These age-related changes are strikingly similar to those occurring in the fixed postmitotic cells of mammals, such as neurons, which suggest that the fundamental mechanisms of ageing may be similar in all metazoans. Thus, although there are no large-scale, age-related decreases in dry weight and protein content of the brain of the blow fly during the whole lifespan, intraneuronal alterations seem to be of crucial importance during the ageing process. Structural changes involve an increase in autophagic vacuoles, loss of ribosomes, the appearance of 'giant mitochondria' and lipofuscin-like material, as well as condensed nuclear chromatin.

THE FUNGAL MODEL

It is generally accepted that four kinds of morphological phenomena arise in the hyphae during development of fungal cultures which have been taken as models of ageing in higher organisms. Three of the changes are determined by the position of the particular structures of the hyphae and governed by the events that have taken place since the culture was initiated. The fourth type of change occurs in the growth and reproductive characteristics of the entire fungus from generation to generation.

Positional Effects

As the fungus develops, growth takes place by a steady extension of the hyphae. At the same time there is an increase in size of the meristematic apex from its position as a small outgrowth of a spore to its final position at the margin of a large colony. Apical changes are manifest mainly when the initial rise in growth rate declines and there is an eventual cessation of size increase. The terminal changes are not necessarily degenerative and are reversible in that excision of a part of the margin of a colony, with or without its transfer to a fresh medium results in a reduction in size and a regeneration of its apices. From this point of view the phenomenon is of questionable value as a general model for ageing. However, when approaching another colony or reaching the physical limits of the medium; the tip region may show degenerative changes that are also a feature of the development of the regions of the hyphae behind the advancing apex. These latter changes are described as compartment ageing, and are to a certain extent analogous to sequential senescence in higher plants.

Compartment Ageing

In higher fungi, as the apex moves forwards, incomplete transverse septa are produced, which indicate cytoplasmic compartmentation of the hyphae. For those species where septa are not produced it is still evident that there is a regional differentiation along the length of the hypha. Thus, there is a well-defined sequence of cytological changes, each phase occurring when the region is in a fixed position relative to the apex. Mitochondria become shorter, small vacuoles appear which enlarge to fuse together and lateral branches may be produced (Figure 7.8). Within each compartment there is a progressive increase in the concentration in the matrix of the cytoplasm concurrent with a progressive increase in the vacuolar volume.

Area Ageing

This term refers to the sequence of gross morphological developments with time of different hyphae within a given area. An area observed from its initiation shows branch production, a narrowing of hyphal diameter, the appearance of hyphae lacking in definite growth orientation, pigment production, the formation of reproductive structures and autolysis and death of hyphae. Area ageing is apparently a manifestation of ageing of the organism as a biological unit.

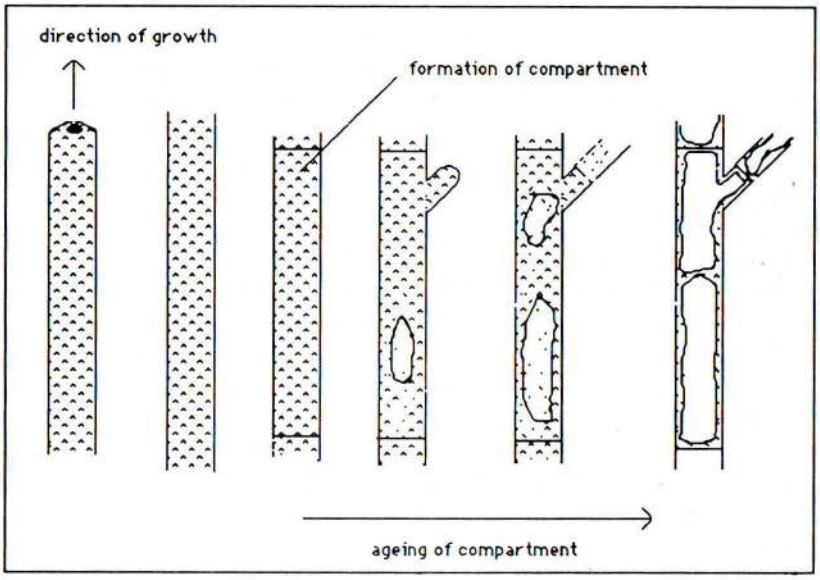


Figure 7.8. Compartment ageing in fungi

Clonal Ageing

Characteristics of a fungus change from generation to generation. Growth rate, pigmentation, antibiotic production and ability to produce asexual spores all show an identical cyclic pattern in successive cultures. This type of temporal change is due to alterations in nuclear constitution or in hereditary extranuclear structures resulting mainly from selection during subculture. This ageing may or may not be reversible.

As already mentioned many of the developmental changes are reversible by changing the immediate environment of the hyphae. The phenomenon of vacuolation appears to be due to the effects of a substance produced by the fungus. This vacuolation factor, as it is termed, may be used to initiate vacuolation in young fungal apices giving a sequence of cytological changes the same as that in natural development.

THE PROTOZOAN MODEL

Protozoa have been used as models of cellular ageing because they are single living cells and, unlike multicellular organisms, they are free from complicating influences of other cells and organs, and can be manipulated in test tubes like

micro-organisms. In ciliates and other protozoans the haplophase is short and the diplophase long, as in the animal kingdom generally. Populations of rapidly multiplying, so-called vegetative individuals consist of diploid organisms. Division is by fission, involving division of the ciliate macronucleus and micronucleus, which gives rise to further diploid individuals. When ciliates are cultured by fission on a large scale, the character of a population, as measured by the mean properties of all phenotypes present at any one time, will characteristically go through a series of temporal changes akin to sexual maturation followed by ageing. Clones derived from single organisms have therefore been likened to multicellular organisms. In the laboratory a particular clone will eventually die out after its component organisms have passed through the fission cycle a certain number of times. New clones may arise from a clone that is undergoing clonal ageing if sexual reproduction, i.e. conjugation or autogamy, takes place.

Conjugation (Figure 7.9) is triggered by environmental changes which stimulate two individuals to come together with the occurrence of cytoplasmic fusion. Normal conditions for conjugation may include alternating periods of darkness and light, exposure to specific substances formed by the cells of complementary types, and direct contacts with individuals of another mating type. The events of autogamy involve the formation and fusion within a single organism of haploid male and female micronuclei involving meiosis. In conjugation, haploid micronuclei are reciprocally exchanged between two organisms and a fresh nucleus is formed in each mate. Such individuals serve the same function as gametes even though they represent the diplophase of the life cycle. Once fusion or conjugation is complete, in *Paramecium* for example, the large macronuclei disappear and the micronucleus of each member of a pair undergoes several divisions, one of which is a reduction or meiotic division. Following this there is a mutual exchange of one haploid nucleus between partners to fuse with the one that remains behind, after which the paired organism subdivides into a number of daughter organisms each equipped with a diploid nucleus of double parentage, from which the macronucleus and micronucleus of each develops. The members of mating pairs of *Paramecia* look alike although there are significant biochemical differences, and mating typically occurs between individuals of different pedigrees.

Age is measured either by the number of fissions already undergone or by actual time that has elapsed since the clone was started, providing it has been maintained under a regular sub-culturing regime. Individuals that arise from autogamy or conjugation are the starting point for new invigorated clones, which, in turn, age if they are kept in an excess of standard medium, under constant environmental conditions (Figure 7.10). Clones exhibiting the life-cycle phenomenon can only be obtained by frequently subculturing them in order to obtain maximum fission rates.

A clone dividing by binary fission changes with respect to several

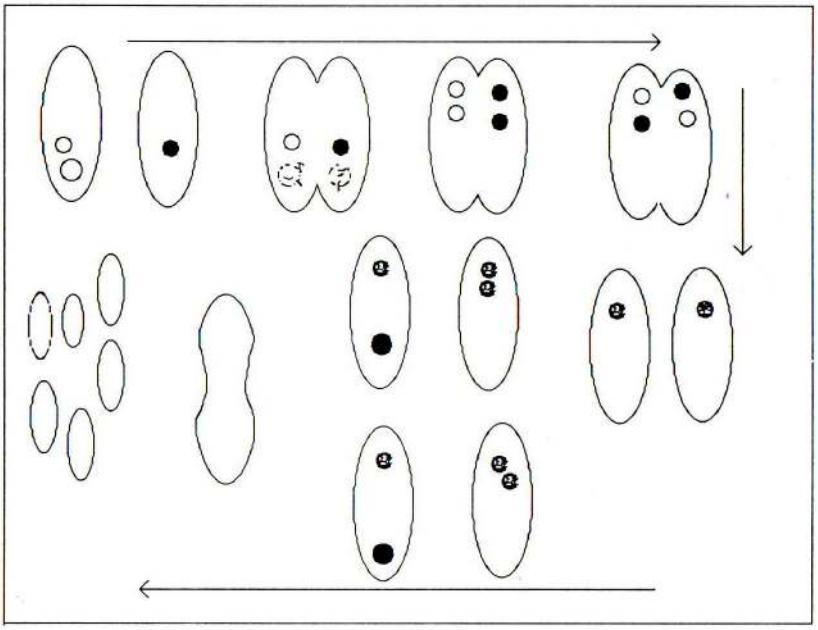


Figure 7.9. Conjugation in *Paramecium*. Beginning at the upper left: two individuals unite lengthwise. In each the micronucleus divides and the macronucleus disappears. After further division of the micronuclei all but two on each side also disappear. Of the two remaining micronuclei on each side, one passes to the other side and fuses with the micronucleus of that side to form a zygote nucleus. Separation of the two sides occurs and individuals are formed containing two micronuclei, one micronucleus remaining as such and the other becoming the macronucleus

parameters. These changes may be conveniently investigated by observing the organisms that remain behind after each subculturing and which are left to grow on in an undisturbed environment. Through this procedure unique cell phenotypes are revealed in these 'leftover' cultures, distinct from those in earlier or later cultures. There is also a fall in the viability of the clone since each leftover culture is less able to form new clones by sexual processes. Also, phenotypic changes occur directly in the mainstream culture. In particular, certain phenotypes persist for a given number of divisions then they are irreversibly lost and new phenotypes appear in a predictable sequence. These changes are not due to the environment becoming impoverished.

When leftover cultures begin to exhaust the available food supply they enter a condition which permits either conjugation or autogamy providing a certain period has elapsed from the origin of the subcultures. Up to about 35 divisions, populations which are allowed to deplete their food, do not conjugate if given the opportunity, nor do they undergo autogamy. Samples removed from the

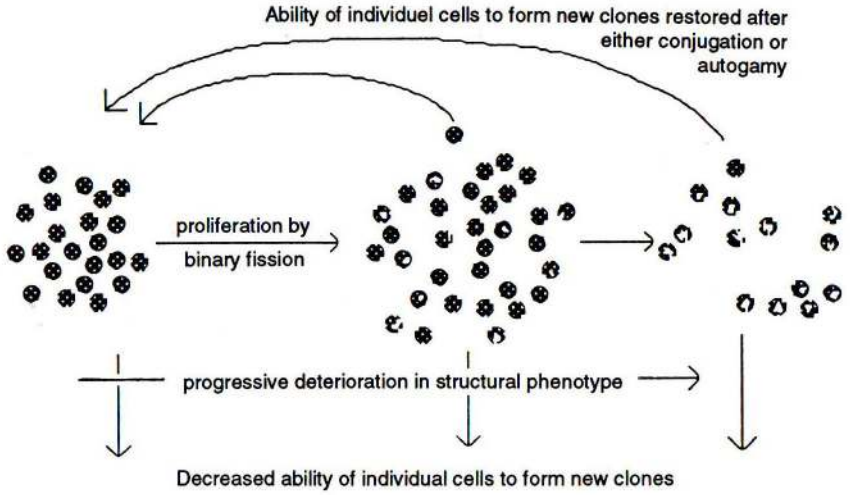


Figure 7.10. Diagrammatic representation of fixed lifespan in cultures of *Paramecium* reproducing by binary fission and the effects of inducing autogamy and conjugation

same clones later consist of cells which conjugate but do not undergo autogamy. After a further transition period, starvation induces autogamy alone.

The three different responses to starvation in leftover populations, namely no sexual reproduction followed by conjugation, then autogamy, indicate successive stages of maturation, maturity and ageing; a midpoint comes at about 70–100 divisions.

Progeny of sexual reproduction may also be used to chart the course of ageing. Products of autogamy, ex-autogamonts, produced late from the mainstream culture develop in three ways; yielding fully viable clones which begin new life-cycles; clones which are either non-viable or divide at reduced rates followed by early death; clones whose fission rates are intermediate between these two extremes. The probability of obtaining a viable clone at a given autogamy is closely correlated with the age of the clone. At, or just after clonal maturity, nuclear reorganization usually results in the production of viable clones, whereas later the process yields progeny which are usually non-viable. In extreme clonal old age new clones always die off or cannot be formed at all. This definition of the life-cycle appears to be a general rule among ciliates.

The 'immortality' of ciliates is connected with the fact that the outcome of sexual reproduction is a pair of rejuvenated young cells, instead of a mother and daughter cell of different ages. However, this very fact sets any clonal model apart from general postmitotic ageing in the higher metazoa because the individual life of a protozoan comes to an end at the moment of its division into two new cells; furthermore this occurs without any detectable cytoplasmic

ageing from the time of its previous division.

Age-related changes in gene expression occur at all levels in ageing clones. In *Tokophrya infusorium*, the number of tentacles in individuals of old clones is 16 or less, compared with about 60 in the founder members. The 'young' organism is able to produce a new cell every 2–4 hours, while 'old' ones lose this function completely. The macronucleus changes from a spherical shape to an irregular body of larger size, in old organisms. Instead of only one macronucleus in young *Tokophrya* there are from 2 to 4 in old individuals. Chromatin bodies consist of a dense network of many fine filaments in young *Tokophrya*, but in old organisms a cavity appears in the body and the filaments become arranged in parallel series. The matrix, in which chromatin bodies are suspended, is homogeneous in young and is fibrous in old organisms. The number of chromatin bodies increases with advancing age.

Because clonal death is such a major expression of cultures, the protozoan model has been used to support the concept that cell death of age involution is not a consequence of multicellularity and that clonal death is the ultimate fate of all unit cells because of the fundamental way in which genetic information is used to regulate biochemical processes that are common to all living things. Ageing, as found in cell systems of higher metazoa, is evidenced by a slowing down of fission rate, decreased general activity, and intracellular degenerative changes in the organelles and cell body.

However, in protozoa, at any point in the path to clonal 'death' the process of ageing may be arrested and individuals returned to a juvenile state by changing the physical conditions of the culture to stimulate sexual reproduction by either autogamy or conjugation. This result has been interpreted as the unmasking through the chromatin rearrangements of sexual reproduction of error correcting codes. On this model, the cell lines arising in cultures of mammalian fibroblasts may result from the activation of a similar type of code. The main contribution of the protozoan model to gerontology, mainly due to the elegant experiments of Sonneborn, has been towards an understanding of clonal ageing on the basis that the finite lifetime of a whole clone may not only be a model for ageing in the whole organism, but indeed, might be the same phenomenon reduced to a lesser degree of complexity.

There is still much that we do not know about the life-cycle strategy of ciliates. Although many clones ultimately die if they do not undergo some form of sexual reproduction, some clones of protozoa apparently do reproduce asexually and indefinitely. In this connection, the prolongation of the life of populations of *Paramecium bursaria* by conjugation varies greatly, even when the conjugants are young. Also, a high proportion of ex-conjugants normally die, and the death rate is highest in those clones that are more closely related. Fifty-three per cent of ex-conjugants die before undergoing five cell divisions and 30% die without dividing at all. Conjugation may produce non-viable clones, clones of limited survival, and some vigorous clones apparently capable

of unlimited asexual reproduction. It is suggested that it is from these latter clones that laboratory cultures are normally obtained.

Clones that do not mate can be said to have a laboratory life-cycle. Is this analogous to that of the soma of the wild mouse brought into captivity? Fundamentally, the answer is probably yes. Sexual reproduction appears to be essential for wild type protozoa to survive. Since survival simply requires the rearrangement of genetic DNA from two individuals, the evolution of conjugation may be regarded as an adaptive response to cellular ageing, which eventually shuts off information from working genes. It has been suggested that this is due to the accumulation of mutations, which evidently do occur because they are revealed by selection from old clones that have been 'rescued' by conjugation. Cells are crossed from young clones to old clones and marker genes reveal that old cells accumulate many dominant and recessive detrimental and lethal mutations. These micronuclear mutations appear abruptly in the fission life history and are not simply accumulated throughout the entire life-cycle. They are first noticed after only 80 fissions and by 220 fissions all of the progeny of clones are non-viable. Under circumstances which permit cells to undergo periodic nuclear reorganizations no increase in detrimental or lethal mutations occurred in one culture maintained for 1250 cell doublings.

Other relevant work on the protozoan model involves nuclear transplantation in amoeba. Clones of amoeba can be stabilized under certain conditions in a non-dividing state, with the complete arrest of protein synthesis for long periods. Food supply seems to be an important factor. Amoebae will multiply indefinitely if kept on a food supply permitting logarithmic vegetative multiplication but, if kept on a limited food supply, then transferred to the optimum diet, they have a variable lifespan. This span of from 30 days to 30 weeks is dependent upon the conditions of exposure to the deficient diet. Division may be induced in these stable clones, which either causes them to exhibit stem-cell behaviour, i.e. they begin to divide with one daughter cell of each binary fission dying without further doubling, or, after a fixed number of divisions the entire clone dies.

Microtransplantation of a nucleus from a clone showing stem-cell behaviour will induce the same behaviour in an individual from a stable clone. Also, the transfer of a small amount of cytoplasm from an individual in a clone with limited lifespan (a spanned clone) confers limited division capability to an individual in a stable clone. These results, in outline, conform closely to other observations on fungi, which show that senescence, which is a normal part of the life cycle in fungi, is preceded by the appearance in the cytoplasm of a material which can transmit premature senescence to young hyphae.

Clonal ageing of ciliate cultures has similarities of fixed lifespan fibroblast cultures, which are discussed in the next section; cultures die out after a characteristic time; it gradually becomes difficult to make subcultures from individual cells of the main culture; the failure of asexual reproduction is

associated with an increased frequency of mis-specified surface and intracellular structures, notably malformed nuclei.

The apparent importance of change in the micronucleus, the appearance of mutations, and the effects of transplanting nuclei all point to genes as a major site of clonal ageing. The fundamental change may be the loss of DNA homeostasis at the level of specific genes, mutations may therefore be an effect and not a cause of clonal ageing. On the other hand, the main effect could be through the loss of mitotic regulation because morphological abnormalities and cell death may arise at any stage of life but have a much higher frequency during the latter stages of clonal ageing when the division rate is much depressed, i.e. there is more chance of things going wrong between mitoses. These difficulties of interpretation show the futility of hypothesizing with respect to our very limited understanding of where the ultimate regulatory mechanisms of cellular homeostasis reside.

SEEDS

Presumably the different seed longevities of various plant species represent adaptations to particular soil conditions, and the different productive mechanisms of seeds are part of particular evolved strategies. Dormancy of seeds has been shown to be heritable, and selection can change the dormancy properties of a population. According to the longevity of its seeds, a single parent is represented in quite distinct generations of decendants, and from this aspect, buried populations of long-lived seed are a memory, both of past genotypes and of past vegetation. In an ecological perspective seed longevity is part of an anti-ageing strategy whereby some of the potential rate of increase of population size has been sacrificed to maintain a potential for growth, by delayed germination, which may not be realized. This can be an advantage in a hazardous environment when synchronous germination would place the whole population at risk. On the other hand most survivorship curves of species with short-lived seeds have a constant mortality risk within the population as a whole, implying that variation between years in environmental stresses are relatively unimportant. These aspects have played a role in the reproductive strategies of plants.

In archaeological field-work, radiocarbon dating techniques have been used to age viable seeds of the lotus, obtained from soil samples at 1040 $\pm$ 210 years. Similar techniques have shown that the seeds of some common weeds may remain viable in the soil for 1600 years. The optimum conditions for longevity appear to be slightly to moderately moist soil deficient in oxygen. Generally, aquatic species, temperate tree species with nut seeds, and tropical tree species from primary forest have short-lived seeds. Even with artificial storage the longevity of seeds of many tropical plants is measured in weeks or

months. The class of seeds living more than 10 years contains grasses, sedges, rushes and many herbaceous weedy species.

Experimentally, seeds have value as models for studying cellular homeostasis because they have the following unique features:

1. No cell division occurs during storage so that if deviant cells arise they are not selected out.
2. Cell division may be easily initiated at any time.
3. The availability of self-pollinating species makes it easy to obtain homozygous material for genetic studies and so test for mutations arising in storage.
4. Species are available with small numbers of large chromosomes so that nuclear changes may be detected on cell division.
5. Seeds are easily subjected to different experimental environments during storage.
6. Large numbers of seeds are readily obtainable for experiment.
7. Conditions of storage may be arranged so that accidental deaths may be avoided.

Many observations on seed longevity have been made with artificially, rather than with naturally stored materials. Two long experimental studies have been made involving the deliberate burial of seed. In 1897, seed of 23 different species was buried in inverted bottles of sand in a field at a depth of 54 cm. Samples have been taken from these stores at intervals of time after the start of the experiment. The species included two trees, a biennial and two perennials. The reminder were all annuals. Some seed of the trees and five of the herbaceous species died out in less than five years, three species remained viable after 80 years and only one germinated after 90 years.

It is a characteristic of all samples of seeds investigated that the percentage of viable seeds falls with age, and this is correlated with a gradual rise in phenotypic variability in plants obtained on germination, particularly with regard to the frequency of mutant characters. Indeed, chromosome abnormalities are readily detected by histological methods in the roots of several species of plants grown from old seeds. Although the proportion of abnormalities increases with temperature and moisture content during storage, there is little doubt that the changes are brought about by intrinsic processes in the seeds. The interplay between the intrinsic process and environmental variables makes it possible to alter the rate of seed ageing and examine the seeds for viability and corresponding changes in the frequency of chromosome aberrations. From such experiments these two parameters are highly correlated.

The cytological evidence is in favour of seed ageing being primarily one of chromosome breakage, which takes place entirely in the interphase state. These nuclear aberrations appear in seeds, and also become detectable under the

microscope when the cells of the embryo begin to divide on germination. Thus it may be postulated that the death of the embryo is the result of the accumulation of cellular damage associated with chromosome breaks. On this idea, for any given percentage seed survival, the mean frequency of aberrant cells in the survivors would be constant irrespective of how rapidly viability declined, and would be independent of the storage conditions. On the whole this is observed except that for the most severe environmental conditions when the chromosome aberrations are less than would be anticipated.

Although chromosome damage is a reliable index of ageing in seeds it is unlikely to be a causal factor in seed deterioration. This is because cells with damaged chromosomes are eliminated during early growth, for example in shoot and root tips, where it is unlikely that even marginally affected cells cannot compete with normal cells. The elimination of aberrant cells in this way throws doubt on the general importance of somatic mutations in dividing cell populations being a cause of ageing generally. Cytological damage is likely to be an extreme indication of generalized damage to chromosomes. In this respect, quite minor alterations in nucleic acids could lead to complete disorganization of cellular function. Selection pressure does not eliminate minor aberrations of this type, which behave as recessive mutants and can be easily detected by breeding experiments.

Explanations put forward to explain the loss of viability of seeds fall into two classes:

1. Storage results in gradual accumulation of mutagens within the seed.
2. Cells in seeds encounter random events which result in chemical ageing of macromolecules.

Experimental tests for the mutation hypothesis have involved testing seed extracts for mutagenic activity on various dividing systems. Results have not been conclusive possibly due either to the lack of specificity in the test situation or to a lack of sensitivity to the particular mutagens.

Random molecular accidents would involve a constant rate of appearance of chromosome damage with time, and may be distinguished from the accumulation of mutagen where the rate of chromosome damage should increase with time. The fact that the number of viable seeds that can be tested for chromosome damage gradually declines through ageing means that it is not possible in practice to ensure the necessary mathematical precision required to distinguish between the two processes where the terminal experimental cohorts are a small fraction of the phenotypes originally available.

Whatever kind of damage occurs on storing seeds it appears to be repairable, providing the environmental conditions are adequate to support aerobic metabolism. Like all seeds, the longevity of lettuce is markedly reduced by increasing the storage temperature under anaerobic conditions (Figure 7.11).

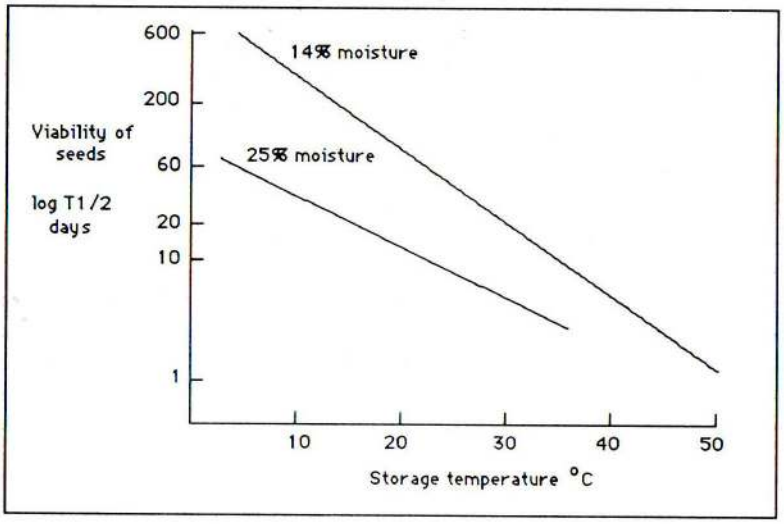


Figure 7.11. Viability of lettuce seeds in relation to storage temperature

The effect of temperature is more marked at relatively low moisture content. This suggests that some protective process is activated or maintained by hydration. Further, at a given temperature, a much higher longevity is maintained under aerobic conditions than under anaerobic conditions (Figure 7.12). These phenomena have been interpreted as meaning the subcellular damage accumulates with increasing moisture contents above 15%, but at this moisture content the above repair mechanisms are sustained by aerobic metabolism. In the experiments maximum repair is obtained at 25 °C in aerobic conditions at 45% moisture. The high moisture content presumably provides for optimum hydration of the cellular system involved.

AGEING OF EGGS AND SPERM

Throughout the living world it appears that ageing results in a decline in fertility. For laboratory rodents this is a well-defined feature of serial reproduction in terms of litters and viable offspring. The ageing of eggs in the ovary makes a contribution to this and in rabbits and hamsters it is concluded that eggs obtained from old mothers have undergone ageing in that very few develop following fertilization and transfer to the uterus of young mothers. In line with this, unfertilized eggs transferred from old rabbits shortly after ovulation and fertilized in young females show a two to sevenfold increase in cleavage failures. The primary defect in old eggs probably results from the ageing of ova in the ovary. This increases the chances of non-viable eggs being

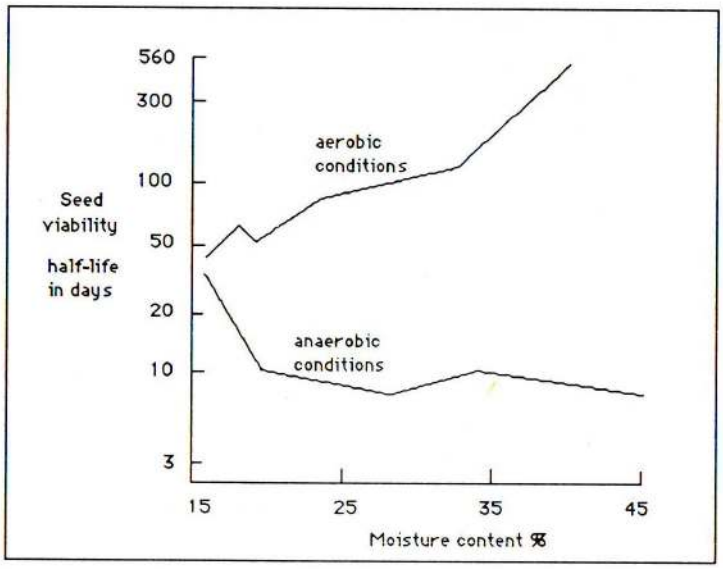
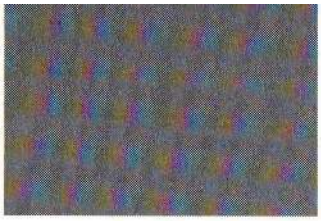


Figure 7.12. Viability of lettuce seeds kept at 25° under aerobic or anaerobic conditions

ovulated as the mother ages. It also results in the maternal age effects appearing in the phenotype of the offspring arising from 'bad' but fertile eggs, that appear more frequently in older females. The best-known example in the human female is Down's syndrome, or mongolism which is due to trisomy at chromosome 21. The incidence of mongolism is of the order of 1 in 2500 for mothers under 30, 1 in 1200 between 30 and 34 years of age and 1 in 300 at 35 to 39 years. This progressive rise continues until the menopause.

Unlike ovogenesis, spermatogenesis continues from puberty throughout life. However, there is evidence for ageing in the testes at a histological level, for example the increase in its collagen content. Also, the stem cell lines appear to deteriorate in that basic fertility of sperm from domestic livestock shows a slow decline in fertility of about 1% per annum after reaching a peak early in maturity. There is also clear evidence for an increase in the number of abnormal sperm in the ejaculate from aged human subjects. Despite this it is clear that the mammalian ovary fails completely in terms of ovulation and is often exceeded in function by spermatogenesis. Both of these phenomena of age effects on eggs and sperm are bound up with the failure of endocrine homeostasis and the feedback rhythms of reproduction.



CHAPTER 8 Ageing as a loss of cellular homeostasis—2

In 1970 I sketched a hypothesis in which the Hayflick limit might apply particularly to the immune system and be related to the early atrophy of the thymus and the progressive inefficiency of immune responses, particularly of the T-cell system, with ageing. The point of view has been strengthened by the evidence of Holliday, Tarrant, and others that in fibroblasts approaching the Hayflick limit there is evidence of defects in enzyme structure compatible with the onset of an error catastrophe; by Williamson and Askona's very clear demonstration that immunocytes in mice show something equivalent to a Hayflick limit in vivo; *and by the work of Fabris* et al. *on the pituitary-deficient Snell-Bagg mouse and the control of its premature ageing syndrome, either by the provision of immunocompetent cells or by hormonal stimulation of thymus function.* (Burnet 1973)

THE TISSUE CULTURE MODEL

Many arguments can be put forward as to the importance of tissue culture in the experimental study of ageing at the cellular level. Cellular phenomena can be studied in tissue cultures without the complicating nervous and chemical influences of the whole organism, and without interrelating actions of organs and tissues. These complications are unavoidable in experiments on the whole organism. Effects of various chemical factors on cells can be easily studied through the addition to, or changes in, the composition of the medium. However, the model suffers from the basic problem of its relevance to whole body cellular ageing, and the question posed by the first critics remains. Does the course of a single cultivation period that isolated tissue cells can traverse represent the whole scale of age processes in a relatively short time? The initial search for suitable cellular systems came up with the fibroblast model, which has become the focus for most ideals and experiments on the connection between errors in the use of genetic information and ageing.

The expression of cellular ageing in the behaviour of cells taken into culture was first studied experimentally using explants of embryonic and adult tissues of the chicken. These early experiments revealed that adult tissue passes through a longer latent period before cell proliferation sets in. Evidence that some kind of rapid ageing occurred in dividing local cell populations was first highlighted by Schilling, who observed that fibroblasts taken from normal

uninjured subcutaneous tissue of guinea-pigs, or from the injured tissue 4–6 weeks after the injury (old fibroblasts), grew in tissue cultures sluggishly, if at all. In contrast, fibroblasts taken from a wounded tissue soon after the injury, i.e. 'young' fibroblasts, grew as vigorously as embryonic or sarcoma cells. Thus, all these results showed a decrease of the growth potential of the fibroblasts with the age of their tissue and possibly the number of divisions.

Evidence for a loss of proliferative potential by *in vivo* transplantation came around the same time from work on grafted fragments of the tail from young rats. The grafts were placed under the skin of groups of rats 20–400 days old, and took successfully in about 80% of the youngest rats and grew to the greatest size in that group. In 400-day-old recipients the grafts grew only slightly, showing usually a poor vascularization and degenerative changes, or did not take at all. Regressive age changes were first observed in the peripheral cells of the tissue implants.

All these experiments suggested the presence of growth-inhibiting substances in old individuals, a concept that was first proposed in the 1920s. Carrel, in particular, claimed to have discovered substances that both inhibited and stimulated cell proliferation. They were named 'trephones'. In the plasma of old individuals the amount of growth-inhibiting substances increased while that of growth-accelerating trephones decreased. This phenomenon was demonstrated on the growth of a pure culture of fibroblasts cultivated in the plasma of chickens 3 months, 3 years and 9 years old. Not only the cell multiplication, but also the life duration of fibroblasts was much decreased in the 'old' plasma.

Other workers found similar inhibitory factors in serum from old people, but not in 'young' serum. 'Old' human serum not only depressed the multiplication of human embryonic tissue culture cells, but also caused degeneration in 52% of cultures by the seventh day of their growth, which increased to 100% on 11th–13th days. These early experiments are commonly dismissed as being irrelevant to modern ideas, partly because of the relatively crude techniques used to prepare the serum factors, but mainly because research into the use of tissue culture techniques in gerontology rapidly shifted towards the use of standardized media and a concentration of effort to gain an understanding of the concept of the fixed lifespan of cultures.

In the light of these findings the question of permanency of cultured cells was clearly a concern of early investigators. In the 1920s and 30s Carrel claimed to have maintained chick heart fibroblasts in culture for more than 20 years. This brought forward claims that cultured cells were immortal, given the correct medium and nutrients. However, despite many attempts by other workers, Carrel's results have not been repeated and it is generally accepted that normal actively dividing chick cells cannot be maintained much beyond a year. To explain Carrel's findings it is assumed that his results were due to the method used to prepare chick embryo extract as a source of nutrients. This was made daily under conditions that probably permitted cell survival so that new, viable,

embryonic cells were constantly added to the culture at each media change. In line with this explanation, it was reported that waves of mitotic activity were coincidental with the periodic addition of chick embryo extract.

LIFESPAN LIMITED CELL CULTURES

Definition of Mortal Cells

It was the careful experiments of Hayflick in the 1960s that established that some cell cultures would proliferate indefinitely while other cultures would die out after a certain number of generations or subcultures, no matter how well they were treated. Cultures that died out consisted of normal diploid cells; cultures that appeared immortal frequently expressed the characteristics of cancer cells with aneuploidy, or structural abnormalities in their chromosomes. The latter were defined as cell lines to indicate their special proliferation capacity whilst those cultures with a limited capacity for division were described as strains of the normal cell type from which they were derived. This variability in division capacity has given rise to the notion that limited proliferation in culture is related to the fact that developmental switches cut off surveillance/repair systems in cells which leave the germ line. Cell lines have undergone a spontaneous transformation during passage which switches on these systems. Some of these lines behave as cancers when transplanted into rats and mice. The propensity for spontaneous transformation varies among species. Human and chick fibroblast cultures almost never transform spontaneously whereas rodent cells often do.

The general system now used for the preparation of cell cultures is to disrupt pieces of isolated tissue with the proteolytic enzyme trypsin to separate individual cells. The isolated cells are placed in a container with a thin layer of cell-free nutritive medium as a serum substitute. The cells attach to the surface of the container and multiply to form a mat, or sheet, on the surface of the container. After forming a mat, multiplication comes to an end and the cells are then subcultured by transferring them to two new containers with fresh medium. When the cells have covered the growing surfaces of these new containers the population is said to have undergone one doubling. Conventionally, the measure of a culture's age is taken as the cumulative number of doublings the population has undergone since the start of the experiment. This is termed the passage number.

Using this technique, it has been established that normal cells derived from almost all vertebrate, and some invertebrate, tissues can be cultivated *in vitro* for various periods of time, but they do not survive indefinitely. At first there is a period during which proliferation is rapid and subcultivations can be carried out frequently. This is followed by a phase of declining capacity for division during

which many of the cells show degenerative changes in their cytoplasm, which becomes granular with odd shaped membranes. Eventually there are degenerative changes in the nucleus. Generally speaking, the process of serial subcultivation of normal cells cannot be done indefinitely, and after a well-defined number of subcultivations, termed 'passages', depending on the source and kind of cells, the strain will no longer respond to transfer by proliferating and the culture dies out.

Fibroblast-like cells in tissue culture are the most extensively studied of these 'fixed limit' systems Serially-cultured diploid embryonic fibroblasts undergo approximately 50 doublings (50 cell generations taking slightly less than a year) then die out, showing well-defined terminal histological changes, often with marked chromosome abnormalities. There is an initial stable phase when the cultures take a constant time to double their population and have no apparent loss of division capability. For human fibroblasts, the doubling time increases exponentially at around the 50th sub-culture which then rapidly leads to the 'death' of the particular cell strain (Figure 8.1).

The resulting curve describing the loss of cells represents the dying out of an ageing population. The probability of dying increases with increasing time.

With respect to the relationship between species lifespan and the doubling potential of cell cultures, a study of eight mammalian species has provided evidence for a positive correlation between longevity and *in vitro* lifespan of fibroblast-like cells.

Fixed postmitotic or highly differentiated parenchymal cells do not multiply in tissue culture, consequently, the kind of cell population derived from

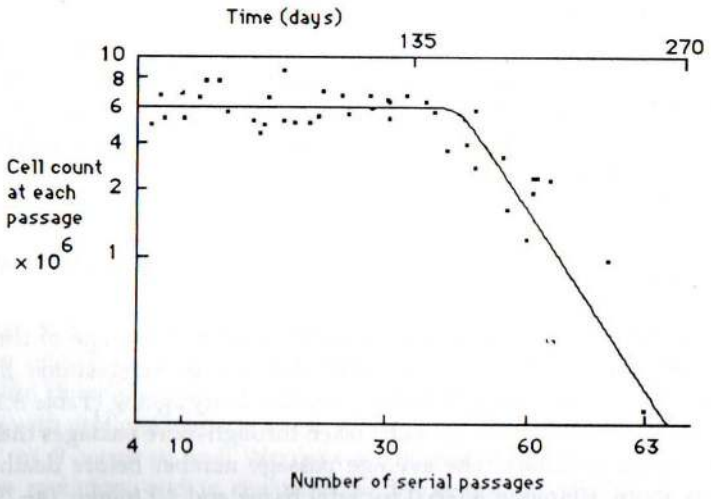


Figure 8.1. Cell counts at each serial passage of diploid human fetal lung cells in tissue culture

postmitotic tissues is most likely to develop from the fibroblasts which are a characteristic of all organs. In the body fibroblasts are critical to wound healing when they proliferate at the site of damage to fill spaces produced by the loss of cellular tissue. Normally, in the body, their potential for rapid division is held in check by unknown homeostatic mechanisms, which are released when tissue is taken from the body and used as an explant. It has been pointed out that expression of 'tissue culture ageing' *in vivo* may be apparent only under certain conditions such as wound healing in aged individuals.

The identification of tissue culture cells as fibroblasts is based primarily on their morphology and behaviour. They may be derived from several kinds of precursor phenotypes such as pericytes and endothelial cells as well as the collagen-producing fibroblasts of general connective tissue. Strictly speaking, because of uncertainties in their origins, these spindle-shaped cells should be called 'fibroblast-like cells'.

The inability of cell cultures in later passages to proliferate indefinitely was at first ascribed to various technical difficulties, such as inadequate nutrition, pH variation, accumulation of toxic metabolic products and micro-contaminants. However, suitable controls to test these various possibilities have shown without doubt that the degeneration of clones is unrelated, at least in any simple, direct way, to any of these factors. During subcultivation, samples of the cells may be frozen in liquid nitrogen. After thawing they will begin dividing again but the total number of doublings they can attain is the same as that of the culture from which they were taken. The reconstitution of frozen human fetal diploid cell strains has revealed that regardless of the doubling level reached by the population at the time it is preserved, the total number of doublings that can be expected is about 50, including those made prior to and after preservation. Cold storage of human diploid cell strains merely arrests the cells at a particular doubling level but does not influence the total number of expected doublings. It appears that the ability to double 50 times is probably a characteristic of each clonable cell in the population and that mixed populations of cells with different doubling potentials do not influence each other.

Donor Age

The major influence on the lifespan of cultured stains is the age of the donor from which they were obtained which has led to suggestions that the phenomenon is a cellular manifestation of whole body ageing (Table 8.1). Cells from fetal tissues, such as lung, can be taken through more passages than those from the lungs of adults. The average passage number before death of the culture is about 50 (range 35–63) for fetal tissue and 20 (range 14–29) adult tissue. Other studies have involved the comparison of a number of parameters of growth in cells cultured from healthy individuals in their 20s and 30s with

Table 8.1. Effects of donor age on the 'longevity' of serially cultured human skin fibroblasts

Age of donor (years)	Number of cultures	Longevity of passage* Range	Average
8	17	66–67	66.5
13	15	57–58	57.5
65	13	32–33	32.5

*Number of serial subcultures before extinction of strain

those from healthy individuals in their 60s to 90s. This work showed that the the average lifespan for the cells derived from the youngest group was significantly greater than that from the older group, and the cell cultures from the older individuals show age changes much earlier than the ones prepared from the younger individuals.

In one of Hayflick and Moorehead's early experiments, a mixed culture of cells was prepared from young female and old male tissues. The different kinds of cells were differentiated by the presence and absence of Barr bodies. Barr bodies are the masses of sex chromatin that appear in the female interphase nuclei. In a mixture of cells from old males and young females the 'old' cells in the mixture stopped growing after the same number of population doublings as did 'old' controls grown individually. The 'young' cells in the mixture grew vigorously and stopped growing after the same number of population doublings as did the 'young' controls. Since the old cells stopped growing when the young cells were proliferating vigorously this rules out any effect of environmental influences on lifespan, such as nutritional deficiencies, microbial contamination, and the accumulation of toxic products of ageing produced by old cells.

By culturing fibroblasts from human upper-arm tissue ranging in age from embryonic to 90 years it has been found that the average number of divisions declines by 0.2 for each year of the donor's life.

Genetic Aspects

Light has been thrown on the genetic aspects of finite lifespan strains by culturing cells from the tissues of people with diseases of accelerated ageing such as the Hutchinson–Gilford syndrome (progeria) and Werner's syndrome. Subjects with progeria begin to age in terms of calcification of the blood vessels as early as 9 years of age. Werner's syndrome results from an autosomal recessive mutation, and is characterized by symptoms similar to those of progeria but which are manifest later in the third or fourth decade. Work with cells from Werner patients has provided further evidence that the lifespan of

cultured fibroblasts is somehow connected with the whole body cellular ageing (Table 8.2). All of these strains display a reduced lifespan in culture of about 14 doublings compared with the normal 50 or so.

Similarly, although there is more variability, and some strains fall within normal limits, fibroblasts cultured from patients with Hutchinson-Gilford syndrome usually have only 2–10 doublings. There are also corresponding reductions in mitotic activity, DNA synthesis, and cloning efficiency in both conditions. In relation to the same viewpoint, cell cultures from diabetics, who also show an early onset of some of the degenerative changes associated with ageing, have lower than normal doubling times.

Morphological and Functional Changes

The shape of the curve obtained from plotting the number of cells obtained against passage number takes a standard form for all cell cultures. The first sign of ageing of tissue culture cells is the formation of fatty granules or vacuoles in the protoplasm. Then the growth of cells and their amoeboid movements start to diminish. Later the fatty granules and vacuoles begin to fill the protoplasm, and gradually cytolysis develops.

The morphological and functional changes associated with the shifts in cell numbers and division potential, may span a period of days, weeks, or months but do not exceed 1 or 2 years. Starting cell populations are called 'primary cultures' and the first stage when there is a build up of rapid multiplication is termed Phases I and II. The period when cells are increasingly failing to divide and when they show an increasing frequency of cytological abnormalities is designated Phase III. Although the karyotype of human diploid cell strains is very stable during Phases I and II, aneuploidy and other chromosome aberrations occur during Phase III. This increase in abnormal chromosomes *in vitro* may be related to the increased frequency of chromosome aberrations *in vivo* evident from scoring anaphase and telophase aberrations in somatic cells of

Table 8.2. Longevity of cultured skin fibroblasts obtained from four patients with Werner's syndrome

Donor	Number of cultures	Range of longevity
1	5	9–10
	4	18–19
2	19	5–25
	4	4–7
3	2	24–25
4	8	10–29

regenerating mouse liver tissue following partial hepatectomy. It may also be relevant that hypodiploid counts in human peripheral blood leucocytes are correlated with the chronological age.

In addition to the histological changes that accompany the decrease in proliferative capacity in ageing populations the cell density maximum in the stationary, or plateau phase, decreases. The signal to enter a stationary phase in cell culture is related, in part, to cell-to-cell proximity or contact and old cells seem to become more 'sensitive' to one another's presence as they get older.

Taken as a whole the changes in normal human fibroblasts ageing in tissue culture do not fall into any particular pattern. Many aspects of cellular function do not change at all (Figure 8.2). On the average, cells from old populations are larger than those from young populations. Nuclear size also increases, although DNA content remains approximately the same. There is evidence that DNA repair may be deficient in ageing cells, and that cells deficient in DNA synthesis may also be deficient in repair. From this kind of data it appears that growth and division become uncoupled, allowing for the accumulation of many kinds of macromolecules. In line with this conclusion many studies have indicated that RNA content increases in ageing cells, although RNA synthesis decreases. Protein content also increases with age while the rate of protein synthesis declines. Cellular lipids also increase with age.

Microcinematography has shown considerable variation of interdivision time of individual cells descended from a single progenitor. The population doubling time, fraction of dividing cells, and total *in vitro* lifespan are not

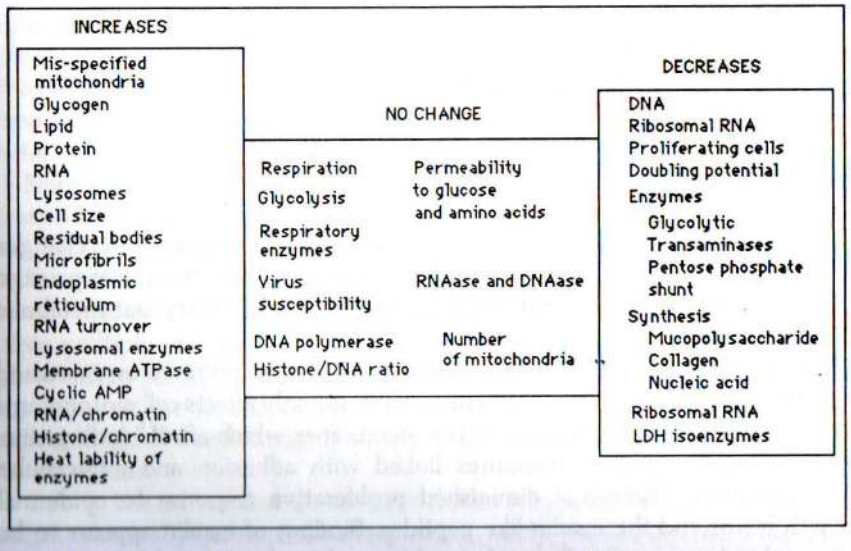


Figure 8.2. Some cell characteristics of normal human fibroblast cultures

affected by cell density. This allows the increased heterogeneity of cells during passage to be explored by measuring the proliferative potential of individual cells after isolation from the culture. The cell cycle transit time increases due primarily to an increase in Gi and possibly G2 phases and it seems that there is an increasing variability in the cell cycle between cells.

The experimenter can establish cultures which reveal considerable variation in clonal growth rate among individual cells isolated during passage. In particular, the proportion of slowly growing clones increases, and it has been found that, at any time, the fraction of cells capable of generating clones of 16 or more cells is linearly related to the remaining proliferative potential of the culture. However, the variation between doubling potential of individual clones is greater than can be accounted for by this variation in division rate between individual cells. Evidence of this kind argues against culture lifespan being determined by a cell clock that counts cell divisions.

Based on quantitative measurements of DNA content and morphological analysis of prematurely condensed chromosomes fixed in time by fusion to mitotic cells, the majority of cells in postmitotic cultures are in the Gi phase of the cell cycle. The state of the quiescent cells in terminal cultures has been investigated by studies using heterokaryons produced by the fusion of cycling cells with senescent cells and cycling cells with nutrient-deprived quiescent cells. The DNA of these heterokaryons has its own response characteristics to the inhibitors of protein synthesis indicating that these two quiescent states are metabolically distinct.

Senescent cells appear to be lodged at the Gi/S boundary.

Senescent cells are defined by the following kinds of morphological changes:

1. Secondary Iysosomes associated with the formation of autophagic vacuoles and residual bodies.
2. Irregular lobulation of the nuclei.
3. Mitochondria with swollen cristae.
4. Gap junctions between cells.

There is correlation between the alteration in Iysosomal structures and changes in hydrolase activity. Also abnormalities such as gap junctions in late passage cells have been correlated with a diminished capacity to carry out metabolic cross-feeding.

These histological findings and biochemical studies point to an increased mis-specification of membrane structures. This not only affects cell morphology but also the biological functions of the membranes which affect binding sites and membrane mediated responses linked with adhesion and intercellular communication. There is a diminished proliferative response to epidermal growth factor and the insulin-like peptides. Binding of insulin appears to be increased in human cell cultures derived from older donors and from patients

with syndromes of accelerated ageing. Concanavalin lectin is not bound so well in late passage and there is an increased clustering of the receptors which appears to be related to changes in the the amount and distribution of a cell surface glycoprotein (possibly fibronectin) which contributes to the spreading and attachment of cells. Fibronectin secreted by late passage foreskin fibroblast-like cells is less efficient in promoting adhesion of both old and young cells. This may be more significant to whole body ageing than the loss of mitotic activity.

CELL LINES

Although Carrel's early results showing that isolated cells are potentially immortal have been dismissed as being due to poor technique the subsequent work which showed 'a Carrel phenomenon' using abnormal cancerous cells, have been confirmed may times. It is clear that the immortality of these cell culture lines, derived from abnormal cells, as distinct from the fixed lifespan of normal cell strains, is a feature of tissue culture using a large number of cell types. These 'immortal' cells occasionally appear 'spontaneously' in tissue culture and are said to be 'transformed'. Some lines have been in continuous cultivation in many laboratories 20 years or longer and have a doubling time of about 24 hours. Lines may also be produced by infecting normal, fixed lifespan cell strains with viruses, exposing them to mutagenic chemicals, irradiating them or growing them on various kinds of solid media.

Lines multiply indefinitely on subculture and have all been shown to have cytological abnormalities. They differ from normal cells of the donor tissue in chromosome number. Even where they are not derived from tumours many lines form tumours when introduced into appropriate hosts. The tendency of tissue culture cells to produce lines with unlimited lifespan depends to a large extent on the animal source.

Fibroblast-like cells derived from chick embryos tend to be stable with a limited lifespan whereas mouse fibroblasts almost always give rise to permanent cell lines. Human fibroblasts are intermediate in this respect.

In addition to their capacity to multiply indefinitely *in vitro*, cell lines have some standard characteristics. When inoculated into suitable hosts, the cells often multiply and sometimes metastasize. Unlike cell strains, most cell lines grow when placed in the hamster cheek pouch which provides a culture environment free of immunological rejection responses. In this respect cell lines appear to stand in relationship to cell strains as transplantable tumours do to normal tissue.

Cells in line culture that do not have the karyotype of the tissue (or strain) of origin, are usually heteroploid, aneuploid or, rarely, psuedo-diploid. Most often they exhibit a distribution of chromosome numbers around a modal value

which in the case of human cell lines is usually in the seventies. Sex chromatin is not retained in cell lines derived from female tissue.

'CULTURE-LIMITED' CELLS IN GERONTOLOGY THEORY

One of the first postulated mechanisms to explain the existence of limited lifespan strains is that the capacity to divide is affected by an accumulation of heritable damage from daughter to daughter. Therefore cells from an adult donor start with more damage before cultivation than cells taken from an embryo. The shape of the curve obtained by plotting the rate of cell production at different times throughout the finite lifetime of a human diploid cell culture is similar to a multiple-hit or multiple-target curve. In this respect it may be that some critical level of damage which affects a vulnerable cellular entity must be reached before exponential death of the culture begins. Hayflick felt that since 50 doublings preclude any simple dilution of an 'activator', or 'accumulation of an inhibitor', the effect is upon some self-duplicating cellular system and consequently must be heritable within the culture conditions.

Another possibility is a rise in DNA errors through faulty copying and, eventually, this results in the inability of the cell to activate cell division after 50 doublings. Alternatively, it is possible that some self-duplicating entity essential for cell division is, itself, dividing at a rate somewhat slower than that of the cells. Such a process would ultimately result in the complete loss of this essential entity after 50 doublings with the concomitant inability of the cell itself to divide. The model for this may be the Kappa factor effect which causes the death of clones in *Paramecium*.

Chromosome anomalies that occur at the time normal human diploid cells cease to divide *in vitro* are also found to occur in the peripheral blood leucocytes of a man in direct proportion to his age. Also, chromosome damage appears to be a feature of the intermitotic hepatic cells, which is only revealed in regeneration experiments. These comparisons provide indications that the processes of 'tissue culture ageing' underlying the limited lifespan of normal fibroblast-like cells may have a counterpart in the normal day-to-day physiological process that can be seen in the donor, so it is important to consider whether or not the phenomenon is really related to whole body ageing.

Obviously the balance between division and death of cells is of great importance in the maintenance of those organs where cell proliferation continues throughout life. In postmitotic tissues, with no possibilities of replacement of faulty cells, the rate of cell death would be expected to govern the decline in organ function. These viewpoints have produced two corresponding lines of research. One based on models of proliferating cell systems deals with alterations in the dynamics of the cell cycle. The other is concerned with detrimental changes in the structural and enzymic proteins of postmitotic

tissues and the systems which detect and replace faulty molecules.

The fact that species exist as stable genotypes over very long periods of time, during which damage and errors in cell function would be expected to accumulate, implies that systems exist for detecting and correcting damage. The long-term stability of grafted varieties of many plants also points to similar systems for correcting errors in the proliferating meristematic tissues of roots and shoots. It has already been pointed out that animal cell lines also appear to have an indefinite lifespan irrespective of whether they are maintained by serial transplantation or repeated tissue culture. From this viewpoint, cell lines may arise because of the unmasking of a surveillance and correction system to maintain perfect cell function.

In both research strategies the tissues that should be compared with the *in vitro* system are those such as skin, gut lining, and the blood stem-cell systems that are continuously proliferating, and those such as cartilage and liver that, although not dividing, may divide in response to physiological needs. These may be explored by cross-sectional analysis whereby tissue samples are examined in different age cohorts. The other approach is to transplant repeatedly pieces of tissue from host to host, testing the capacity of transplants, containing mitotic cells and reverting mitotics, to survive repeated movement to standard age hosts.

Burnet was one of the first to incorporate the phenomenon of the Hayflick limit into a general theory of ageing. He started from the point of view that if there is any reasonably simple clue to the nature of ageing there must at some point be a nexus between information in the genome and some key process in the organism which sets the tempo of the ageing process. This led him to propose that the steric configuration of the key enzymes associated with DNA replication and repair is the most likely form in which the genetic requirement for a lifespan is primarily expressed. The central mechanism was somatic mutation which is basically similar to germ-line mutation except in regard to the ways in which it can be phenotypically expressed through having an opportunity to develop a large enough clone of mutant descendants. One possibility is that a somatic mutation during embryonic life involving cells that will be concerned with skin pigmentation or hair formation can produce grossly visible abnormality. The other, which is relevant to ageing, is that a sequence of secondary mutations may become possible as a result of the initial mutation and lead to what Orgel called 'error catastrophe', with the elimination of the clones concerned. Burnet felt that that an error catastrophe of this sort is the most likely explanation of the Hayflick limit. A third outcome of somatic mutation he considered to be the development of neoplasia and in this category he also included the development of autoimmune clones of immunocytes as essentially a form of conditioned malignancy. His aim in formalizing these ideas was not to imagine a possible mechanism of ageing in detail but to devise a simple working hypothesis to account for results of research that were emerging in the late 60s

and early 70s which could stimulate potentially productive experimental work on ageing.

TRANSPLANT TESTS OF DIVISION POTENTIAL

Some differentiated animal epithelial tissues may be serially transplanted from old to young animals. For example, skin grafts from young donor mice remain in a satisfactory condition through five repeat transplants. In this type of experiment the maximum lifespan of grafted mouse skin is five years, compared with the mean lifespan of about two years for the donor animals. The pioneering work on transplants and ageing was carried out by by Krohn and a selection of the results of his work is given in Table 8.3.

Krohn demonstrated that the survival of transplanted skin in mice was related to the age of the graft only. Grafts from young donors remained in satisfactory condition for about 650–1000 days and survived two to five transplantations. The limitation of making transfers beyond this lifespan was the technical problem of the ever-decreasing size of the grafts, which between 850 and 1750 days had become too small to move. Krohn's skin grafts from certain types of old mice grew as well as did grafts from young controls. With successive transplantations, the grafts became progressively smaller and many were lost, but this problem occurred with both old and young grafts. Compared with the longest recorded lifespan of 3.5 years for any mouse, the maximum lifespan of skin transplants ranged from 4.5 to 6.7 years. No clear functional tests were carried out to determine whether old skin was rejuvenated by the young recipient environment. Furthermore, there was no way to prove that the functioning cells had in fact come from the original donor and had not migrated in from the successive recipients.

Table 8.3. Survival of serial skin grafts in mice

	Host 1		Host 2		Host 3		Age*
	Grafts	Good	Grafts	Good	Grafts	Good	(yr)
Donor							
1	14	13	11	3	3	2	6.66
2	30	30	25	16	13	5	6.00
3	10	10	10	6	1	1	6.33
4	13	11	10	9	8	6	5.00
5	30	25	23	18	11	7	4.83
6	26	24	20	20	2	2	4.75
Total	123	113	99	72	38	23	

*Total time for graft survival (not all experiments are completed)

Although this result might suggest that normal mouse skin shows an unlimited proliferative capacity, in the absence of any direct evidence, the actual decrease in graft size may have resulted from gradual failure of the proliferative response of graft regeneration. Nevertheless, eventual death of the transplants appears to be due to the technical problem of maintaining a suitable size of graft for surgery rather than the loss of proliferative potential.

Krohn also showed convincingly that young ovaries or fertilized eggs function much less well in old than in young recipients, indicating that other local and/or systemic factors besides the ovary affect reproductive ability with age. Furthermore, fertilized eggs from old donors were as viable as those from young donors in young recipients. Later work has indicated that ovarian impairments are a major factor in the onset of reproductive senescence. For example, ovaries from middle-aged mice support very few cycles after grafting into young ovarian hosts.

In general, the transplant method has also been useful for studying intrinsic age changes in proliferative capacity of other organs. For example, mammary gland tissue in rodents survives transplantation and proliferates in the fat pad of hosts up to two years of age (Table 8.4). There is no fall in the percentage number of 'takes', although the time taken to fill the available space in the fat pad increases linearly with age of the transplant. The growth rate is dependent upon the number of cell divisions previously undergone.

Transplants of all ages grow best in very young hosts. However, in these very young hosts it is evident that the proliferative capacity of transplants from the oldest animals has decreased by about 30% in the second year of life (Figure

Table 8.4. Growth of mammary transplants from donors of various ages transplanted into different aged recipient mice

			Transplant Growth (%) Generations	
	Donor age	Host age	1	2
Experiment 1	3 week	3 week	92	
	3 week	3 week	77	
	12 months	3 week	93	
	26 months	3 week	58	
Experiment 2	3 week	12 months	49	3
	3 week	12 months	26	
	12 months	12 months	44	4
	12 months	12 months	19	
Experiment 3	3 week	25 months	37	
	3 week	25 months	31	35
	25 months	25 months	28	
	25 months	25 months	29	31

8.3). Transplants taken from donors of all ages grew much more slowly, and at about the same rate in 1-year-old and 2-year-old hosts (60% decrease in maximum growth rate observed in 3-week-old hosts). This result indicates that although intrinsic ageing of the mammary gland has reduced its maximum proliferative capacity, systemic changes have also placed severe limits on proliferation. This limit seems to relate to events during the first year of life and may be nothing more than a drop in growth hormone associated with the cessation of general growth.

Transplantation experiments have been undertaken with a range of other tissues and all definitely support the ideas of age-related limited proliferation. This has been found to be the situation with bone marrow cells and for single antibody-forming cell clones.

Marrow cells are easily transplanted by injection as a suspension into the recipient's bloodstream. The recipients must be lethally irradiated to kill all their own stem cells and the bone marrow is rapidly repopulated with the donor cells. In such experiments, it is essential to have an unambiguous method for identifying donor cells, because if even small numbers of recipient cells survive the irradiation, they are able to multiply and repopulate the recipient. Then they may function normally, giving the false impression that the donor cells are still functional. For example, proliferating haematopoietic cells, identifiable by a chromosome marker, are unable to repopulate the organs of heavily irradiated recipient mice after the third transplant generation. Cells from normal fetal mouse liver, spleen or marrow, lose their capacity to form colonies in the spleen during repeated passage in irradiated hosts, and there is a gradual loss of the

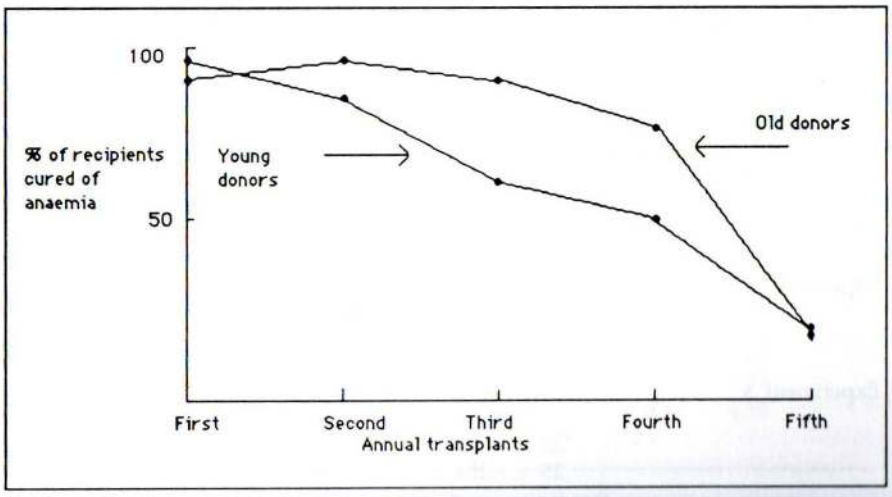


Figure 8.3. Growth of rodent mammary transplants in relation to host age

ability of these cells to prevent the death of the host through counteracting the radiation damage. After one year in the recipient, marrow cells were transplanted into a second irradiated recipient. Some recipients were checked to be sure that they contained cells with abnormal chromosomes, proving that they came from the original donor. This procedure was repeated every year, and after about 40 months and three or four successive transplantations, the recipients had lost their original capacity for repopulating the marrow. One recipient still had a few cells containing the abnormal chromosome after five successive transplantations over 60 months, but that was the maximum observed. This phenomenon, termed 'decline' is taken to indicate new colony-forming cells have lost the self-renewal capacity of the starter inoculum. These studies suggest the capacity of haematopoietic colony-forming cells to proliferate decreases, either with whole body age, or because they were intrinsically timed to age. In contrast, leukaemic cells retain their proliferative capacity to proliferate through successive transplantations.

In all of the early work on cell transplantation it was assumed that the methodology of handling the cells did not damage them. Evidence that transplantation damages stem cells comes from experiments where stem-cell proliferation was measured in the same animals after repeated irradiation that killed most but not all of the cells. Stem-cell proliferative capacities were much higher than those reported by workers who transplanted stem cells into successive irradiated recipients.

In fact, very few transplantation experiments meet the criteria necessary to support an unambiguous result. The minimum criteria for setting up an experiment are that the transplanted tissues should be unambiguously identified, and, in particular, they should function normally and show a temporal loss of this functional ability compared to identically treated younger controls. In an attempt to meet the latter criterion, instead of using lethally irradiated recipients, mice with a hereditary anaemia which caused stem-cell defects were used. The anaemic mice were populated by donor cells that restore red blood cell production, which is thus taken as a measure of the functional integrity of the transplanted cells. Donors with a different kind of haemoglobin from the recipients were used to identify the progeny of the transplanted stem cells. The anaemic recipients produced only donor-type haemoglobin, proving that their red blood cells were now being produced by stem cells from the donor. Since the hereditary anaemia was never cured spontaneously, both the donor haemoglobin type and the cure of the anaemia identified the donor cells. Red cell production can be rigorously measured in the recipients, and this is a good test of the functional ability of the transplanted old stem cells.

The results from this experiment showed that the loss in proliferative capacity depended on the number of serial transplantations not on whole body age. For example, the percentage of anaemic mice cured by both old and young stem-cell lines declined in a parallel fashion with repeated serial transplantations

at either 1 year or 3 month intervals. This decline became significant after only three successive transplantations (Figure 8.4).

From this kind of experiment it has been concluded that although normally functioning tissues transplanted in animals cannot proliferate without limit, there is little or no difference in the performance of cells from old or young mature donors using the best-defined *in vivo* transplantation models. These include 'red blood cell producing', or immunologic stem-cell lines and mammary gland cells. Even the limited proliferative capacities of such tissues appear sufficient for them to function far beyond their donor's maximum life expectancy.

IS BODY AGEING RELATED TO CELL DIVISION LIMITS?

These studies may be taken together with descriptive evidence from mitotic counts in bone marrow, intestinal epithelium, and basement layer cells of the skin which appear to divide at the same rate until animals die in old age. In order to support the Hayflick model in terms of whole body ageing being due to the tissues 'running out of cells' it is necessary to obtain an estimate of the actual number of divisions that these cells undergo in a lifespan. If they greatly exceed the maximum 50 doublings obtained using *in vitro* systems it is unlikely that these tissues age because they run out of divisions.

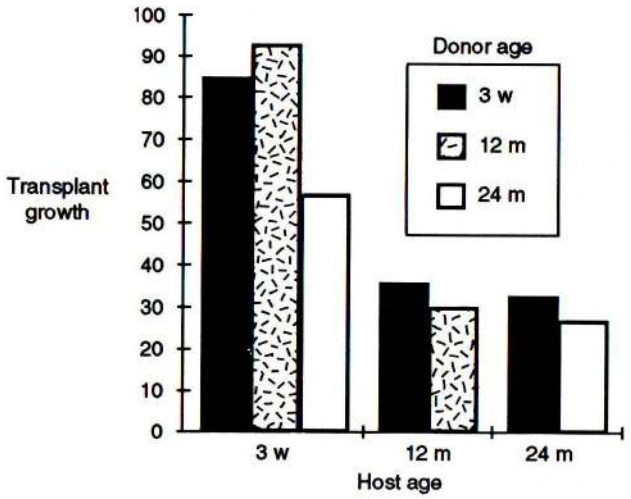


Figure 8.4. Decline in the ability of transplanted stem cells to cure genetically anaemic mice

In summary, cell cultures derived from normal tissues typically undergo a period of vigorous proliferation and then enter a phase of cytological and biochemical degeneration, which is characterized by a reduction in proliferative ability, that ultimately leads to death. At any point in their life history, cells may become cytologically abnormal. This change may be induced by viruses or other agents, or it may occur spontaneously. These abnormal cells always acquire an indefinite lifespan.

Considerable speculation has arisen on the possible role of limited cell division in stem-cell division and survival in the body. In particular, calculations have been made to determine if any cell lineages *in vivo* are likely to go through more than 50 generations. Different conclusions may be reached depending on the starting assumptions about the kind of division system that applies. Do all stem cells divide regularly, to what extent is a group of stem cells homogeneous in terms of past number of generations? One cell may be the result of three past divisions while an adjacent cell may have had 20 divisions in its past history. It would certainly appear that 50 generations per fetal cell is greatly in excess of what would be required during an animal's lifespan. Starting with two cells, 50 generation's would give around 1000 litres of cells!

There is a common feeling amongst biologists that rapid and prolonged proliferation does not occur *in vivo*, where no population of stem cells is known to die out in one year. Also, stem cells do not give rise only to more stem cells. Probably close to 50% differentiate into fixed postmitotic cells of one sort or another.

Probable explanations for the ultimate cessation of division of normal cells *in vitro* involve multiple-hit or multiple-target phenomena. Alternatively, it is possible that some self-duplicating entity essential for cell division is, itself, dividing at a rate somewhat slower than that of the cell. Normal bone marrow, intestinal epithelium and basement layer cells of the skin apparently divide at high rates and seemingly undergo greater than 50 divisions. However, models of stem cell proliferation exist which indicate that the entire complement of cells produced from these tissues during the lifetime of the animal can be encompassed by 50 or fewer cell doublings. Thus, although they may not reach the number of divisions required to show cytological and physiological deterioration of fibroblast-like cells *in vitro*, each division may still leave its mark.

A central point of theoretical discussion concerns the way in which stem cells build up their progeny. The two relevant models for cell division are termed 'tangential' (or 'maintenance') succession, and 'clonal' succession (Figure 8.5). In the tangential model it is postulated that there is a pool of homogeneous stem cells multiplying so that one daughter differentiates to a specialized cell and the other retains its stem-cell characteristic. In this model the size of the pool is constant, and cell division replaces cells that die or are removed at the end of their differentiated life, e.g. red blood cells and gut epithelial/mucus cells. The clonal succession model assumes all differentiated cells would be produced by

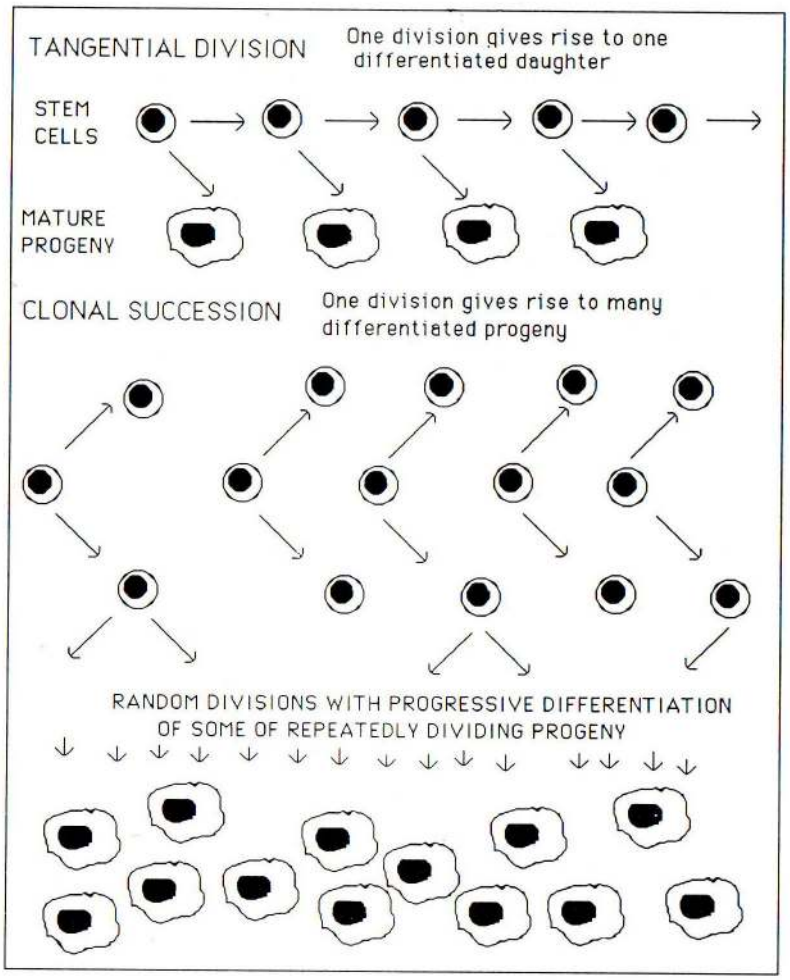


Figure 8.5. The tangential and clonal succession models of cell division

logarithmic cell division with asynchronous mitosis, so as to produce a variation in the rate of division of the primitive stem-cell pool. This would provide for the regular release of differentiated cells, and, in comparison with tangential division, there is a reduction in the number of stem-cell generations necessary to produce a given population of differentiated cells. It provides many populations of dormant ancestral cells at various doubling rates which could be varied through a succession of environmental controls. Kay, who first discussed these models, envisaged that clonal succession would apply, for example, to the intestinal crypts of man, where a high rate of mitosis implies a

large number of cell generations. Cell turnover in the gut, where a source of slowly reproducing cells within the crypts has been described as a candidate for the clonal succession model, has a frequency of one per 100 hr. The tangential model cells in the crypt bases may undergo only 5000 mitoses in a 60 year human lifespan, whereas clonal succession suggests that the entire population of cells shed by the intestinal epithelium may be achieved by a stem-cell population in about 50 doublings or less.

Although there are a number of reservations that must be voiced concerning the experimental design used in these studies, the overall thrust of the evidence suggests that the dividing cells do age intrinsically. But, as yet there is no evidence for any causal connection between this phenomenon and age involution of tissues, or the increased force of mortality in old organisms.

ANALYSIS OF THE CELL CYCLE

Cell Fusion

Time-lapse cinematography may be used to measure the interval between one mitosis and the next. This has revealed much overlap in the interdivision time of cells from both young and old cultures but on average, cells from cultures prepared from old populations have longer interdivision times than cells from young populations. A fundamental problem of clonal ageing in tissue culture is whether the decline in the proliferative capacity of ageing cultures reflects a uniform decline in all the cells, a decrease in the fraction of cells in the population that are proliferating, or a combination of these factors. This involves investigating the influence of ageing on the cell cycle. In particular, information is required on age changes in the percentage of cells traversing the Gi phase (the pre-DNA synthetic phase), the S phase (the period of DNA synthesis), the G2 phase (the post-DNA synthetic phase) and the M phase (the period when cells are in mitosis).

In young animals nearly 100% of the cells in young cultures synthesize DNA while only about 20% of the cells do so in old cultures. As the population ages, there is an exponential decline in the cells that are cycling. Many appear to to be arrested in the pre-DNA synthesis or Gi phase of the cell cycle.

The relative roles of nucleus and cytoplasm in cellular ageing may be investigated using the techniques of cell hybridization. The usual assay for cell ageing is the rate of incorporation of H_3 thymidine into nuclei or isolated DNA (Figure 8.6).

The early cell fusion studies were based upon work which showed that nuclei from differentiated cells, such as the chick erythrocyte could be reactivated after fusion to actively proliferating cells. It was found that the fusion of old fibroblast cells with proliferating cells did not induce DNA synthesis in the old

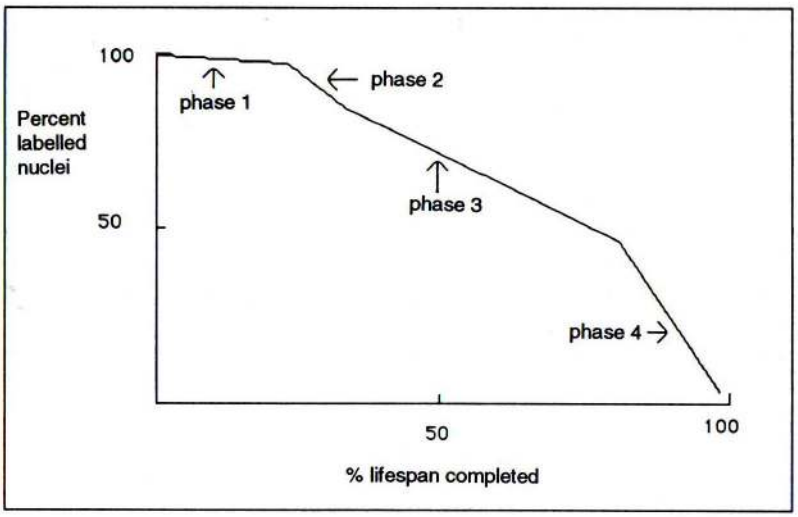


Figure 8.6. Characteristic phases in the decline in DNA turnover measured by H3 thymidine incorporation into normal human fibroblast-like cells in tissue culture

nuclei but inhibited that in the young parental cell nuclei. In this procedure, cells of different types are incubated together in the presence of Sendai virus, which enhances the fusion of cells. The fused cells contain two nuclei, one from each cell, and are called heterokaryons. In some cases, nuclear fusion also occurs to form a synkaryon, or nuclear hybrid. Neither heterokaryons nor synkaryons prepared from mixtures of old and young cells acquire the characteristics of the young component, indicating that cellular ageing is a 'dominant' characteristic. The growth potential of synkaryons recovered from the fusion of two skin fibroblast-like strains of differing proliferative lifespans was intermediate to that observed in hybrids derived from the short-lived strains and the long-lived strains. Similarly, hybrids derived from the fusion of a neonatal foreskin strain with a strain from a 68-year-old donor displayed a division potential intermediate between the old and young parental stains. However, if senescent human cells are fused with HeLa cells, which are a tumour-derived cell line, or with virus-transformed cells, DNA synthesis is reinitiated in the normal old nuclei.

More detailed analyses of this phenomenon have revealed that inhibition by the old cell occurs only when the younger, cycling parental cell is fused in the early or midportions of the Gi phase or when it is in the G2 phase at the time of fusion. When the cell is in late G2 or in S phase, DNA synthesis is completed in the cycling nucleus. Similar observations were reported in studies in which

parental cells were made non-replicative by serum deprivation or by exposure to amino acid analogues. Thus, a variety of extrinsic or intrinsic stimuli may activate a common metabolic pathway which shuts off the mitotic cycle.

Some cell lines are capable of stimulating DNA synthesis in postmitotic nuclei, for example in fusions of HeLa cells and SV-4O transformed human fibroblast-like cells. Chromosome cytology indicates that complete replication of the genome of the old cells is occurring in these heterokaryons. It has been suggested that the capacity to reinitiate DNA synthesis correlates with the mechanism by which the established cell lines are transformed; DNA viral transformants are dominant, while carcinogen transformed cells are recessive with respect to the capacity to reinitiate DNA synthesis in senescent nuclei.

One criticism of these heterokaryon studies is that only DNA synthesis is examined, and not the complete cell cycle activity. Earlier studies on proliferative behaviour of synkaryons resulting from the fusion of proliferating cell types and older cultures appeared to complement the heterokaryon studies. Proliferating hybrids cannot be found following fusion of young and old human fibroblast-like cells. In contrast, hybrids derived from the fusion of old normal cells with established cell lines possess an apparently unlimited growth potential. On the other hand, fusion of young or middle-aged fibroblast-like cells and HeLa cells gave rise to a majority of hybrid clones with a growth potential of fewer than eight population doublings, while a few displayed a more extended growth potential. In both kinds of experiment, 1% foci of rapidly dividing cells formed in the old hybrid clones, which gave rise to rapidly dividing cultures possessing an extended and probably infinite growth capacity. This indicates that the hybrids are less stable than the normal diploid parent, and easily transformed.

The recovery and characterization of proliferating synkaryons resulting from intraspecific hybridization of human strains are clearly of relevance to the problem of tissue culture ageing, but in the absence of selective markers in human fibroblasts, efficient isolation of such hybrids has proven to be difficult. It may be concluded from the changes in growth rates that the limited lifespan phenotype appears to be dominant in hybrid cultures. The observations are in apparent contrast to the heterokaryon studies in which certain transformed cell types reactivate DNA synthesis immediately following fusion. A possible explanation for these observations is that the factors involved in the initiation of DNA synthesis are elevated in the cell types which are dominant in the heterokaryon assay. This could result in a transient ablation of the action of the postulated inhibitors in senescent cells following fusion. In this connection there is a correlation between the levels of DNA polymerase-alpha in S-phase cells and the capacity to stimulate DNA synthesis in old cells in heterokaryons. In the proliferating hybrids, the levels of the initiators of DNA synthesis would be regulated by the non-transformed or 'normal' parental genome and thus exhibit a finite growth potential.

Division Models

In summary, individual cells proceed asynchronously from a rapidly proliferating state gradually becoming more and more slowly proliferating until there is a total inhibition of DNA synthesis. These arrested cells then undergo histological and behavioural changes.

Although the age of a culture is expressed practically by the number of doublings undergone, an important question is, 'do cells measure age by the passage of time or by the number times they have divided? This question may be answered by slowing down division by reducing the serum in the medium, then replacing the serum to allow the cells to resume dividing until the strain dies out. Controls, in this experiment, were typically kept in a continuously proliferating state for about 32 doublings. Those maintained in a non-dividing state for 177 days became old after about 36 doublings. The total number of population doublings in each culture was essentially the same, yet those where division had been suspended lived 287 days and those kept in continuous division lived for only 77 days. This type of experiment shows that chronological time is less important than the number of divisions.

Several experiments have indicated the importance of environmental conditions in the control of doubling times of cell cultures. Reducing oxygen tension produced no significant difference in the lifespan of cells but increasing it to twice-atmospheric was extremely toxic, and shortened lifespan drastically. Other theoretical work suggests that this effect could be due to the oxidative effects of free radicals, but the natural anti-oxidant vitamin E has no effect on the lifespan of cultures.

The only agent that has been consistently shown to extend cellular lifespan is hydrocortisone, a human adrenocorticosteroid hormone. At a concentration only twice as high as that in human blood, cortisol extends the lifespan of cultures of human fetal lung fibroblasts 30–40%. DNA synthesis, measured autoradiographically, is also increased significantly by cortisol, although it does not reverse cellular ageing. On the other hand, cortisol added at different phases in the lifespan produced changes in lifespan in direct proportion to the amount of time the culture is grown in its presence. Cells appear to have precise recognition sites which identify the position of the atoms that are important to the stimulatory action of the hormone. Compounds which have these atoms stimulate DNA synthesis, whereas those which lack one or more are ineffective.

Autoradiographic and spectrophotometric analyses of cortisol-treated young and old populations of fetal lung fibroblasts have revealed that the hormone may act primarily in the Gi phase of the cell cycle, and delay the transition of cells from rapidly proliferating to the slowly or non-proliferating states. Cortisol maintains a higher percentage of cells in the actively proliferating pool at any passage but does not prevent the overall decline in cell division.

Because cortisol requires serum factors to stimulate DNA synthesis and cell division it may act by amplifying a primary systemic blood signal. This hypothesis is supported by the discovery that hydrocortisone significantly increases the uptake of labelled serum protein by young, but not old cells. This kind of experiment gives some insight into the difficulties of extrapolating from simple culture situations to multifactorial systems in the body. An extreme critical viewpoint is that the *in vitro* system is so simple that it cannot possibly give information that is relevant to the whole body state where there is an interplay of so many factors on the division mechanism.

Bearing in mind the vast amount of research on tissue culture ageing it is not surprising that several theoretical models have been proposed to provide a conceptual framework for the *in vitro* expression of the Hayflick limit. So far none of these theories can assimilate all of the phenomena.

The 'commitment theory' of Kirkwood and Holliday proposes that the natural condition for human embryonic fibroblasts is an immortal state. Upon division, cells can become committed to ageing at a relatively high frequency of about 0.275 per cell generation. Once the cells are committed to becoming senescent they can only go through a limited number of divisions. The chance process of commitment, and the relatively few uncommitted cells in young–middle-aged cultures can account for the variability in the lifespan between cultures. Holliday reported changes in the proportion of non-dividing cells with age that were consistent with the commitment theory.

The model of Prothero and Gallant proposes that the probability of commitment increases with increasing cell division. In their model they propose that once a cell is committed it is then able to undergo about seven additional doublings. This is brought about by an increase in the concentration of an antimitotic protein mediated by a positive feedback mechanism.

A similar molecular control mechanism has been proposed in the model of Shall and Stein. This model predicts a gradual rise in the concentration of a protein called the 'mortalization', or M protein, that competes with another protein, the D protein, for a binding site of an initiator gene for cell division. If the D protein, which is produced at a constant concentration, binds to the initiator site, the initiator is produced and the cell divides. However, if the M protein binds it prevents binding of D and irreversibly blocks production of the initiator, preventing cell division. Using appropriate values for the rate of increase of M, Shall and Stein obtained good fit to the growth of human and mouse fibroblasts as a function of time and also to the data on the mitotic labelling index of human fibroblast-like cells. In addition, this model can account for the immortality of cells through mutations occurring in the M protein or its binding site. It is also consistent with the heterokaryon results.

CELL CYCLES IN TISSUES

In the last year of life in the mouse intestine there appears to be the introduction of limiting factors into the G0 phase of the cell cycle (Figure 8.7). This conclusion comes from measuring the cell cycle dynamics in the mouse oesophageal epithelium. The experiment involved making a single subcutaneous injection of tritiated thymidine followed by autoradiography of mitotic figures at different times after the injection.

Similar changes in the cell cycle of human skin epidermis may be responsible for the 40% increase in the mean time taken for the horny layers of the skin to be replaced (Figure 8.8). Transit times of epidermal cells were calculated from the time taken for a dye applied to the skin surface to disappear in relation to the number of horny layers, determined by blister biopsy.

It could be that the increased time in G0 is related to failure of signals to activate the cell cycle. If so this could account for the slower responses of proliferation in response to the need for regenerative responses of wound healing. A general block in G0 could account for the delay in mitotic responses to external stimuli in old animals, such as the delay in activation of DNA

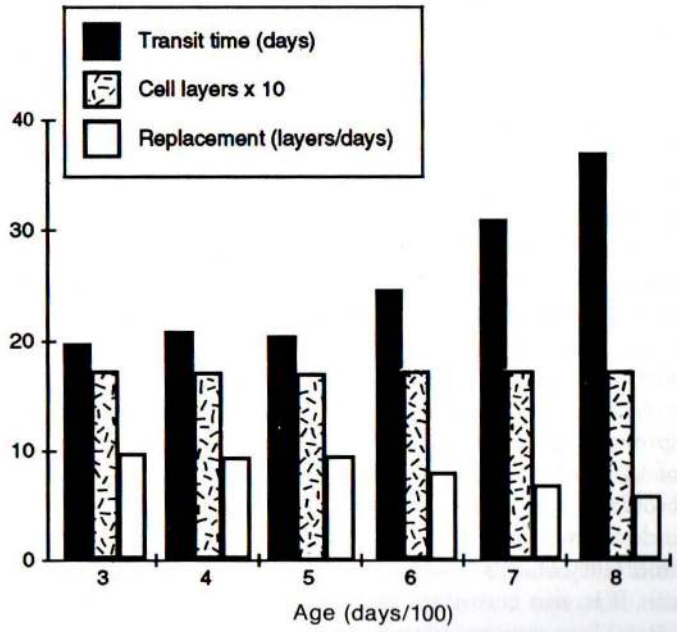


Figure 8.7. Mean duration of the cell cycle and its phases in the oesophageal epithelium of the mouse

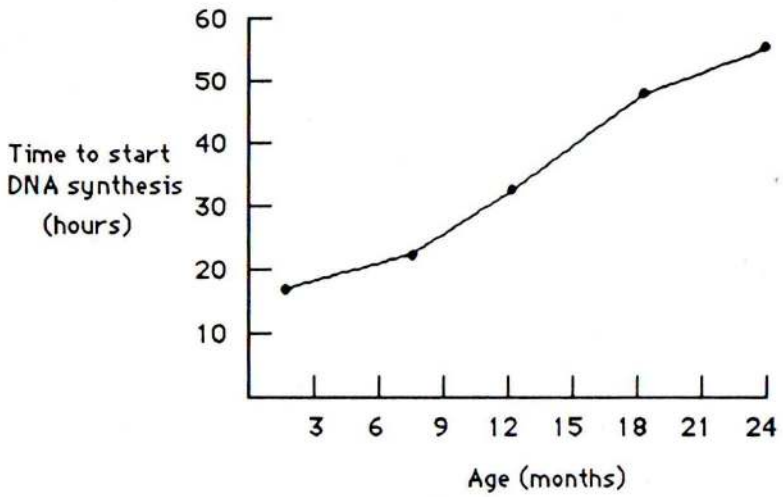


Figure 8.8. Age-changes in the turnover of human skin epidermis

turnover in the rat submandibular gland in response to the drug isoproterenol (Figure 8.9).

SUMMARY

Taken together, experiments on mammalian cell cultures and protozoan clones indicate that dividing cells as a homeostatic system may take up one of three different forms of behaviour. The rate of proliferation may decline to a point where the rate of cell death exceeds the rate of division, giving a fixed lifespan clone. The rate of division may slow down but still exceed cell death, giving cloned transplants that exceed the lifespan of the donors. Limited lifespan clones may be transformed by some kind of genetic release of error detection and repair systems, to give cell lines that appear to be potentially immortal. It is significant that some of the invertebrates, such as sea anemones, which do not appear to age, are maintained by the continuous proliferation of groups of embryonic type cells which also appear to be capable of forming gametes.

The difference between cells that conform to models in the first and second categories may be more apparent than real in that the cells may differ only in the rate at which their respective division mechanisms deteriorate. That is to say mammalian cells conform to a general pattern of development which involves a counting mechanism which transmits to the genome whole body development time and number of divisions already undergone to repress internal error detection and/or repair mechanisms. Furthermore, the gearing of the counting

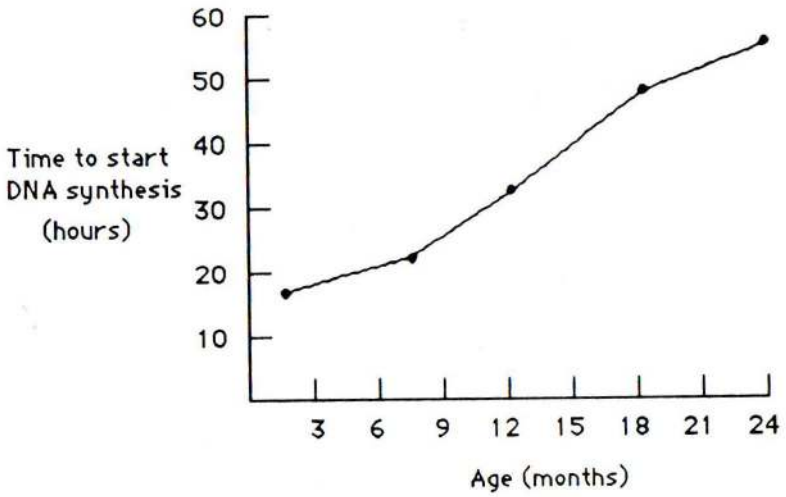


Figure 8.9. Age-delay in initiation of incorporation of tritiated thymidine into DNA of rat submandibular gland in response to isoproterenol treatment

mechanism appears to be under the control of genes which govern lifespan.

Normal human diploid embryonic fibroblasts (cell strains) undergo approximately 50 doublings *in vitro*; adult fibroblasts undergo about 20. Those cell populations which are capable of indefinite proliferation *in vitro* (cell lines) have abnormal properties and may behave like cancer cells. Thus, transplantable tumours are to cell lines as normal somatic tissue is to cell strains. The former populations have indefinite proliferative potential and the latter a finite lifetime. Fibroblast-like cells seem to age in the body in a way that causes them to lose division potential. This involves nuclear and cytoplasmic changes which may be transmitted experimentally to younger cell types to inhibit their proliferative capacity. Normal tissues such as skin, bone marrow, and mammary tissue, serially transplanted as their hosts age, have generally been found to lose the division potential of their mitotic and intermitotic cell populations. Thus, an *in vivo* counterpart for the *in vitro* results are known, but it does not seem as though the tissues in the body run out of division potential before death occurs from old age. The problem remains as to the relevance of tissue culture models and transplantation models to ageing of mitotic cell systems in the body.

CHAPTER 9 Ageing at the molecular level

Understanding the mechanisms of biological ageing must ultimately depend on knowing the changes that occur with time to molecules that comprise living systems. Furthermore, it is most likely that these chemical changes are of types that have been studied and about which quite a bit is known. That is, there would appear to be little justification at this point in looking for a new type of chemistry to explain ageing at higher levels of organization. The ways in which substances can change with time are quite limited.

It would be difficult to avoid the conclusion that the primary, or most fundamental, changes most likely occur in non-dynamic or non-renewable substances. Or more simply, for something to age it must remain present—or at least the rate of ageing must be greater than the rate of replacement. The only exception might be the accumulation of alterations in a self-replicating molecule or structure. (Kohn 1971)

MOLECULAR CONCEPTS

Ageing is a difficult area of knowledge to conceptualize. Concepts are aids to understanding and are therefore personal views. The ultimate in personal concepts is a textbook, which is structured to reflect the starting points and terminations of an individual writer, and is limited by the author's practical experience and his or her, depth and breadth of intellect. That is why Chapter 1 sets out an information network to help learners navigate the information in subsequent chapters, and extend it to build their own body of knowledge. It is based on a wide range of broad starting concepts within the experience of most people who have taken elementary courses in science. Every reader will no doubt have a preference for a particular entry point.

This chapter, in summarizing current molecular views of ageing, provides the opportunity to focus attention on what might be called the primary concepts. All subjects have a list of simple concepts which define the items of information available for conceptualizing more complex processes, systems and hypotheses. This elementary list represents a kind of inventory of primary concepts which define the simplest kinds of organization that are required to assemble useful secondary and tertiary ideas. Every personal body of understanding results either from the clustering of primary concepts to produce higher level

formulations, or the analysis of secondary, tertiary, etc. phenomena to identify the primary concepts which explain them (Figure 9.1).

Primary concepts of biology are 'species' together with the conceptualizations of planetary structure and solar radiation which govern natural selection in ecosystems. In as far as ageing is a biological phenomenon, 'species' and 'ecosystems' are also primary concepts in gerontology, particularly if ageing is regarded as the ultimate outcome of an evolved environmental strategy of resource utilization. In this respect, the genetics of ageing is moving towards a central theoretical and practical position. Whether ageing is due to programmed developmental events, or information loss, species and environment are seen to be central to the very existence of ageing. At a molecular level the operation of a programme and the need to cope with a progressive accumulation of faulty macromolecules gives a key explanatory role to DNA, its evolutionary history, its chemical integrity, and its functional biochemistry. Any molecular explanation of ageing has to connect DNA blueprints with the gradual appearance of a variety of evidence for age-dependent loss of the genetic control of chemical, structural and functional integrity (Table 9.1).

DNA AS A CONCEPTUAL STARTING POINT

Life expectancy and many other measurements of ageing must be determined from a consideration of species at the population level. Such species/environment viewpoints have also lead to several higher level conceptual models for predicting the effect of genetic alterations on length of life. These theoretical

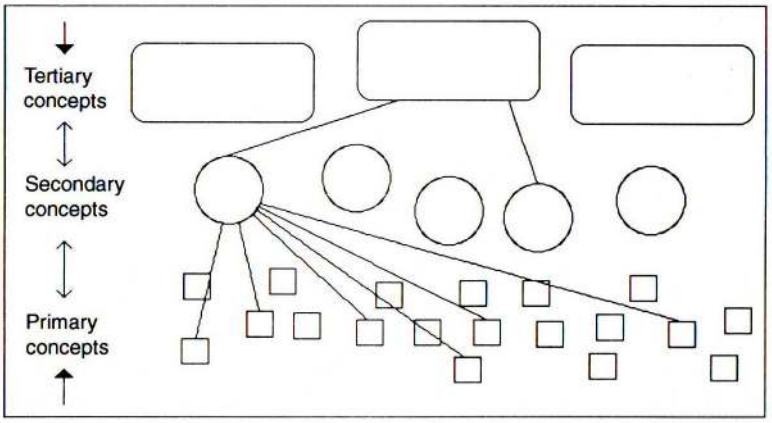


Figure 9.1. Diagrammatic representation of pathways of conceptual learning by bottom-up clustering (primary concepts) or top-down analysis (secondary/tertiary concepts)

Table 9.1. Some of the evidence for age-dependent loss of cellular control systems

Chromosomal aberrations
Increased binding of chromatin proteins to DNA
Indirect evidence for the accumulation of DNA–protein and Protein-protein covalent cross-links
Increase in the number of DNA breaks or nicks
Modifications of chromatin protein ie phosphorylation and acetylation.
Decrease in ability of ribosomal RNA to hybridize to purified DNA preparations.
Decrease in transcription ability of the chromatin, shown by *in vivo* and *in vitro* tests
Abnormal proteins probably resulting from post translational reactions
Accumulation of fluorescent pigments in a number of postmitotic cells
Accumulation of amyloid in tissues
Finite dividing potential of cells *in vivo* (serial transplants of mouse lymphocytes, alveolar duct cells of the mammary gland, and mouse bone marrow cells) and *in vitro* (human skin fibroblasts)
Loss of cells
Slower cell cycles
Slower rates of regeneration
Collagen cross-linking

treatments of ageing and evolution have already been reviewed and will now only be summarized.

Selectionist models take the view that either length of life is selected directly, or that selection for fitness directly results in lengthened life. For example, if there were significant investment in maternal or grandparental care, a direct selective force to extend lifespan beyond the reproductive period might exist expressed via 'lifespan prolongation' genes. On the other hand, length of life may be specified or determined primarily by the action of genes whose primary mode of action is to increase fitness during the child-bearing period but which still function post-reproductively to maintain the organism after the end of reproduction.

In contrast, non-selectionist concepts suggest that length of life is not adaptive and is not selected directly. Senescence and limited life result from reduced selection pressure at older chronological ages. In species such as mammals, the force of natural selection is a decreasing function of chronological age and leads to failure to eliminate genes that are deleterious only late in life.

Non-selectionist theory is widely accepted as a rallying point among evolutionary biologists who are interested in the evolution of ageing. On balance a mode of evolution through the actions of life-limiting 'gerontogenes' is favoured where the genes have positive actions on fitness in early life but negative actions that decrease the probability of survival at later times. An alternative model of 'mutation accumulation' postulates the existence of genes with age-specific action that have no role in early-life fitness but which act deleteriously late in life to limit lifespan.

Although, as yet, we cannot define the precise role of any gene in setting a species lifespan we do know that to survive, every species has to evolve ways of overcoming a variety of chemical processes which work against the existence of the complex substances and processes of living cells in all tissues. From this perspective a molecular interpretation of ageing should encompass the sum total of all reactions that contribute to the destruction of cellular organization together with those DNA systems that prevent these reactions prevailing. A selection of these reactions which have a bearing on the progressive accumulation of faulty macromolecules is presented in Figure 9.2.

They fall broadly into two categories according to whether the damage arises because of the inherent thermodynamic instability of the molecules themselves, which makes them sensitive to local physical and chemical impacts, or because synthesis errors occur during the assembly of nucleic acids and proteins. The latter possibility defines an important area of 'error theory' because the structural integrity of DNA, RNA and proteins rests on the accuracy of maintenance and use of interdependent templates which govern their assembly. Thus, synthesis of a DNA template requires a protein enzyme; protein synthesis requires an RNA template; the synthesis of an RNA template requires both an enzyme and a DNA template. Errors in making templates, which because they are the machine tools of the assembly line, will yield defective enzymes which may result in more errors in templates.

MOLECULAR AGEING BY INTERNAL FACTORS

Crystalization and Aggregation

An inorganic crystal is defined as lattice in which the largest number of ions and water molecules surround each ion of the opposite charge. For example, in a sodium chloride crystal every sodium and chlorine ion is surrounded by six sodium ions. The three-dimensional arrangement and the periodicity of each type of ion and associated water molecules depends on the charges and the size of the ions. Organic molecules also form crystals in which molecules are held together by covalent bonds or electrostatic van der Waal's forces. This is the situation with large and complex molecules such as protein, nucleic acids, lipids and carbohydrates. They contain parts with configurations of active groups and charged atoms that enable them to form regular crystalline arrays with corresponding charged segments of other polymer chains. In a stable environment crystals tend to grow (racemization) and their centres become isolated from exchanges with the environment. With time, crystalline regions will inevitably appear within organic polymers and these will alter their biological activity in terms of solubility and exchange reactions of surface charged groups. This defines crystallization as a basic process of molecular ageing.

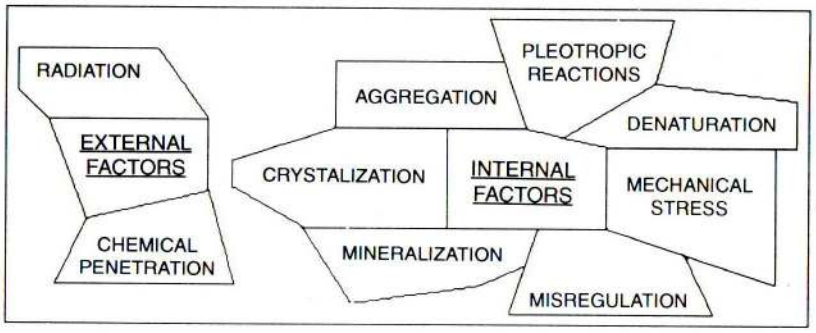


Figure 9.2. Conceptualised information clusters about the chemistry of ageing

Once started, crystallization is a progressive process. It results in a decreased solubility, loss of flexibility of chain polymers, an increase in ordered structures with cross-links, and terminates in aggregations of large units. Ageing of organic substances by crystallization outside the body takes place naturally in accord with thermodynamic principles and is responsible for paint hardening in a tin and the loss of extensibility in elastic and leather. This prompts manufacturers to increase the useful life of such products by adding inhibitors to the system. Cells are faced with the same problem and would be expected to have evolved 'anti-crystallization' strategies.

Autocatalytic aggregation of proteins is thought to occur by a variety of interactions between reactive groups on amino acid residues to form cross-linked chains. One of these possibilities, particularly relevant to living cells as sugar-based energy systems, involves the interaction of reducing sugars with amino acids (the Maillard reaction). A primary reaction is initiated between protein amino groups and the aldo and keto groups of sugars to form a Schiff base. A 'glycation sequence' is then propagated via the formation of reactive intermediates called 'Amadori products'. This produces more reactive secondary products which then interact and terminate in a variety of insoluble macromolecular complexes (Figure 9.3).

Denaturation

Chain-like macromolecules are first characterized by their chemical composition. This is their 'primary structure'. When such molecules possess repeating 'backbone' zones capable of forming electrostatic links within each chain, the chains themselves may become coiled. This is their 'secondary structure'. Where there are reactive groups scattered along the periphery of a chain, the coils may become folded or twisted into specific three-dimensional configurations. This is their 'tertiary structure'. In proteins the amino acid sequences of the

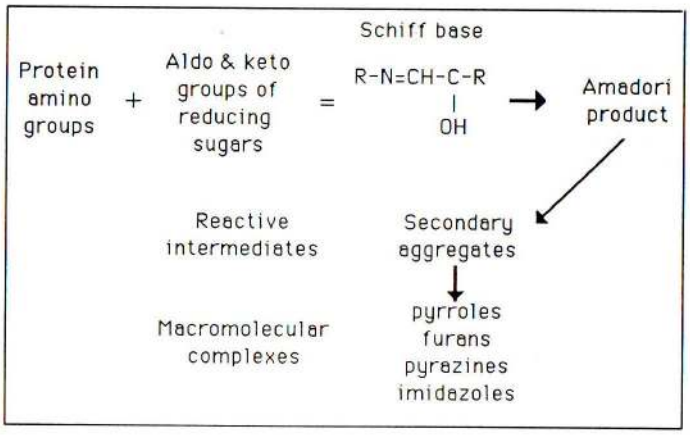


Figure 9.3. Formation of macromolecular aggregates by the Maillard reaction

polypeptide chain are the primary structure, the repeating peptide linkages comprise the secondary structure and the interactions between amino acid residues define the tertiary structure. The biological properties and functions of proteins and nucleic acids depends upon the maintenance of specific structures at all three levels.

Denaturation is usually defined as the loss of tertiary structure due to the rupture of bonds that hold components of the molecule in ordered, folded configurations. The macromolecule unfolds assuming a random state with increased entropy. Biological properties are lost and there is often a loss of solubility. Compared with the natural or 'native' state, denatured proteins are more easily degraded by proteases and may demonstrate active groups that were previously masked. Their catalytic activity depends on the tertiary structure so enzyme activity is lost. A certain level of abnormal cytoplasmic proteins may always be present in cells which results from post-translational modification involving spontaneous denaturation and deamination reactions.

Denaturation tends to occur naturally with the passage of time and it can be greatly increased by agents such as heat (thermal noise), light, increased pressure, physical stresses, surface tension, dehydration, change in pH, and interaction with chemicals that rupture hydrogen bonds or remove groups, such as NH and SH from the outer regions. From this point of view, the turnover of macromolecules, and maybe cells, could be part of a strategy to replace substances that have aged by denaturation occurring, either as an inevitable consequence of their structure, or because denaturation is an outcome of their function. The selection of 'replacement' genes for macromolecules must have occurred early in the formation of cellular systems based on protein enzymes.

Pleotropic Reactions

Denaturation is bound up with the existence of pleotropic reactions. These are defined as harmful side-effects of processes that are nevertheless vital for cellular survival. For example, the capacity to produce carcinogens from an inactive hydrocarbon decreases with increasing lifespan for different mammalian species. This is fundamentally a side-effect of the detoxifying enzyme systems for dietary hydrocarbons. Potential pleiotropic damaging agents, or by-products of useful metabolic processes, found in all mammalian cells are aldehydes, oxidizing agents such as O_2 and H_20_2 and free radicals such as 0′ and HO′. Particular attention has been focused on autoxidation, and the formation of various types of free radicals as a side-effect of cellular respiration and this has brought to prominence the free radical theory of ageing. However, all metabolic processes have a potential for producing collateral damage and the more enzymes and their products that coexist in a cell, the higher the probability of a harmful side reaction or pleiotropic effect. These are potentially available for forming highly reactive peroxides. Lipid peroxidation is a key secondary reaction, producing malonaldehyde, which can cross-link with the primary amine group of proteins, nucleic acids, or phospholipids.

Lipofuscin is thought to result from free-radical reactions of this kind, and in this respect it may reflect the presence of intrinsic pleiotropic ageing processes that have the potential to do serious cellular damage. As a marker for free radical damage it indicates the importance of metabolic rate in producing molecular damage and this is borne out by experimental work across mammalian species. A study of the accumulation of lipofuscin in non-human primates suggests that sexual maturity is a starting point. The first appearance of lipofuscin correlates well with brain weight. Thereafter the rate of cardiac accumulation in three species of primates correlates with their maximum lifespans. Rate of accumulation is also positively correlated with specific metabolic rate and inversely with brain weight. The relationship between the rate of lipofuscin accumulation and specific metabolic rate holds across a range of laboratory mammals. All of this is indirect evidence for pleotropic effects of cellular oxidation being an important determinant of mammalian lifespan. Work on insects also points in the same direction and will be discussed below in relation to repair strategies.

Amyloid is a fibrous protein associated with a large number of unrelated diseases where it accumulates often replacing cells that appear to be lost by age involution. Amyloidosis shows a general increase with age and is particularly prominent as a component of senile plaques in the central nervous system. Its proteinaceous nature and high content of fibrous structures indicates that it may be formed by some kind of crystallization process involving the formation of cross-links.

In the early 1960s, Szent-Gyorgyi and his coworkers found evidence of the presence in various plant and animal tissues of a substance which could retard

growth (retine) and of another one which could promote growth (promine). Initially retine was determined in thymus, aorta, muscle and tendon. Its isolation showed it to be a 4-hydroxy-2-ketobutyraldehyde belonging to a group of ketoaldehydes that are derivatives of glyoxal. These substances would result from normal metabolic processes by a non-enzymic conversion of trioses and triosphosphates. A series of ketoaldehydes was synthesized and found to inhibit cellular growth with a specific action on protein synthesis. The inhibition can be reversed by adding an equimolecular amount of cysteine. The known stochiometric reaction between cysteine through its thiol and amine groups and methylglyoxal explains these experimental data. Szent-Gyorgi suggested the idea that there may be a rise in cellular ketoaldehydes as a function of age, and this has been confirmed in liver and brain of rats at a level of about 0.03M. There is a 60% increase between 2 and 24 months of age. Treatment with folcysteine for 42 days reduced the level of liver ketoaldehydes in old rats by 50%.

The importance of regulating the pleotropic action of ketoaldehydes, particularly in relation to their action as inhibitors of protein synthesis, is suggested by the following facts:

1. Methyl glyoxal inhibits protein synthesis, an action that can be reversed by thiol compounds such as cysteine.
2. The existence of the ubiquitous glyoxylases which, as a system, consist of two enzymes with essential SH groups, and a coenzyme, glutathione, which transforms the ketoaldehydes into biologically less active oxyacids such as lactic acid.
3. There is a significant increase in oxidized glutathione and a decrease in active SH groups with age.

Mineralization of Tissue

Many tissues and blood vessels accumulate a number of minerals, particularly calcium, reaching levels likely to interfere with normal function. The classic tissue in which this principle was revealed is the human aorta (Table 9.2). On the basis of histological and biochemical studies it is concluded that changes in the elastic tissue of the arterial media, in particular its calcification, precede atherosclerosis of the intima. A large proportion may be bound to elastin because purified elastin isolated from the blood vessels contains calcium which increases with chronological age of the subject. Mineralization is also a feature of arterial ageing in laboratory rodents and mice in the wild.

Progeria victims, who probably have only one or a few abnormal genes, show an accelerated mineralization of tissues, in addition to other accelerated ageing processes, indicating that a simple regulatory process controls the rate of mineral deposition.

Table 9.2. Calcium, cholesterol and elastin content of atheroma-free human aortas

Age	Medial			Intimal	
	Calcium	Cholesterol	Elastin	Calcium	Cholesterol
18	0.26	0.74	41.4	0.05	0.75
19	0.32	0.58	36.4	0.07	1.51
21	0.72	0.78	48.6		0.87
24	0.77	1.20	44.5		
35	1.14	0.99	47.6	0.10	1.94
37	1.89	1.09	43.3	0.13	2.42
44	2.35	1.47	42.1	0.15	1.82
46	2.04	2.51	45.6	0.26	1.54
56	7.10	2.51	40.2	0.26	3.11
57	3.14	1.58	38.8	0.18	2.94
65	4.59	2.60	40.6	0.17	1.19
68	9.22	2.89	45.8	1.46	5.72
70	9.75	1.92	40.2	0.39	3.74
78	9.84	2.92	39.2	0.31	1.45

Medial calcium is calculated as percentage of dry fat-extracted elastin; intimal calcium as percentage of dry weight of intima; cholesterol as percentage of dry weight of either intima or media; medial elastin as percentage of dry, fat-extracted media.

Misregulation of Enzyme Systems

Enzyme activity is a highly regulated process characterized by very tight controls on the concentration of those that are rate limiting. For many enzymes there is also feedback regulation of activity in relation to the levels of both substrate and product. The former sometimes involve the activation of genes for enzyme synthesis, and these so-called induceable enzymes may be augmented as the result of a complex pathway of information transmission from substrate availability, through the endocrine system, to the hormonal activation of genes in a target organ that make more enzyme. Certain liver transaminases responding via the adrenocortical system fall into this category of induced enzymes.

Measurements of enzyme activity in relation to age have not shown any consistent pattern, either between tissues or between enzymes in a particular tissue. A collection of 95 sets of data on rodent enzymes is shown in Figure 9.4. Although again, it is difficult to make suitable comparisons because of differences in the age range of animals studied and the various techniques used, this number of experiments probably reflects the best possible random sample available across age and strains. It indicates that most enzymes do not change their activity, expressed, either per unit protein or DNA. Those that show an increase are just about balanced by those showing a decreased activity. These shifts in the specification of enzyme patterns, although not indicative of a

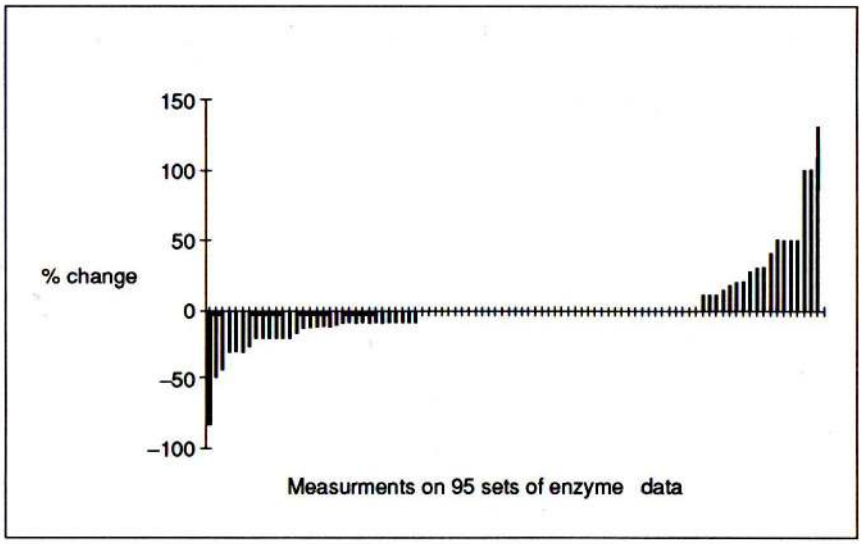


Figure 9.4. Age-changes in various enzymes in rodent tissues

general loss of the capacity to synthesize enzymes are consistent with the view that the youthful genetic specification of tissue biochemistry cannot be maintained.

Liver tryptophan peroxidase in rodent liver is an adaptive enzyme and is synthesized above a baseline in response to an injection of tryptophan. Both this enzyme and tyrosine transaminase are increased after the injection of adrenal glucocorticoid hormones of the cortisol type. The maximum responses are shown in Table 9.3, which also shows that there is no age-dependence in either the basal levels, or the responses to substrate and hormone. However, no general principles are revealed because another hormone-induced rat liver enzyme, fructose 1,6 diphosphatase, shows an age-related decline in response to its inducer glucocorticoids. Between the ages of 1 and 15 months a twofold increase in diphosphatase is reduced to a 30% increase to a standard injection. Putting these differing trends of enzyme induction into an evolutionary perspective would imply that some advantage acrues to the selection of a gene, or genes, which gradually reduces the sensitivity of fructose 1,6, diphosphatase synthesis to adrenocortical regulation. The difficulty in assessing the survival value of such an advantage, particularly in the context of one out of thousands of proteins, is an indication of the weakness of our understanding of the evolution of ageing.

The search for an explanation of age-related failures to maintain a youthful pattern of protein catalysts must, on the one hand, involve examining ways in

Table 9.3. Response of two induceable liver enzymes in rats of different ages

	Treatments*					
	12–13 Months of age			24–26 months of age		
Enzyme	None	Amino acid	Corticosterone	None	Amino acid	Cortisol
Tryptophan peroxidase	11.1	70.4	35.6	12.7	71.4	35.1
Tyrosine transaminase	103.0	—	338.0	96.0	—	423.0

Activities are expressed as μmoles of enzyme product formed per gram dry weight of liver per hour. None of the differences between groups is statistically significant.

which ambiguity can be introduced into the flow of information from DNA which specifies the type and quantity of proteins, and on the other, the postsynthetic inactivation of catalytic centres by denaturation.

Much of the debate about misregulation of polymer synthesis has centred on the idea that if errors arise in the transcription–translation machinery of DNA they become self-perpetuating. An inevitable sequence follows in which there is a gradual but irreversible and accelerating breakdown in the correct coding of DNA leading by positive feedback to what has been termed an error catastrophe. There are other worthwhile hypotheses which take different viewpoints, one of which, presented next, directs attention to the ossibility of errors in protein assembly involving changes in the ribosome matrix.

The Polysome 'Faulty Matrix' Model

Neither the rate of synthesis, nor the sequence of bases or amino acids that are assembled to produce specific polymers, is determined absolutely by the genetic code. There is an opportunity therefore for ambiguity arising through variation in the properties of the polysomes containing assembly templates for both protein and RNA synthesis. The mainline of practical evidence is that, under certain strict experimental conditions, the polysome matrix influences the specificity of amino acid incorporation. For instance, it has been shown *in vitro* that the accurate translation of synthetic messenger RNA depends on the conformation of RNA as it is presented in ribosome-messenger systems. Furthermore, distortion of this conformation results in altered rates of amino acid incorporation, and gives rise to incorporation errors. An incorporation error is where, for example, leucine is inserted in place of phenylalanine in systems which code exclusively for phenylalanine. Other experiments have shown that gamma irradiation of RNA polymerase, which is assumed to produce structural distortions, results in transcriptional miscoding.

Age-related changes in the matrix of polysomes have been inferred from spectrophotometric analysis of isolated suspensions of ribosomes separated into size classes in sucrose density gradients. This experimental dimension of ribosome size coincides with a marked and progressive change in the density of RNA. The larger the ribosomes, the more open the RNA structure and the less the hypochromicity of the separated fractions measured by the absorbance ratios at 254 and 300 μ. Using this procedure it appears that there is a change in the variability of ribosomal structure favouring a more open configuration (an increase in the organizational entropy (Table 9.4). Confirmation of this comes from the increased susceptibility of RNA in ribosomes of old rats to hydrolysis by RNAase. It may be of practical importance that an inosine compound belonging to a family of drugs which improve learning ability in rats appeared to produce ribosomes with a less variable, more youthful, structure. This type of model may have a bearing on questions concerning the loss of cognitive function in old age and ideas that protein synthesis is involved in supporting and fixing the flow of conceptual information in intact brain.

MOLECULAR AGEING BY EXTERNAL FACTORS

The major external factors that could be rate limiting for lifespan are radiation (heat, UV, cosmic rays and sources of radioactivity) and damaging chemicals that enter the body in food or via the skin. Increasing temperature within the normal range of life accelerates denaturation and in this context normal temperatures of about 37° C are harmful. Homoiothermy may represent a trade-off for increased efficiency of enzymatic reactions against a high rate of thermal denaturation of cellular components. Work on insects shows that adult lifespan is inversely related to environmental temperature but there is no evidence that deaths at high temperatures are due to an acceleration of a normal pattern of ageing. Ultraviolet radiation is particularly damaging to skin where it

Table 9.4. Changes in the polysome matrix of rate brain ribosomes in relation to age and treatment with inosine-dimethylamino isopropanol (NPT)

	Organizational entropy index*
Young	6.2
Old	17.3
Old treated with NPT	7.4

*Standard deviation of the mean orthochromicity in the largest 40% of polyribosomes (expressed as % of mean). Orthochromicity of the RNA of the separated fractions is measured experimentally as the optical density/μg RNA in each fraction in the native state relative to that after KOH hydrolysis of the fraction.

appears to damage DNA, and is the cause of increased skin cancers in habitual sunbathers. This skin model has proved useful in investigating DNA repair mechanisms. However, most experimental work has been carried out on the life-shortening actions of ionizing radiation. This area of research became popular in the mid-1960s, coinciding with the popularity of studies in radiation biology, when it appeared to offer a way of controlling ageing in the laboratory.

The administration of sub-lethal doses of whole body radiation early in life appears to increase the rate of ageing. This is evident in the mortality curves which appear to be shifted towards the left, the amount of displacement being dose-related (Figure 9.5). It was observed that those who did not die of acute injury appeared to recover but with the passage of time they lost hair, looked debilitated, demonstrated decreased activity and died at increasing rates. All of these changes occurred earlier than similar changes associated with natural ageing.

The age at radiation is a major determinant. Mice are at their least sensitive at about one year old. From weaning to one year of age the sensitivity of mice given 100r radiation decreases from 6% to 3% shortening of remaining lifespan. At ages above 1 year the sensitivity increases, reaching 6% shortening of remaining lifespan at about 100 days. This curve resembles that for acute deaths at higher doses when mortality in young animals is due to effects on stem cells. In older animals loss of stem cells is associated with increased sensitivity to all kinds of stress.

The search for unambiguous evidence that low doses of radiation accelerate normal ageing processes has been unsuccessful. One complication is that the

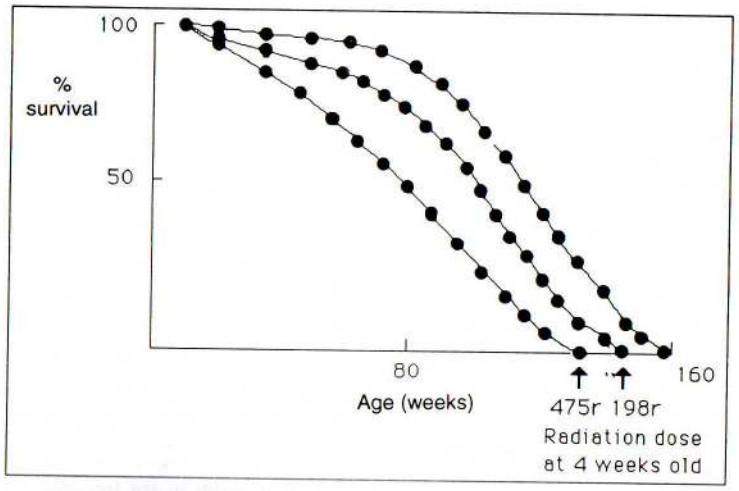


Figure 9.5. Survival curves of mice given two doses of whole body radiation at four weeks of age

incidence of all kinds of cancers, particularly leukaemias, is increased. Also, diseases characteristic of normal ageing appear earlier, but their incidence pattern varies from normal. Chromosome damage visible in liver cells is increased as an initial response, but there is no correlation with the altered mortality pattern, and the chromosome aberrations gradually decline with age. The latter phenomenon supports the idea that there is a norm set for a permissible level of chromosome damage. Connective tissue cross-linking, taken as an indicator of age in normal mice, is not affected by doses of radiation that have long-term life-shortening effects. All of the evidence indicates that the effect of radiation is not a straightforward stimulation of some kind of central timekeeper.

POSSIBLE CHANGES IN THE WORKING GENE

Examples of internal factors producing DNA damage are:

1. Normal cellular metabolism, which generates free radicals and reactive metabolites capable of cross-linking DNA to proteins, RNA, and itself.
2. Metabolism of certain externally originating molecules, producing excited molecules which form DNA adducts.
3. Heat from body temperature, which causes a loss of bases and subsequently single-strand breaks and single-stranded regions in cellular DNA.
4. The action of enzymes which can degrade DNA.

Examples of external factors producing DNA damage include:

1. Ultraviolet light, which joins some adjacent bases to form a bulky product which distorts the helix.
2. Gamma rays and x-rays, which generate free radicals and thereby alter or remove bases.
3. Chemical mutagens and carcinogens, which either bind to DNA and form adducts, produce DNA strand breaks, or slip between bases to form non-bound intercalations.
4. Ultraviolet light used in conjunction with certain chemicals causing chemically mediated DNA–DNA, DNA–RNA, and DNA–protein cross-links; and viral DNA can insert into the host DNA, altering the information content.

Both internal and external factors affect the working genes either by calling into action a gene-regulated process to counteract the change or by receiving damage which affects its normal working. Molecular ageing factors therefore

have to be considered against the structure and mode of working of the genome.

The activation of structural genes in higher organisms is assumed to proceed by mechanisms whereby the genes are regulated by adjacent parts of the genome broadly defined as chromatin. There may be several of these special regulatory sequences of DNA governing a particular structural gene. The regulator sequences are, in turn, controlled by activator proteins that are coded by integrator gene sets. Particular activator proteins can activate several regulatory sequences (Figure 9.6).

An integrator gene set is responsive to a special effector which activates its gene set by combining with a complementary sensor gene. In this way it is envisaged that a range of environmental variables all acting at the same time, by changing the pattern of hormones and metabolites, which are likely to function as effectors, could produce subtle changes in the pattern and rate of gene expression. On this model it is possible for a change in one specific metabolite to alter the expression of a number of gene loci.

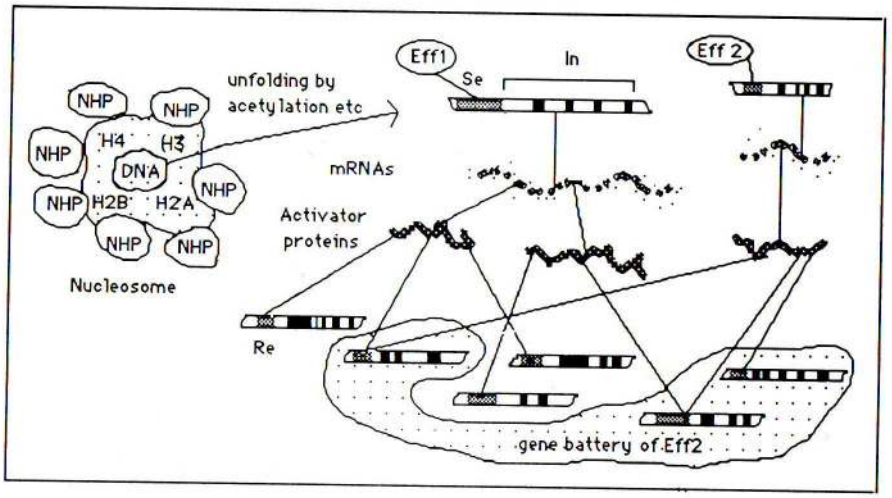


Figure 9.6. The Britten-Davidson model of gene regulation. DNA is made active by reactions that release it from the nucleosomes. Effector molecules (Eff1, Eff2) are then able to interact with sensor regions (Se) of the extended DNA. The sensor regions then activate an adjacent set of integrator genes which produce corresponding messenger RNAs. These code for a range elsewhere on the DNA, thereby activating a linked set of structural genes (St). In this way it is possible for a particular effector molecule to activate a battery of genes.

Potential Sources of Molecular Errors

Taking the Britten-Davidson model as representing the present consensus of the way in which genes are expressed at the biochemical level enables several potential sites to be identified where ageing could affect the pattern of tissue enzymes. Some of these potential sites are associated with the complex structure of the gene, which is a functional combination of DNA with several well-defined histone proteins and other non-histone proteins that are not so well characterized. Other possibilities may be defined in terms of what we know of the way in which the DNA code is expressed and regulated (Figure 9.7).

Unfortunately, all current models of working genes are tentative in that, although the main properties of DNA and its associated structural and enzymic components are not in doubt, the details of the controls and feedback systems are far from clear. In this context, work on the ageing genome awaits information from cells in the early stages of development. Also, it must be said that we know very little in principle about the means used by cells to detect damage to the components of the genome and the ways in which repair or replacement are undertaken. Nevertheless, despite these uncertainties, it can be said that, so far, research on ageing has not brought to light any outstanding evidence for a major disturbance to the model.

Actual DNA Damage

With regard to structure, many studies have been made on the possibilities of modification of nucleosome histones, cross-links between DNA strands, nicks in

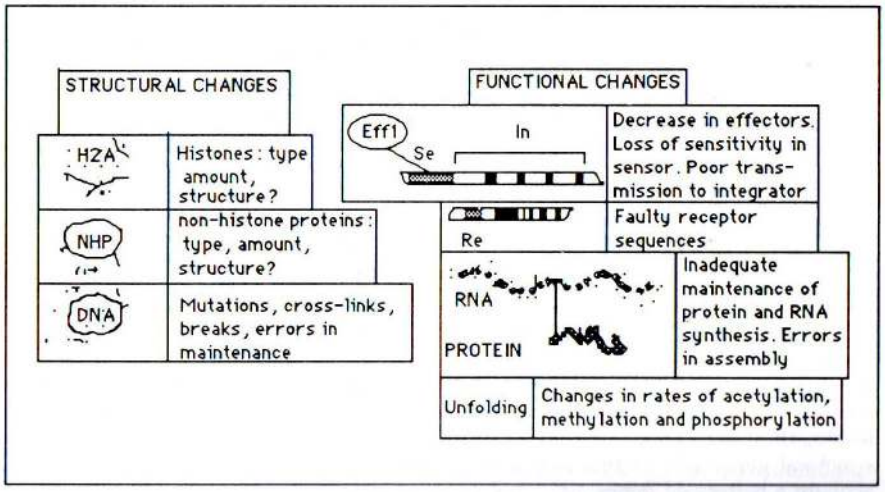


Figure 9.7. Sites of potential deterioration in the biochemistry of gene expression

the base chains, alterations in template activity, and changes in sequences measured by melting temperatures, etc. At the functional level, measurements have been undertaken to define the activity and fidelity of DNA polymerases, and DNA repair by directing attention not only to the biochemical systems centred on the nucleic acids but also on the properties of the proteins which they produce. As outlined in the beginning of this section, DNA can be damaged by physical, chemical, or biological agents generated internally or externally. These cause DNA breaks (breakage of one or both strands), distortions (kinking or opening up of the intertwined strands), and adducts (addition of extra molecules to the DNA).

The potential effect of these lesions relates roughly to the size of the distortion of the DNA helix. Small adducts interfere with DNA replication only slightly, whereas bulky adducts, joined bases, intercalations, cross-links, and strand breaks are the source of many cellular dysfunctions. Nevertheless, small non-distorting damage will have a substantial effect if it alters the information content of the DNA, as do base modifications which cause the wrong RNA bases to be matched with DNA bases.

Many of these types of DNA damage have been shown to accumulate with age: DNA-protein cross-links, single-strand breaks, single-stranded regions, and chromosomal aberrations. The lack of a breakthrough could be because the correct site for a fundamental age-dependent failure of the genome has not yet been identified. On the other hand, it could be that that the expression of genes in the bulk of old cells is close to normal. Another possibility is that any failure quickly results in cell deletion or that the age-changes are so small that they have escaped detection by the relatively crude methods presently available for locating damage points. Reports of changes in both chromatin structure and function in old animals that have subsequently been contradicted by further work, emphasizes clearly that there are problems of technique.

MOLECULAR AGEING IN CULTURED CELLS

Nuclear Regulation

From transplant research and *in vivo* assessment of normal proliferating systems it is clear that people and experimental animals do not die because their cells 'run out'. From this point of view cell cultures cannot answer the question why do people die of old age. On the other hand cell cultures can be used as models to learn about the molecular aspects of senescence.

In particular, tissue culture experiments have been set up to find answers to the following questions.

1. Does the error rate increase with age?
2. Can premature senescence be caused by translation errors?
3. Do enzyme activities or numbers of molecules decrease with ageing?
4. How much translational error is necessary to cause cell death?

From this kind of work it is now accepted that results are consistent with the idea that errors occur during the flow of information from genes to proteins. However the peculiar characteristics of cell cultures, which make it difficult to set up definitive experiments allowing extrapolation of results to cells in organs, means that so far the questions, although they have produced precise results, have not provided definite answers.

On the whole, cell fusion studies appear to support the idea that the termination of cell division is a regulated process and not simply the outcome of accumulated random errors. It appears that overall control of proliferative potential is regulated by nuclear functions, because the growth potential of young cells fused to senescent cytoplasts is no different from controls. There appears to be an intrinsic cytoplasmic inhibitor of DNA synthesis in senescent cells, but the evidence is indirect. Cell reconstruction studies have been carried out after exposure to cytochalasin B, which allows the nucleus to be freed from its cytoplasm. After centrifugation, and fusion of old or young cytoplasts to old or young nuclei surrounded by small residual amounts of cytoplasm (karyoplasts), the behaviour of the new cells indicates that the nucleus is primarily involved in determining the fixed lifespan.

There is a diminished growth potential in cells containing cytoplasmic or nuclear components derived from senescent cells, indicating that the *in vitro* lifespan is also partly under cytoplasmic control. The nature of such cytoplasmic regulatory activities has not been defined, but it is known that specific genes are activated following fusion to cytoplasts.

Error Catastrophe

The error catastrophe theory has dominated discussion of the theoretical possibilities for template changes. Direct measurements of the accuracy of protein synthesis are difficult to make and the few direct tests for sequence alterations in late passage cells have failed. Experimental work has involved the search for altered enzymes using indirect measurements of changes in primary structure, such as heat lability and immunochemistry as markers. Heat-labile glucose-6-phosphate dehydrogenase has been detected in normal senescent human fibroblasts as well as fibroblasts from donors with the Werner and Hutchinson–Gilford syndromes. Also, increased levels of enzymically inactive immunologically cross-reactive lactate dehydrogenase has been detected in late passage fibroblasts from normal donors. Other work has failed to confirm these findings and prompted discussion about whether the difference can be reconciled by postulating that increased thermolability may be associated with developmental differences in enzyme structure.

Another approach has been to measure the rate of viral replication as a test for accuracy in replication of the nucleic acid system. Results showed that both

RNA and DNA viruses were able to replicate in old cells to the same extent as in young cells. New viruses produced in old cells were as viable as those produced in young cells, and their thermostability and mutation rate were not affected by the age of the host cells. Other tests with herpes virus have revealed wide differences in the reversion frequency between temperature sensitive mutants. The reason for these apparently contradictory results is unknown.

The present consensus is that the molecular basis is much more broad based that the Orgel theory although it is not possible to disprove or prove it.

Somatic Mutations in Cell Cultures

With regard to ideas about somatic mutations it has been argued theoretically that if about 95% of the critical genes are located on the autosomes, then mutation rates about 100-fold higher than those known in fibroblast-like cultures would be necessary to limit the lifespan to 50–60 population doublings. Further, mutations on the X chromosome would result in a lower fraction of dividing cells than has been reported.

Experimental studies suffer from the same difficulties as the search for mis-specified proteins and have not been conclusive. Several workers have approached the problem by trying to increase the level of somatic mutations using mutagens. Again the results are not clear-cut. The response to these mutagenic agents with respect to growth potential and proliferation rate behaviour is influenced by a range of variables; the species and age of the donor, the passage number, the method of assaying growth potential and the fact that decreased viability is a relatively non-specific biological test. Cell hybridization work shows that somatic mutations are not a primary cause of tissue culture ageing. Fusing young cells with old cells cannot restore cellular morphology in old cells and has no effect on cellular proliferation. Hybrid cells do not have greater proliferative potential than the parental cells as would be expected if youthful chromatin was able to complement random recessive mutations. Also, diploid and tetraploid human fibroblasts appear to have the same *in vitro* proliferative potential.

Strand Damage

There are several reports of increased damage in the DNA of late passage cells. Other research on late passage foreskin cultures has demonstrated an increased number of sites sensitive to *Micrococcus luteus* extracts in purified chromatin preparations. There is also evidence for increased single-strand breaks in fetal lung cultures. In no case have these changes been linked to the loss of proliferative activity.

All of these results might only apply to single copy genes, and loss or inactivation of reiterated DNA sequences may be extensive and of greater significance as a primary ageing mechanism. Some evidence for the latter suggestion comes from work on postmitotic tissues showing a loss of sequences in ribosomal RNA, and from a specific family of repeated sequences of DNA in old cultured fibroblasts. Extrachromosmal circular DNA derived from sequences situated between clusters of repeated (alu) sequences is amplified. The loss of repeated DNA sequences has been proposed as the timing mechanism for expressing a DNA synthesis inhibitor which appears in old cell cultures. The basis of the mechanism is a gradual loss of repeated DNA sequences that maintain gene repression. Olovnikov's 'marginotomy' model proposes that end regions of DNA, defined as 'telogenes' may not be replicated. These small buffer sequences are lost during replication and when all of the buffer DNA has been lost, the ends of actual structural genes can then be lost and the cell division is no longer possible.

PROTECTION AND REPAIR

Protection repair and replacement act as anti-ageing processes to reduce the rate of accumulation of cellular damage. The following systems have been taken as evidence for the existence of anti-ageing strategies:

1. Skin systems that repair UV damage to DNA. These have been localized in a specific region of the XPAC repair gene using the xeroderma pigmentosum skin model.
2. Microsomal mixed-functional oxidative systems.
3. Protein and turnover of cells and their organelles.
4. Free-radical scavengers and antioxidants such as vitamins E and C, selenium, and glutathione. Superoxide dismutases inactivate the O radical, and glutathione peroxidase destroys lipid peroxidases. Experiments have also demonstrated that dietary antioxidants inhibit the development of amyloid in mice.

There is also experimental evidence indicating that membrane instability limits the viability of cells in culture and the whole body lifespan. A number of drugs, particularly corticosteroids of the cortisol type, salicylates and antihistamines prolong the life of fruit flies. Corticosteroids, aspirin and polyvinylpyrrolidone prolong the *in vitro* survival time of several cell types. These diverse compounds have little in common at the chemical level but practically all drugs which produce significant life-prolongation actions in fruit-flies and isolated cells are known or suspected stabilizers or protectors of cell and organelle membranes. It may be that membrane stabilizers, like antioxidants, protect

cellular membranes from free-radical damage and lipid peroxidation.

It has been shown that genes in the brown locus (b) segment of the mouse chromosome-4 influence longevity. The b region also contains the mouse homologue of a gene that complements the human excision repair defect associated with the xeroderma pigmentosum complementation group A (the XPAC gene). Work implicating this locus in DNA repair may be connected with the genetic factors that seem to confer immortality to cell lines in tissue culture. By forming hybrids between various immortal lines and screening these hybrids it has been shown that there are at least four distinct complementary groups for indefinite division. Human chromosomes 1 and 4 each harbour genes for at least one such complementation group.

One important argument against unrepaired damage being a determinant of ageing is the commonly held multistage hypothesis, particularly as developed by Burch to explain baldness and patches of skin pigmentation, as well as cancer. This requires a point effect, such as a somatic mutation, to initiate a sequence of changes which eventually produces a visible or measurable outcome. It was developed as an explanation of the log-linear relationship between age and probability of a relatively rare disability. The most commonly used mathematical models for the increase in the incidence of degenerative diseases with age are all various forms of the multistage model. These postulate that a disability requires several independent rate limiting steps and that the change from normal to the disease state can take place in distinct stages, each with its own causes. However, the insistence on using a multistage model produces a difficulty in that it is necessary to postulate similar numbers of rate-determining stages for very different outcomes. An alternative model assumes the amount of physiological damage in members of the population at a given age will follow a probability distribution characteristic of the population and the process. An individual having a value above a critical cut-off level will have a specific disability. The model relates the amount of deterioration at a given age to the corresponding probability of impairment, and can give a log-linear relationship between age and probability of a relatively rare disability. It fits the incidence of a wide range of disabilities including hearing, vision and mobility and sits more comfortably with ideas of the statistical amount of unrepaired damage in particular cellular systems being a causal factor.

This approach, coupled with demographic ideas that longevity genes set a slower rate of ageing, and a high threshold for acquiring chronic disease, may help explain why people over the age of 90 often survive in good health until shortly before death. Their mortality rates are much lower than would be predicted by extrapolating from the death rates of younger individuals. Morbidity and disability appear to be compressed into a shorter period, there is a very low incidence of conditions such as Alzhiemer's disease, and a tendency for men to live longer than women (gender crossover).

EXPERIMENTAL MODELS

Amidst the mass of uncertain and often contradictory evidence, five carefully organized series of experiments stand out as pointing to fundamental connections between DNA biochemistry and ageing.

1. The first is the finding of a positive linear relationship between the lifespans of mammals and the ability of species to detect, excise and repair faulty DNA.
2. The second approach involved using the technique of molecular hybridization to test for the loss of nucleotides and led to the conclusion that rRNA genes are lost from human myocardium at the rate of about 0.5% per year, with a 50% higher rate of loss for the brain hippocampus.
3. The third experimental approach used temperature denaturation of chromatin to reveal changes in the association of non-histone proteins with DNA.
4. The fourth approach using isolated hepatocytes and hepatocyte nuclei has shown that the molecular lesion responsible for a progressive age-related repression of genetic information is an impaired ability to initiate transcription because of a loss of chromatin binding sites for RNA synthesis.
5. The fifth approach is an exploration of the relationship between repeated reproduction at an old age and its effect on the stability of longevity of offspring.

The next section summarizes findings in the fifth approach which points to the importance of extranuclear influences on the rate of ageing.

Extranuclear Factors in Lifespan Shortening

Beginning in 1947 Lansing working with different species of parthenogenic rotifers observed that extinction of a strain could be induced when that strain was reproduced generation after generation at an old age. The bearing of these results on ageing was clear from the signs of precocious ageing which accompanied a progressively decreasing longevity. Since then this 'Lansing effect' has been confirmed in several insects, and a crustacean. A similar phenomenon has also been reported in the experimental vegetative reproduction in the pond weed *Lemna minor* where mother frond age affects daughter frond area. Daughter fronds produced by old mothers also have shorter lives and fewer offspring than their 'sisters' produced when the mother frond was younger.

In Lansing's original experiments he started with a wild strain of the rotifer *Philodina citrina* with a mean lifespan of approximately 23 days. He established

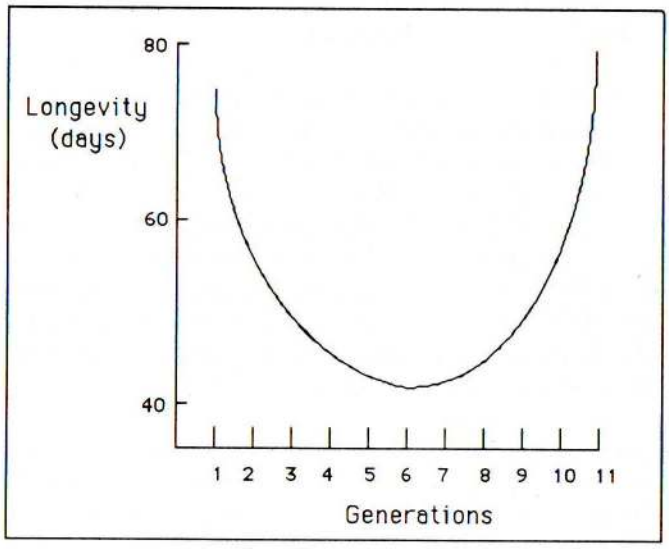


Figure 9.8. Diagrammatic representation of the influence of repeated reproduction at an old age of lifespan of offspring in female *Drosophila*

orthoclones from eggs laid in the 5th, 11th and 16th days of life. In the 5-day orthoclone maintained for seven generations he noted a small increase of the mean lifespan. In the other orthoclones, although the mean lifespan and the mean total number of eggs laid did not decrease significantly, the 11-day orthoclone became extinct after four generations and the 16-day orthoclone after only two generations. In both orthoclones the extinction was due either to the non-development of eggs, or the early mortality of individuals hatched from the eggs, laid on the llth or 16th day respectively. His deduction was that the age of the mother determines the longevity through the action of a 'transmittable and cumulative ageing factor'.

In a second series of experiments Lansing showed that the extinction of the 11 and 16-day orthoclones could be prevented by reproducing them at 5 days. By adopting this procedure the mean expectation of life increased again. From this he deduced that the production or action of the postulated transmissible ageing factor was also reversible and was thus cytoplasmic.

In a third series of experiments he repeated the results and also demonstrated that the extinction of the old orthoclones was preceded by a decrease of both the mean lifespan and mean fecundity of the successive generations.

Later experiments by Lints and Hoste on six lines of *Drosophila* with different

selection programmes, whilst confirming the existence of the Lansing effect, added two new dimensions to it (Figure 9.8):

1. Constant reproduction at both young and old age decreased the mean longevity of the offspring. However, the decrease in longevity per generation was much larger when the reproduction occurs constantly at an old age.
2. The decrease in longevity does not culminate in extinction of the lines but only takes it to a point after which the downward trend is reversed. That is to say, as in Lansing's experiments, the phenomenon is reversible but the reversibility involves the operation of some kind of feedback mechanism in the strain itself. These features bring new insights into the Lansing effect.

All interpretations of the results start with questions about ways in which, as a function of parental age, modified flows of information are passed from one generation to the other through the ova, or through the ova and sperm. The experimental transplantation of differentiated nuclei into enucleated eggs has indicated the influence of composition and configuration of the ovum on gene expression. From this standpoint these results raise questions about the relevance to the Lansing effect of work on embryonic cytoplasmic determinants.

Embryological research has provided many examples showing that the genomic expression of a cell can be changed by the cytoplasm very successfully. Attempts to connect embryological principles with phenomena of ageing were made in the late 1960s, particularly by Muggleton-Harris. This research showed that transplanted nuclei from embryonic and adult lens cells in the leopard frog, *Rana pipiens*, have a limited capacity for participating in cleavage and development. Experiments with isolating media and conditions under which the donor cells were selected, and nuclei were transplanted, showed that some of these restrictions can be diminished or rectified. One important factor was that the ability of a nucleus to support cleavage and development did not depend entirely on the differentiation, or age, of the donor cell. The one necessary factor leading to consistent success was to ensure prior selection of the donor cell from a group of cells which had recently undergone mitosis and were synthesizing DNA. One of the major reasons suggested for failure of amphibian nuclear transplants to complete normal development was restrictions in the form of condensed or compact chromatin of the nuclei.

Experiments with protozoa show that self-replicating macromolecules contained in a small amount of 'aged' cytoplasm is sufficient when injected into a 'young' cell to bring about a form of behaviour typical of the 'aged' cell from which the cytoplasm was obtained. The concept of cellular 'homeostats' was put forward to explain this, and may apply, in reverse, to the above amphibian experiments, and have relevance to the fact that the environmental stimulation of dying clones of *Paramecium* to conjugate rejuvenates them.

This raises important questions as to the nature of differences and similarities between mortal somatic cells and immortal cells of the germ line. Work across a wide range of living organisms has demonstrated that ageing of reproductive DNA is counteracted by periodic rejuvenation. This takes place by a variety of processes such as recombination and meiotic haploidization, cyclic regeneration of systems for transcription and translation during gametogenesis and early development, and the selection of stable, viable genomes at various stages of the reproductive cycle. The most important opportunities for the rejuvenation of germ cells are created by meiotic recombination and repair. These seem to be unique processes for eliminating lesions and alterations in the DNA system which, at the moment, appear to be irreversible in somatic cells.

An important general proposition is that different cell types may exist in a variety of alternative metastable, steady states, each of which is controlled, not only by chromosome genes, but also by complexes of macromolecules, or processes which are self-reproducing in the presence of an appropriate chromosome genotype. These complexes or 'homeostats' may be disrupted by various environmental changes. Similar cells will behave differently under the influence of different homeostats. Maybe in the case of the amphibian nuclear transplantations, a similar mechanism occurs whereby, with replication, the aged differentiated nucleus can be altered by the non-differentiated cytoplasm sufficiently for it to participate competently in its new environment. In other words the molecular arousal of the genome depends upon its activation by cytoplasmic agents.

Temperature and Rate of Ageing: an Insect Model

Two theories have been proposed to explain the sensitivity of insect lifespan to environmental temperature. The rate of living theory suggests that the greater longevity at low temperatures arises because the rate of ageing processes like those of chemical reactions are greater at higher temperatures.

Experimental results show that:

1. Flies kept first at a high temperature and then transferred to a lower one die at the same age as do flies kept continuously at the lower temperature. This statement is only true of males and ovariless females, since the lifespan of normal mated females is increased by a preliminary period at a high temperature.
2. Flies kept first at a low temperature and then transferred at various ages to a higher one initially have a reduced expectation of life at the high temperature of one day for every day spent at the lower temperature until the expectation of life at the high temperature has fallen to approximately half its initial value in young flies. Subsequently the expectation of life at the high temperature does

not alter substantially with increasing age at the low temperature.

3. Flies first kept at 25.5 °C and then transferred to 20 °C can reproduce at both these temperatures although the former is close to the maximum. F_1 hybrid males between the B and K inbréd lines were raised at 20 °C, and 4 days after emergence, with the exception of a control group, transferred to 25.5 °C. A group of 110 males which were kept continuously at 25.5 °C until they died survived for a mean of 43.1 days at that temperature. Other groups, each consisting of 20 flies, were kept at 25.5 °C for 12, 16, 24, 28, and 32 days, and were then transferred to 20 °C and kept at that temperature until they died. The mean ages at death with standard are plotted in Figure 9.9 against days spent at 25.5 °C.

The threshold theory assumes the rate of ageing to be independent of temperature. On these assumptions all groups returned to 20 °C should die at the same chronological ages as the controls regardless of how long they had previously spent at 25.5 °C. These results are incompatible with the rate of living theory. To explain them Clarke and Maynard put forward the 'threshold theory'. The essential feature of this theory is that if at a lower temperature an individual starts to die when it reaches a state (a1); then at a higher temperature a similar individual will start to die at an earlier state (a2) In more detail it is assumed that:

1. The lifespan is divided into two phases—'ageing' and 'dying'.
2. The rate of the ageing process is approximately independent of

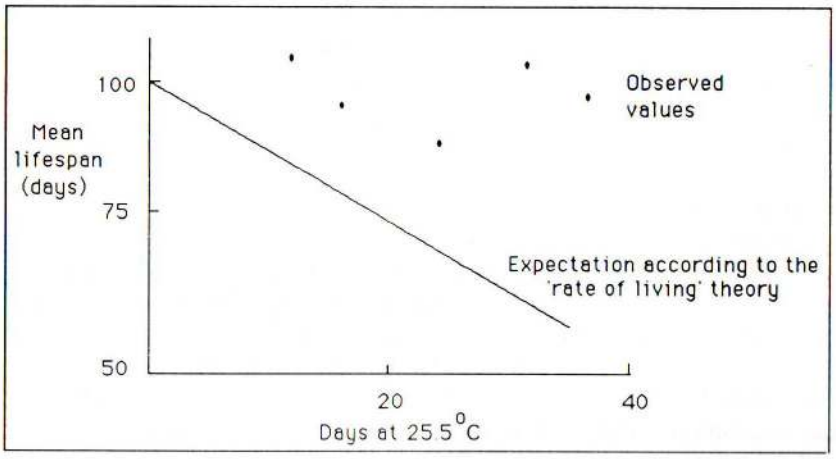


Figure 9.9. Longevity of male *Drosophila* kept first at 25.5 °C and then at 20 °C as a function of time spent at 25.5 °C

temperature. It continues until the vitality of the individual has fallen to a threshold level below which the individual can no longer maintain itself at the temperature at which it is living. This threshold is higher at higher temperatures so that individuals which can maintain themselves at a low temperature may be unable to do so at some higher temperature.

3. In individuals whose vitality has fallen below the threshold appropriate to the temperature at which they are living, a second process, 'dying', commences and terminates in death. This dying process is temperature dependent in its rate, and provided it is not allowed to terminate in death can be reversed at lower temperatures.

Later experiments using different species of fruit-flies, mosquitoes, rotifers, and tropical fish, indicate that neither the rate-of-living theory nor the threshold theory adequately explains results of temperature-transfer experiments. Some experiments with different species and strains of fruit flies gave results that agreed with the rate-of-living theory, whereas results with with other strains supported the threshold theory. The lifespans of males agreed with predictions of one theory, while lifespans of females in the same experiment agreed with those predicted by the other theory. In other cases, results obtained did not agree well with predictions based on either theory. Furthermore, fish reared at a high temperature for about half their life and then transferred to a lower temperature lived 76% longer than those kept at the lower temperature continuously.

One important aspect of the metabolic regulation in poikilotherms is that it incorporates features that enable adaptations to be made to changes in environmental temperature. A form of adaptation involving the synthesis or activation of enzymes which operate more efficiently at the new temperature, could explain some aspects of temperature-transfer experiments. A new pattern of enzymes created in response to a change in temperature would in effect mean that the organism responded from a new metabolic baseline with an enzyme pattern enabling it to adjust to a new temperature.

'Induced Repair': an Insect Model

Throughout life, insects lose cells from all postmitotic tissues because of random or stochastic damage. This loss of cells must compromise the ability of the organism to maintain homeostasis and would therefore be expected to play a role in mortality.

The main implication of Pearl's rate of living theory is that rates of metabolism and ageing are linked together. This is supported by much experimental evidence. For example, the data for two insects, the milkweed bug (*Oncopeltus fasciatus*) and the housefly (*Musca domestica*) is given in Table 9.5. It is possible, but as yet not directly proven, that the connection is through the

Table 9.5. Lifespan, metabolic rate and metabolic potential in two insects

Inesct	Temper-ature	Lifespan[1]	Metabolic Rate[3]	Metabolic potential[3]
Milkweed bug	18	93.9	0.7	1.6
	25	60.2	1.3	1.7
	30	23.9	2.7	1.6
Housefly	18	4.81	4.6	5.3
	25	20.4	9.4	4.6

[1]days; [2]μl/O_0/hr/mg wet wt; [3]μl O_2/mg wet wt/lifespan

deleterious effects of oxygen-free radicals and hydroperoxides produced as by-products of oxidative metabolism.

Since the rate of generation of oxygen-free radicals is directly related to the metabolic rate, the steady state levels of free radicals and the actual damage they inflict will depend on the efficiency of antioxidant protection systems and the mechanisms to repair free radical damage. It follows that for each species, under particular environmental conditions, there is a definite sum of aerobic energy expended per unit body-weight during life. This 'metabolic potential' is determined by the balance between the rate of free radical production, the rate of free radical removal, and the rate of repair. Therefore the rate of ageing is directly related to the rate of unrepaired molecular damage inflicted by the by-products of oxidative metabolism and inversely related to the efficiency of antioxidant and repair mechanisms.

Enhanced longevity has been observed after irradiation of adult flies and moths which lack somatic cell renewal, as well as in beetles and crickets. If the phenomenon results from changes in somatic tissues the beneficial consequences of irradiation in terminally differentiated tissues could be a widespread occurrence. It would not be observed in mammals which are highly dependent on somatic cell renewal, particularly in the haematopoietic tissues. The phenomenon may be accommodated within the 'induced repair theory' which states that:

1. Processes which repair DNA damaged by experimental radiation also repair DNA damaged by agents such as UV, alkylating agents and endogenously generated free radicles.
2. DNA-repair enzymes and cofactors are kept at genetically determined high levels in proliferative cells and decline to low levels in terminally differentiated tissues as organisms age.
3. DNA damage to postmitotic cells increases the repair capability at the tissue level.

The predictions from the theory are:

1. The decline in repair capability would lead to accumulation of DNA lesions. These would reduce those adaptations to stress involving postmitotic tissues which require activation of transcription as part of the homeostatic mechanism.
2. In insects, and other organisms predominantly made up of postmitotic cells, moderate doses of radiation or of DNA-damaging chemicals evoke a response which involves increasing DNA repair capability. If this response was a general one, previously accumulated damage would also be repaired, and this would give irradiated organisms better homeostatic responses, thus increasing longevity.

In line with these predictions, irradiation of female *Tribolium* beetles enhances their longevity and they display increased resistance to oxygen poisoning and heat, both of which are believed to act primarily on terminally differentiated tissues. Experiments to date have been insufficient to demonstrate whether the increased resistance stems from enhanced ability to adapt as originally predicted by induced repair theory or from an intrinsic resistance associated with a persistent increase in the level of stress proteins. Furthermore it has not been demonstrated that increased stress resistance actually accounts for enhanced longevity although there are similarities in the time courses of the two phenomena.

Two important lifespan correlates in insects are the mode and frequency of reproduction, and the ambient temperature. The general picture at present is that reproductive activity in insects does reduce lifespan of both sexes. Which aspects of reproduction are of importance in producing this effect has yet to be demonstrated. In particular, more work is needed to investigate whether reproductive activity reduces longevity because it generally depresses overall homeostatic efficiency or because it accelerates ageing by diverting resources from general repair and maintenance.

Damage and Repair: Mammalian Models

DNA repair systems fall into three broad classes:

1. Strand-break repair.
2. Excision repair.
3. Postreplication repair.

Strand-break repair rapidly rejoins broken single strands and, in some organisms, broken double strands. Excision repair systems remove the damage

from the DNA; an enzyme called endonuclease nicks the DNA near the damaged site, after which the damaged region is removed and replaced.

There are several separate endonucleases for recognition of DNA distortions. A given cell type may be more proficient in one of these types of repair than in others, and several disease syndromes, including some which prematurely exhibit facets of ageing, have a deficiency of one repair system while the other DNA repair systems appear to be normal.

The consequences of damage and repair are likely to be very different in mitotic and postmitotic cells, due to the consequences of entering DNA replication or cell division with unrepaired damage still present.

Minor DNA damage, such as small adducts and viral incorporation, often have no effect on replication or division and will be passed on to the new cells during cell division. Minor damage can thus accumulate in the DNA. On the other hand, if strand breaks or DNA–DNA cross-links are present during cell division, the chromosomes will be unequally distributed. The result of such major alterations in the integrity of cellular DNA is usually reproductive death of the cell.

DNA damage may be assessed experimentally by *in vitro* and *in vivo* methods. In the *in vivo* method DNA damage is produced by injection of benzo (a) pyrene which forms a specific adduct with liver DNA. The rate of removal of this adduct from its bonding with DNA is measured by isolating the DNA and assessing the amount bound at different times after the injection. In the *in vitro* method repair is measured in cultured cells after treating them with a DNA damaging agent in the present of hydroxyurea. Using one or other of these approaches, the following results support the idea of a causal role for insufficient or erroneous DNA repair in mammalian ageing.

1. There is a positive association between the longevity of *M. musculus* and *Peromyscus leucopus* and their accumulation of DNA damage.
2. The repair of UV-induced DNA damage in a short-lived congenic mouse strain is lower compared with a long-lived hybrid, whereas UV-induced repair was not different from that in longer-lived inbred partner strain.
3. There is a strong correlation between longevity and UV-induced DNA repair in inbred mouse strains with mean longevities of 300, 600 and 900 days.
4. The rate of removal of an experimental adduct from liver DNA in a short-lived BALB mouse strain (lifespan 600 days) is about half that in a longer-lived C57B strain (lifespan 900 days).
5. Dietary restriction, which increases the mean survival age of rats from 31 to 39 months, promotes increases over the lifespan of enzymes thought to be part of the body's antioxidant system (Table 9.6).

Table 9.6. Levels of three liver enzymes thought to be involved in cytoprotection against free radical damage in rats: effect of dietary restriction

	Superoxide dismutase[1]		Catalase[2]		Glutathione peroxidase[3]	
	N	DR	N	DR	N	DR
Age (months)						
7	57.7	43.3	1.67	1.23	1.38	1.04
18	55.2	47.2	1.24	1.41		1.47
30	45.1	55.9	1.17	1.48	1.41	1.42
% Change (7–30 months)	-22	+29	−30	+20	+2	+37

N = normal fed: DR = dietary restricted.
[1]U/min/mg protein; [2]U/min/mg protein; [3]nmole/min/mg protein

CHAPTER 10 Ageing as a model of 'systems thinking'

An explanation should be as simple as possible, but not simpler (Albert Einstein)

SYSTEMS THINKING

Most of the mysteries facing gerontologists involve unravelling systems of one form or another. In fact it could be said that 'ageing', as a body of knowledge, consists of many interconnected systems nesting one inside the other that can take the learner from 'evolution' to 'gene transcription' or vice versa. In Chapter 1 there is a section dealing with the issue of gerontology as a distinct body of knowledge. A body of knowledge emerges from a mass of data and information when a pattern of concepts arises which forms the scaffold or 'structural steel' for thinking about it and communicating it. A pattern of concepts in the form of an array, or cluster is the logical filing system for fitting data and information into the architecture of the knowledge system to which it is related. The construction of this type of navigation structure is particularly crucial for learning about ageing, where the aim is to assemble a personal viewpoint by selecting data and information from a very large cross-subject database. It is the first phase in thinking about any subject and involves defining a vantage point, panoramic view, or outlook, in which a concept of current interest can be related to other concepts.

To apply systems thinking to any phenomenon, a basic understanding is obtained by first expressing some assumptions about it in the form of a conceptualized structure summary. This can be a tabulation, diagram or map summarizing pathways for following through the outcomes of the assumptions. Starting from this conceptual vantage point detailed models of a system can be compiled:

1. By constructing a boundary around a set of elements and interrelationships such that the cause of the dynamics exhibited by the system resides in the boundary ('causal models').
2. By arranging, or connecting up, the elements within the boundary in such a way as to define what is causing the dynamics to occur (closed-loop models).

3. By identifying the limiting factors, or operational levers, which will allow the investigator to 'run' the system so as to make and test predictions about how it works (operational models).
4. By defining common patterns of system behaviour over time which, for example, cause temporal phenomena, such as progressive build up, loss, and oscillation, which are common to a variety of systems (comparative models).

Together, these four ways of thinking about how a system works, are developments of constructing a panoramic conceptual map. The act of creating the various kinds of model defines 'systems thinking'.

Systems thinking about ageing advances, like any other kind of scientific investigation, first and foremost by 'leaps and bounds' into the unknown, but also by 'return visits' to old incontrovertible truths that remain tantalizingly inexplicable. In fact, the first quarter of the 1990s has been characterized by excursions both backwards to old truths, and forwards into largely unknown areas. The following brief descriptions highlight three new outlooks on humoral communication, stress and population which in the early 1990s emerged as important conceptual vantage points for the systems analysis of ageing.

Humoral Communication Systems

One of the first pieces of evidence that raised a possibility that specific factors could trigger a system of ageing in a youthful system was the interaction between human T lymphocytes and interleukin. Normal adult human T lymphocytes activated *in vitro*, and maintained in the presence of interleukin 2, a factor involved in the normal differentiation of lymphocytes, cease to proliferate after undergoing about 20 doublings. This fixed lifespan is similar to that of human adult human fibroblasts multiplying *in vitro*. Subsequently it was found that failure of division is not associated with a general deterioration in other cellular functions and many normal functions are retained in the senescent lymphoctyes, as a system. As intracellular systems come under scrutiny it is becoming clear that decreased replicative capacity *in vitro* can be an inappropriate secondary response to a range of alterations in other cellular characteristics which transmit intracellular messages to the division mechanism. For example, the *in vitro* failure of replication in preadipocytes, which in adipose tissue either produce fat cells or maintain a mother clone, may be contrasted with the situation in the body, where the numbers of adipocytes do not change, or may even increase. This work is beginning to conceptualize the largely unknown intercellular and intracellular factors that coordinate tissue and cells to function as a system, which may malfunction *in vitro*.

The endocrine system has a part to play in this holistic model of tissue

ageing. Hormones are now being reexamined as proximal factors in tissue ageing. Here, for example, a possible endocrine mediation of the effects of dietary restriction in protecting against tumorigenesis is beginning to emerge. Effects of adrenalectomy on the production of experimental tumours in mouse skin suggest that it is possible for increased levels of corticosterone and dehydro-epiandrosterone in dietary restriction to suppress tumorigenesis and retard ageing.

A primary decline in hormones seeems to be linked to the age-related loss of muscular activity. Deteriorations of motor function appear to be largely due to impaired dopaminergic regulation. In rats, a low muscle response is primarily a consequence of striatal receptor loss and is restored by oestrogen or prolactin, with improved motor performance. It has also been found that falls in muscle mass and reserve strength can be conteracted by β-2 adrenergic agonists. Experimentally, one of these pharmacological agents, clenbuterol, increases muscle mass and carcass protein to a similar extent in young and old rats, and hastens recovery of muscle mass following protein-energy malnutrition in old rats. This work points to practical rewards for the pharmacaological increase in fitness in the elderly by focusing on the holistic system of hormones/tissue/cells and organelles.

Stress Systems

Stress and related cellular responses are beginning to form a conceptual framework which brings together a number of research areas that have been traditionally isolated. When facing environmental challenges that produce tissue damage, organisms from bacteria to mammals elicit the synthesis of anti-stress proteins that limit the extent of damage incurred. They also repair or remove conformationally altered protein. It is to be expected that more effort will be made to unravel the molecular basis of this system, not only to understand the universal failure in responses to stress in old organisms, but also to define the state of tissue turnover, which must be part of any general 'stress system'.

For example, the response to cold stress is being pobed at the molecular level where cellular factors, less precisely defined than hormones, appear to be involved in the loss of non-shivering thermogenesis and diet-induced thermogenesis. A special protein is a key factor in this system which uncouples mitochondrial oxidation from the formation of ATP, releasing the energy as heat. This uncoupling protein (UCP) is located on the inner mitochondrial membrane. The age-related failure in heat production seems to occur on, or after, translation of the UCP, causing impaired thermogenesis in brown fat.

Oxygen metabolism of cells is also regarded by many as imposing a chemical stress on the intracellular environment. One manifestation of this is the free-radical theory of damage. Recent work on oxygen free-radical theory in

relation to the genesis of tumours has reopened the classical 'Rubner theory' which postulates that species lifespans are related to total 'aerobic lifespan energy'. The new work has shown that the incidence and onset of leukemia appears to relate to the total cumulative energy intake of rats (i.e. age × mean daily energy intake). This places a likely origin of age-dependent tumorigenesis in the mitochondrial system, and points towards a unified systems approach to a biochemical explanation of Rubner's lifespan rule. Clearly, the 'stress system' nests within the 'humoral communication system'.

Population Systems

A unified view of ageing applied to all living things is hampered because of the selection of inbred strains of rats and mice as the main experimental material for laboratory work. However, the exclusive use of inbred strains of rodents is now being widely questioned. There are two reasons for workers turning away from these dominant population systems. First, there is the increased homozygosity of inbred strains which results from inbreeding. This breeding regime favours the increased expression of deleterious recessive alleles which may lead to a drop in reproductive capacity, physiological efficiency, and variability, known as 'inbreeding depression'. A more serious practical limitation in the use of pure strains is that testing the response of homozygous animals to an experiment is the equivalent of repeated testing on a single individual. Therefore, studies on a single strain, or on a small number of strains, will produce results which do not necessarily characterize the general pattern of response.

With regards to the alternative use of F1 hybrids, they provide a genotype that is equally replicable, and also offer a more broadly based test system where inter-individual variability may be significantly lower. This not only produces economies in the numbers of animals required for statistical tests, but also increases the sensitivity of the rodent model to experimental manipulation.

Part of the problem is that the commonly used inbred laboratory stocks may age in idiosyncratic ways. This is evident from the peculiar dominant causes of death in certain strains, e.g. mammary tumours in Sprague–Dawley rats, and non-human patterns of physiological ageing. From the latter aspect, the brown Norway rat has been recommended as a research model for studying ageing of the male reproductive system because, unlike the laboratory rat, it shows a trajectory of testicular failure similar to that in humans. It is therefore a more realistic model for clinical problems of the ageing male reproductive system.

Alongside the re-evaluation of rodents as ageing models, more attention is being paid to the laboratory models invented by geneticists. The fruit fly, *Drosophila*, is being used more frequently to study the genetics of lifespan, both in the labortory and in the wild. These classical genetical systems are being supplemented by more exotic organisms, such as nematode mutants which are

hypersensitive to high oxygen concentrations. As novel laboratory stocks they offer a population system suitable for studying the effects of oxygen free-radical damage. New methods of synchronous culture will make the nemoatode model a more powerful tool;

Questioning the experimental systems available to study ageing is long overdue. At the same time, questions are also being asked about the meaning and application of the Gompertz function applied to analyse population systems through their mortality curves. Standard methematical models like that of Gompertz often fail to describe mortality at extreme ages, where large individual variations in ageing might be revealed. Actually, risk factors explain 70% of the age-dependence of mortality, and the concept of fixed lifespan due to 'an ageing system' is being replaced by a more dynamic and realistic concept, where physiological heterogeneity probablistically determines lifespan. This is the situation in ecosystems where many, apparently unrelated, physiological parameters have to be measured to get reasonably close to predicting the lifespan of wild rodents.

Nevertheless, analysis of mortality curves is only as good as the methodology used to obtain the data, which usually involves cross-sectional sampling. Fundamental problems are now beginning to emerge around the latter approach. This is because some careful longitudinal laboratory studies have revealed that there are more age-related variables in the system than those defined in longitudinal studies on the same population. For example, in a longitudinal study of non-human primates, only about 20% of the variables were predicted from a larger cross-sectional study.

Causal Models

A conceptual map is a navigation system through a mass of information. It is also a crucial and integral step in creating a model to guide research. The most common type of research model involves depicting relationships between causes and effects by linking components of the system diagrammatically with a one-way flow of arrows. The aim is to show 'what causes what'. As a distinct paradigm it may be described as a 'causal' model because it expresses the system in terms of a one-way causality. An example of this approach to model an important aspect of human ageing is given in Figure 10.1. It summarizes Mcgill's histological concept of human cardiovascular disease in which the cause is defined as fatty accumulations in the walls of arteries. These accumulations, shown in longitudinal and cross-section, are detectable very early in life and progress to an age, termed the 'clinical threshold', where they eventually interfere fatally with blood flow in a range of organs. It is a graphic summary of a great deal of evidence about temporal histopathological changes in blood vessels, and clearly demonstrates the principles of 'damage amplification'

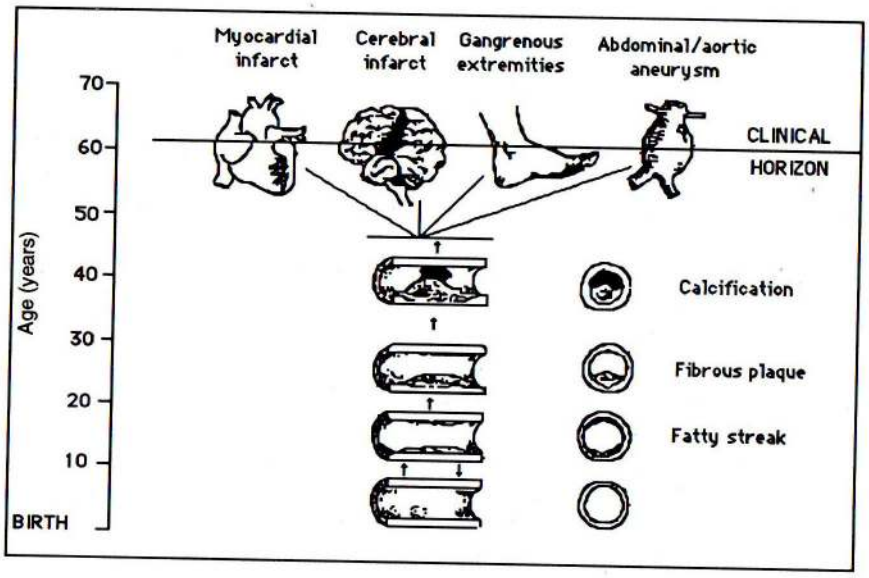


Figure 10.1. Mcgill's histo-pathological model of human cardiovascular disease

and 'variability in organ susceptibility' which are characteristic of many degenerative diseases confronting geriatric medicine.

McGill's model illustrates the major characteristic of causal models. They have actual, or implicit, lines of causality running from each of a set of causal factors to the effect, or effects.

Models with a one-way causality are commonly expressed in the form of lists. A tabular causal model of osteoporosis is presented in Table 10.1. It summarizes evidence from many different research areas in the form of an

Table 10.1. Factors contributing to osteoporosis in the elderly

Age
Chronic renal failure
Drugs, e.g. corticosteroids, anticonvulsants
Eating habits
Endocrine disorders
Failure of calcium uptake mechanism
Immobility
Inadequate calcium intake
Post menopausal hormone changes
Rheumatoid arthritis
Vitamin D
Gender

inventory of causes of the loss of calcium from long bones.

Both of these examples illustrate a common limitation of 'system as cause' models. They seldom give a weighting to each causal factor. To prioritize the factors listed in the 'laundry list' of Table 10.1 requires more information about the available evidence. The sex of the subject is the most important factor because osteoporosis is a ubiquitous disorder affecting up to 25% of elderly women in the USA and Europe. Age is also important because bone loss commences around the age of 40 years and accelerates in the postmenopausal period. A major physiological cause is thought to be a lack of ovarian hormones because replacement therapy after the menopause can reduce the risk of brittle bones. It is difficult to apply a weighting to other factors in the list. Diet may play a role in the aetiology, either through inadequate calcium intake, or through exaggerated urinary losses due to high protein intake. But there is controversy as to whether dietary habits as early as the second or third decade may dermine susceptibility to bone loss in later life. It is known that calcium absorption diminishes with age, and that patients with osteoporosis ingest less calcium in their diet than control subjects. But little information is available on the effects of dietary vitamin D supplementation. Paradoxically the current vogue for increasing the amount of fibre in Western diets may be counter-productive in terms of calcium balance as there is good evidence that high-fibre diets enhance the faecal excretion of calcium. Bed rest and immobility increase mobilization and excretion of calcium and might be expected to exacerbate existing osteopenia.

On the basis of 'hard evidence', as a first approximation, it is only worth constructing a predictive model for osteoporosis using sex, age and hormone production as the operational levers.

This discussion highlights many of the impediments in building a model of degenerative disease where physicochemical events have to be disentangled from the effects of a predominant deterioration in a specific tissue. In this connection, laboratory modelling is limited because of our reliance on a limited range of rodent genotypes. Furthermore, the lifespan and pattern of degenerative disease depends on the regimes for breeding, production and maintenance.

SYSTEMS THINKING ABOUT STRUCTURE

It was Warthin in the late 1920s who first sought to gain an understanding about ageing as a system. His perspective was that ageing is one of several factors that produce more or less severe atrophies and degenerations, termed involution, in all organs. Warthin emphasized the rapid development of involution from the age at which the first loss of cells is seen. Since every organ controls other organs, tissues and functions, if a controlling organ degenerates or atrophies, the organs controlled by it become impaired. The latter, in their turn, and in the same way, produce new 'vicious circles'; and so on. In this

Table 10.2. Standard parts of units of biological systems

Molecules
Giant molecules
Ultrastructures
Organelles
Cells
Organ unit-parts
Tissues
Organs
Groups of organs
Segments and metameres
Individuals
Colonies

manner several vicious circles may be formed. Consequently, specific effects on regressive changes in the controlled organs are added to and superimposed upon others.

In Warthin's model, which is now encompassed by the multifactorial theory of ageing, the combined effect of ageing and any superimposed environmental factors will be greatest when the changes produced by them are similar. Korenchevsky defined this phenomenon as the 'summation' or 'duplication' of the effects of degenerative changes. The number of vicious circles would depend on the number of organs impaired fundamentally by ageing, and also by environmental factors. At this time, in Europe and North America, infectious diseases were important environmental aggravators in the path of individual human development.

Fundamentally, the degree of progression and aggravation would depend on the vital importance of the organ and the degree of regressive changes in it. Although our knowledge has increased greatly since Warthin's time a modern total systems analysis of ageing has to be true to these original ideas and incorporate the variability in the expressions of ageing between individuals, and the underlying general concepts of 'summation' and 'duplication'. Today we would define the latter as positive feedback loops.

Sadly we are still groping in the dark with respect to the organs and functions that have to be interconnected and the biochemical initiators of involution. From the point of view of systems theory, ageing of an organism may be defined as the loss of youthful order that originated from the regulated and predictable sequences of cell migration and differentiation that have been built into early development by natural selection. This order is expressed in the following five basic patterns.

The first pattern comprises the 'standard part or unit'. These exist in a limited number of types but in an unlimited number of identical replicas. They range in dimensions and complexity through more than two dozen orders of magnitude from biological molecules, through the single individual, to the colony (Table 10.2).

The second pattern is that all standard parts of living structures are fitted

inside one another in a system of frameworks which mutually require and determine each other. This is the pattern of 'hierarchy'.

The third pattern of order can be called the pattern of 'interdependence'. It is defined as the mutual dependences of features which extend beyond the dependencies of standard parts or hierarchical sequences. Examples are:

1. The coordinated development in the same organism of features selected from different ancestral species with different phylogenetic origins.
2. The allometric relationships involving size, structure and survival.
3. The directionality of growth.
4. The goal seeking functional directionality of clonal attenuation, regeneration, and homeostasis.

The fourth pattern of biological order is the fact that no biological condition is conceivable without having an inheritance in evolution. Examples are:

1. Ativism, expressed as the 'little tail' in man.
2. The recapitulation of embryonic development within the vertebrates.
3. The irreversible loss of an anatomical homologue such as the fish's fin, a structure which reappears later in evolution, in dolphins, with totally new origins.
4. The switching on of a complete pattern of interconnected determinative decisions by a single genetic command, e.g. the regeneration of a crustacean limb.
5. The existence of a limited number of types of epigenetic system, each corresponding to a major phylum.

The fifth pattern of biological order is that of 'precise macromolecular replacement', or 'turnover', which underlies all other patterns of order. Most standard parts are dismantled and replaced on a time-scale of hours or days without any loss of functional efficiency at a higher level.

To summarize, if we are to understand the biology of ageing as a set of interdependent systems, the observed expressions of ageing have to be related to one or more of these patterns of order:

1. Existence of standard parts.
2, Hierarchy of parts.
3. Interdependence of parts.
4. Phylogeny of parts.
5. Turnover of parts.

Each of these patterns can be defined, and modelled as a system, but the only area that has been subject to concentrated effort by gerontologists is the allometric relationship between longevity and size. The early work for the most part concentrated on morphological parameters, e.g. establishing the regression statistics relating species body and organ weights to lifespan. More recently, emphasis has shifted towards seeking regressions between longevity and functional parameters, such as cytoprotective enzymes, and DNA repair rates. Unfortunately, the establishment of a mathematical relationship between two biological measurements does not define a direct functional connection. So far, allometry has not produced any breakthroughs in systematic zoology where it was first defined at the start of the century. Working on the premise that the size of a body and the relative proportions of its parts is the outcome of survival in competition for limited resources, the starting point should be the animal's life history strategy. Only if it is applied in the context of ecological mortality can allometry contribute fundamentally to our understanding of the origin and maintenance of particular patterns of order involving growth, reproduction, and laboratory ageing.

'Order' is not a common research concept and there are great gaps in our knowledge, not only with respect to defining order in ageing organisms, but also in our understanding of the developmental mechanisms selected by evolution to produce and sustain order. However, we can make a generalization, albeit from a limited range of data, that the loss of order is most obvious with respect to standard parts, and may be commonly seen in the loss, and malformation, of organelles, cells, and organ unit structures. Examples of this kind of evidence are the wrinkles and senile purpurae of human skin, fatty deposits in arterial walls, missing motor end-plates, and misshapen nuclei and mitochondria.

The fact that ageing is universally expressed in organ unit structures from man to protozoans brings questions about ageing up against questions about the evolution of these structures. In the mammalian gut for example, an important unit structure is the glandular pit (Figure 10.2). Pits have evolved a very high level of longitudinal differentiation which coexists with rapid cell turnover. The instructions for cell replacement appear to be pit-specific and pits age on an individual basis. Kidney nephrons also show a similar individual unit structure pattern of ageing with a high rate of cell replacement. In both organs a degenerate unit structure can often be found sandwiched between two normal units. Also, in the kidney, a normal looking tubule may be found below a degenerate Bowman's capsule.

These examples present the problem of ageing and the loss of order in terms of questions surrounding the fourth pattern of biological order. How does a unit structure 'know' its existence, and what goes wrong with this very localized holistic histological perception/replacement system, residing within a community of cells, with the passage of time?

The complexity of the system for maintaining biological order in unit

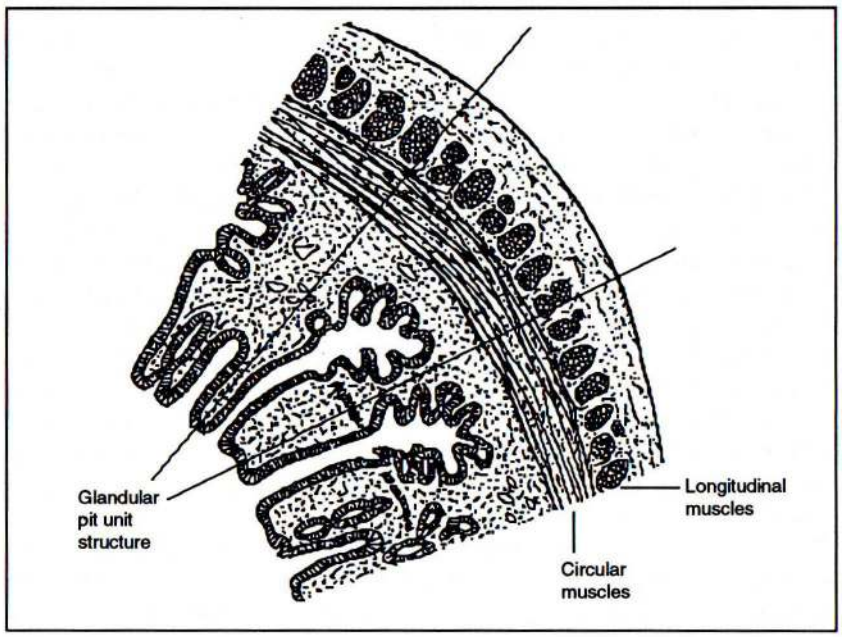


Figure 10.2. Generalised histological cross section through the mammalian gut showing the glandular pit unit structure

structures is well illustrated in the stomach (Figure 10.3). The self-contained secretory unit structure produces a mixture of hydrochloric acid, proteolytic enzymes and mucus lubricant. Each product is produced by specialized cells with cell turnover times of 2 to more than 48 hours. In addition to four types of cells that produce acid, enzymes and mucus, the pit unit is self-contained in terms of its blood capillaries and its three-dimensional collagen lattice which supports, and may direct, the differentiation of the pit cells.

Ageing of this complex, dynamic, cellular community results in a loss of pits, a thinning of the epithelium, an increase in variability of the ratios of one cell type to another and a diminished expression of genes for enzymatic and structural proteins. There is an increase in stem-cell proliferation and a failure to respond adequately to injury of the mucosa. These two aspects may be related in that the age-related failure to reach the structural goal of repair may be driving proliferation at a rate characteristic of a partial repaired state. In this situation we cannot decide if a particular cell in an older animal is different from one in a younger animal. Also, factors such as alterations in feeding habits, and in blood supply to the gut, which might influence the rate at which the experimental isotope labels are taken up, might have a decisive influence on

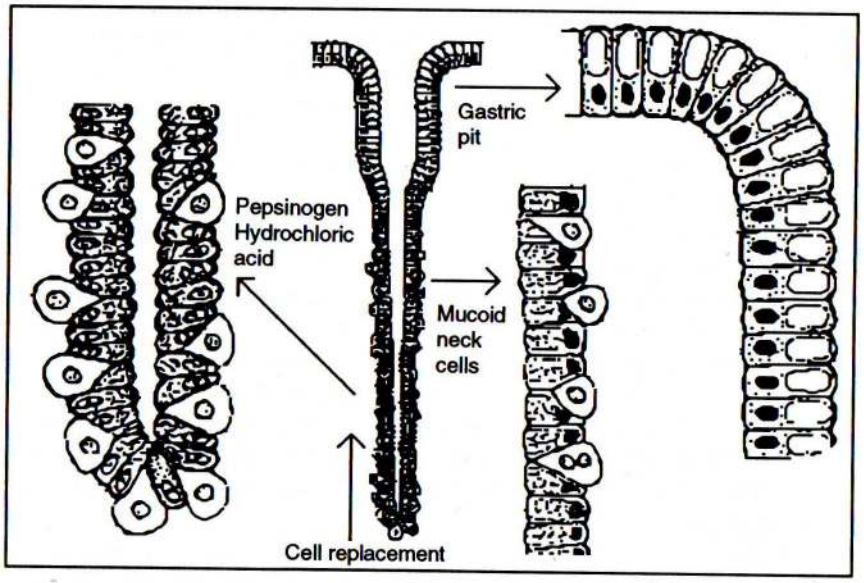


Figure 10.3. Main cellular components of the gastric pit unit structure

what the histologist records.

From many studies on the generation cycle of gut cells it is clear that changes in cell turnover are not the most important key to understanding ageing of the gastrointestinal tract. Cell proliferation is underpinned by a self-contained humoral system which is broadly defined in principle, but not in relation to the age-related loss of structural fine tuning. But more fundamentally, before systems thinking can be applied to gut structure we need to know for each kind of self-regulated structural unit, where the instructions reside for its self-maintenance. Until this question of 'the pit as an entity' is answered, we are groping in the dark for a Warthin vicious circle of degeneration.

The clear and universal manifestations of the ageing of parts raises the following questions in systems thinking:

1. Are replaceable parts dismantled because they contain damaged components, or because they have reached a perceived 'sell-by' date?
2. Do misshapen replaceable parts become more evident with time because of the gradual loss of precision in replacement tools, or because they are no longer regularly checked and/or serviced?
3. Why does the deterioration in chemical specification of replaceable parts appear to follow a progressive trajectory which extends from late development into old age?

4. Are substandard replaceable parts allowed to predominate because of a survival decision to use the savings in materials and energy to meet more pressing problems, or because damaged blueprints accumulate that are fundamentally irreplaceable?

We have no answers to these questions, and until they are answered the study of ageing cannot be connected with what Rupert Riedl has called the two great theorems that have a bearing on evolution, the law of entropy in physics, and the law of descent in biology.

Fundamental order in living things is implied by the mechanisms of selection (Darwinism), of mutation (Neodarwinism), and of population dynamics. These together make up the synthetic theory of evolution and Reidl characterizes 'self-creative order' as the causal connection between these three mechanisms. He believes that self-creative order produces both the ordered, predictable, morphological diversity within individuals, and the regularities of macro-evolution. The existence of ageing is particularly relevant to questions about the meaning of 'self-creative order'.

Reidl, who is one of the few modern theorists revisiting old problems of evolution at the morphological level, defines self-creative order in terms of the concepts of 'self-action', 'goal-building', and 'self-ordering'.

Self-action includes self-steering biochemical/physiological feedback, and examples of self-regulation where the steering parts are being steered and the steered parts are steering.

Goal-building asserts that the paths of evolution run towards particular conditions. These are defined specifically as particular patterns, combinations of events, and decisions, all of which result in particular assemblies of parts. A goal only originates when it is set by an evolutionary adaptation to environment. It can neither be foreseen, nor given up afterwards. Reaching a goal both limits and enhances possibilities for further evolution.

Historically, the study of ageing has not taken this particular route of biological systems thinking. Instead, a beginning was made by defining higher level end-points of damage in a medical context, of which the ultimate expression is the mortality curve. Even so, gross structural damage to cellular components cannot be connected with the acceleration of mortality of populations with Gompertsian characteristics.

The molecular targets within structural systems which are sensitive to ageing and lead to microscopic degeneration are only now being identified. Paradigms of physiological self-action have moved on historically, with improvements in technological capability, to focus on failures in the molecular expression of genes. New paradigms for ageing, and a few visits to old ones, have also bent minds towards considerations of molecular disorder (Table 10.3). Hopefully research is now being directed towards the study of self-ordering as a dynamic system of pattern maintenance. Patterns, all susceptible to ageing, evolve from

Table 10.3. Ageing: some major experimental paradigms

Period	Assumptions
1900–40s	The rate of ageing is inversely related to the rate of metabolism The rate of ageing is directly related to the amount of food eaten Degenerative diseases are expressions of 'vicious circles' of regressive changes due to the interaction of organs, particularly the ductless glands, that are impaired by ageing
1950s	Ageing is caused by the accumulation of somatic mutations Ageing is due to the accumulation of damage done to irreplaceable macracracracracromolecules by highly reactive intermediates of normal metabolism Ageing results from the expression of late acting deleterious genes, and/or pleotropic genes which have a beneficial effect in early life
1960s	Ageing is caused by mistakes in assembly of blueprints Ageing is caused by chemical damage through cross-linking of macromolecules, of which skin collagen was a common model Ageing is due to a mechanism programmed into cells which limits the number of proliferations
1970s	Ageing is controlled by neuroendocrine pacemaker cells which set a clock of ageing Ageing is caused by the failure of DNA repair systems Ageing and age-related pathologies are causally related to increased lysosomal activity due to leaky membranes destabilized by free radicals
1980s	Ageing occurs because the force of selection in environments with limited resources and high mortality from disease and predation, will ensure that investment in maintenance of the soma will be less than that required for its full integrity Oxidatively damaged DNA is the underlying cause of many age-related diseases Lifespan is linked with the genetic control of the superoxide dismutase (SOD) antioxidant system. It does this by maintaining a low steady state level of life-shortening superoxide free radicals generated as transient intermediates in normal oxidative metabolism

the symmetries and ranks of a limited number of biological parts up to products of the human intellect, i.e. the composer's and architect's scores and blueprints.

To summarize, systems thinking about ageing as the loss of structural, and therefore functional, integrity, which began with Warthin's generation of scientists over 50 years ago, is a thread that also runs through Reidle's three propositions about the ways in which living things are put together. It brings a temporal viewpoint to bear on those apects of genetic control and regulation of self-action which are concerned with making, assembling, checking, and replacing

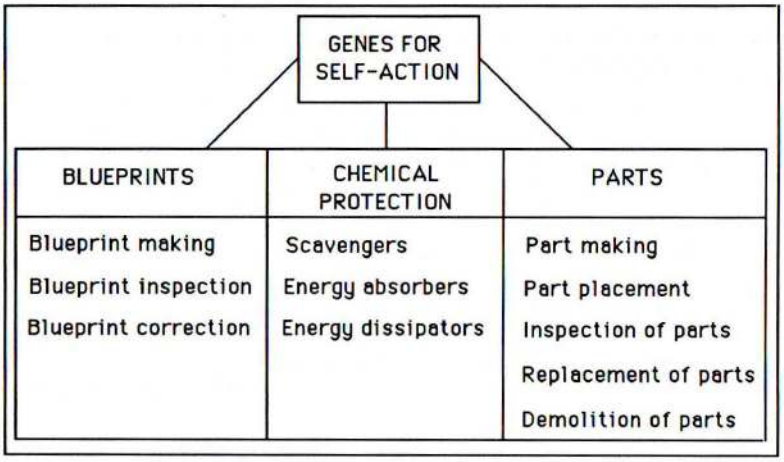


Figure 10.4. Structure summary of a genetic system for self-action which is relevant to ageing

parts, and for protecting them against chemical and thermal damage (Figure 10.4).

So far the discussion has been confined to departures from morphological plans. Changes in chemical plans of parts also occur. For example, during the second year of life there is a steady drop in the concentration of phosphatydyl choline in the membranes of rodent microsomes. At two years of age this important chemical building block is about 50% of its maximum, which was attained at the end of the first year of life. There is a similar rise and fall in the concentrations of two other 'membrane bricks', the phospholipids, lysophosphatydyl choline and sphingomyelin. But these important components follow trajectories that are phase shifted to manifest earlier maxima. Arguing from changes in these particular lipids there appear to be at least three clock-like mechanisms, each responsible for triggering a series of late epigenetic pathways of membrane development which continue with a powerful momentum beyond the ecological lifespan of rats and mice, well into laboratory old age. Similar changes occur in the lipid composition of mitochondrial membranes in many animal phyla. From insects to man, there is a rise in the ratio of cholesterol to phospholipid and changes in fatty acid composition. These changes would be expected to alter the physicochemical properties of organelle membranes regarding the transport of ions and metabolites, and increase their susceptibility to chemical damage. This kind of universal evidence for alterations in the chemical specification of unit structure cell membranes is important because it demonstrates the operation of what might be called a 'sliding scale blueprint'.

By analogy with the system of a construction worker building a wall, it is as if the first plans specified the use of solid bricks and that subsequently each day's

supply of building materials arrived with a new specification to use bricks made of more porous materials. To bring this analogy in line with the high rate of turnover of biological membranes, each new specification would state that every day a certain number of bricks of both kinds that had already been laid should be removed and immediately replaced by the current ratio of solid bricks to porous bricks in the wall as a whole.

The idea of 'changing blueprints' highlights a problem of classifying such systems as examples of 'ageing'. The changes cover a long time-scale. They proceed from an origin in the time of youth when they occur alongside other changes characteristic of epigenetic processes that are clearly adaptive and expressions of an evolutionary programme of development. Some gerontologists consider that such 'active' systems should be considered part of 'development' and not considered as 'ageing'. Here, the distinction between development and ageing is that ageing is 'passive'. It does not enhance functional capacities and is steadily progressive with a strong random pattern of expression. Development is not steadily progressive, is not random, and features a state of 'maturation', when the system slows down or stops, having enhanced natural selection of organisms in which it featured. According to this distinction growth, as the primary developmental system, is 'active' in that it is terminated when clock-like mechanisms trigger the exit of cells from the cell cycle. These clocks are coupled with a programme of stimulation and inhibition of DNA replication, an epigenetic system which has evolved to regulate species body size. This is achieved by the segregation of the progeny of mitotic clones with diminished growth potentials. The goal is to bring about a gradual loss of cell division that characterizes the species' pattern of growth. This slowing of the growth of individual clones, and of their subclones, has been described as 'clonal attenuation'. Many gerontologists claim that fixed lifespan clones of fibroblasts in tissue culture are a special example of this developmental system because the mitotic cycle appears to be an active process and is associated with the predictable appearance of inhibitors. Fixed lifespan fibroblasts are therefore models of cellular development and not cellular ageing!

OXIDATIVE DAMAGE AS A MODEL FOR SYSTEMS THINKING

Taking the numbers of researchers and the size of project grants as indicators of research momentum, the period from the late 1980s into the 90s was dominated by ideas that the rate of ageing is somehow driven by random impact of highly active byproducts of cellular redox systems. These ideas have a long history and the evidence and arguments in support of them have been developed from an evolutionary point of view in Chapter 3, from the point of view of the failure of physiological homeostasis in Chapter 6, and in relation to the free radical

theory of ageing in Chapter 9. They are now brought together, and summarized as a system. The central postulate is that molecular oxygen, which is taken into cells for the main purpose of producing energy via oxidation of glucose and fatty acids, causes damage to other molecules because some of it trickles out of the redox chain in the form of highly reactive free radicals. There are some who believe that the generation of free radicals is the single common process, modifiable by environmental factors and genetic selection, that is responsible for the ageing and death of all living things. However, despite almost 40 years of research since a free radical theory of ageing was proposed by Denham Harman there is no definite evidence that free radical damage is a major cause of ageing. However, it is a centrepiece of systems thinking because the generation of free radicals is the most obvious connection between normal metabolism and cumulative random structural damage to cells.

Whether or not oxygen-generated free radicals are a major cause of ageing there can be little doubt that living things exist because of the evolution of systems to combat and/or deflect chemical damage (Figure 10.5). Furthermore, free radicals exist and their formation and damaging interaction with cellular structures is likely to have resulted in the evolution of protective mechanisms to prevent, or repair, such damage. Uncertainties exist as to the scale of free radical production, and the effectiveness of the protection mechanisms.

A common type of structural diagram for this system is illustrated in Figure 10.6. It shows the causal connections between free radical reactions and cytotoxicity, the systems which protect against it, and the bearing these systems have in preventing failures of homeostasis, which are seen as the main cause of ageing.

There are three avenues of approach to gain an understanding of the model:

1. From the sources of free radicals, which are produced as byproducts of a range of enzyme systems in a variety of sites (Figure 10.7).
2. From the free radical detoxification system, which consists of a range of antioxidant enzymes.
3. From the compartmentation of sensitive targets, which prevents free radicals from wreaking havoc throughout the intracellular environment.

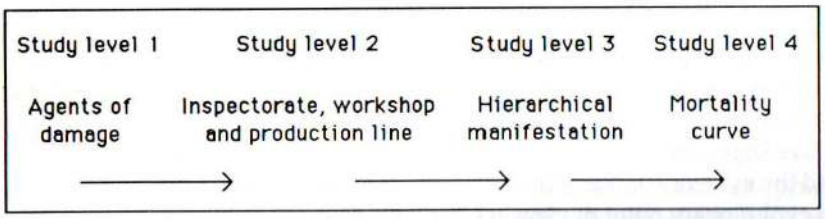


Figure 10.5. A causal diagram to represent the need for an evolved system to oppose damage at the molecular level

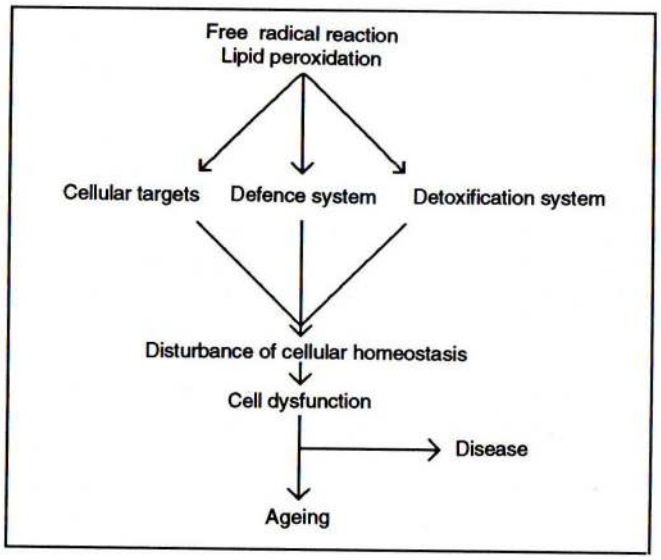


Figure 10.6. A structural diagram of a free radical model of ageing

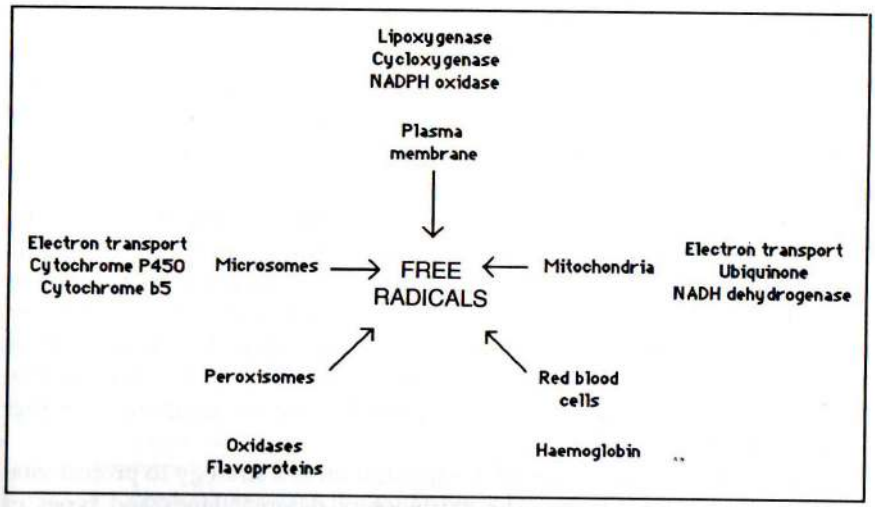


Figure 10.7. Sources of free radicals

Highly reactive oxygen compounds are produced as the result of incomplete reduction of molelcular oxygen during electron transport. One of its paired electrons is removed leaving the other of opposite spin unmatched. Compounds containing oxygen in this state are called free radicals. They are highly reactive because they are not in a stable spin state. They readily give up or accept an electron to stablize their unpaired electron. Since a free radical can either accept or donate a free electron they react with other compounds to produce another free radical. Chain reactions can therefore be set up which can be terminated when free radicals interact together. A chain reaction can also be stopped when a free radical comes up against a compound which is converted into a very stable free radical after it has combined with a reactive free radical. Such compounds are known as 'spin traps' because they effectively 'mop-up' or 'neutralize' potentially cytotoxic substances with an unpaired electron.

Reactive semi-reduced compounds of oxgygen include the superoxide anion radical, the hydroxyl radical, hydrogen peroxide, organic peroxide radicals, and singlet molecular oxygen. Hydrogen peroxide is formed in several normal aerobic pathways. It is potentially dangerous because it can interact with iron to form hydroxyl-free radicals which then react rapidly and indiscriminately oxidizing almost any available nearby molecule. If the iron taking part in this reaction was Fe(III) bound to a macromolecule, this type of reaction would result in the loss of the macromolecule's biological specificity. Most of the damage caused by free radicals is probably due to the formation of hydroxyl species.

There are several kinds of protection agents, including enzymes, vitamins, sulphur-containing reducing agents, and polyunsaturated fatty acids. These may act in a combined fashion to protect biological systems against oxidative damage by destroying oxygen-free radicals or their precursors. In this respect they may collectively form part of an evolved system of cytoprotection for aerobic organisms.

The main protective enzymes are superoxide dismutase (SOD), catalase and peroxidase. There are several types in each category some of which are restricted to certain parts of the cell. For example, a manganese type of SOD is present in mitochondria, and a copper/zinc type is located in the cytosol. There is also a small amount of extracellular SOD in the extracellular matrix. These enzymes convert the superoxide radical to water. The existence of several differentiated types of antioxidant enzymes in different locations is further evidence for an evolved system.

Little is known about intracellular sequestration as a strategy to protect vital components against damage. The existence of different kinds and types of enzymes, positioned in different locations, which destroy free radicals, suggests that they are part of a complex compartmented, antioxidant strategy. The destruction of cellular integrity by homogenizing tissues, and isolating the different structural components, unleashes a whole range of oxidative reactions upon membrane components, reactions which are contained in the intact tissue.

Having defined oxygen cytotoxicity in terms of its biochemistry and potential for damage it then has to be linked with the accumulation of damage to cells and molecules which appear as manifestations of ageing. Some lines of experimentation which might define such a connection between cytotoxicity and ageing are expressed in the following questions:

1. Is the kind of damage in old cells compatible with free radicals?
2. Is there an increased emphasis on aerobic metabolism in old cells?
3. Is there an age-increase in the steady state level of oxidants?
4. Is there an increase in the level of oxidation products in old cells?
5. Do antioxidants increase lifespan?
6. Does increasing partial pressure of oxygen reduce lifespan?
7. Is there a correlation between lifespan and antioxidants?
8. Does increased aerobic metabolism cause tissue damage?
9. Do spin-trapping agents restore youthful characteristics?
10. Is free-radical production implicated in degenerative diseases?

Some of the answers to these questions are presented in the structural diagram of the 'redox arena' in Figure 10.8. This diagram indicates that the arena is a focus for many diverse areas of gerontological research where an understanding of oxidative mechanisms is central to the various research strategies. A breakthrough from any direction would provide a link between metabolism and

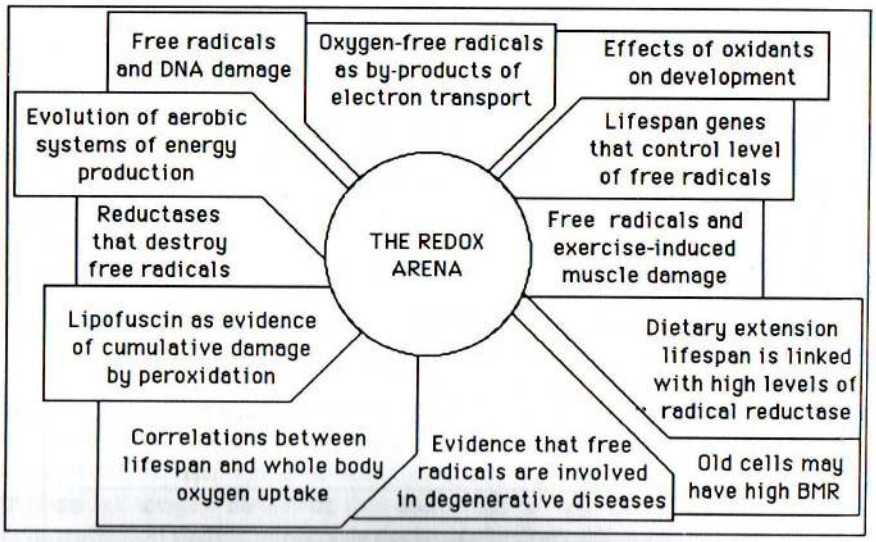


Figure 10.8. A structure diagram to show the main research areas that have a bearing on the relationship between electron transport and ageing

longevity with implications at all levels of biological order. Much experimental evidence supporting the statements in Figure 10.8 has been considered in previous chapters and the following sections introduce new points of discussion.

Old Cells May Have High Basal Metabolic Rates

On average, heat production, oxygen consumption and carbon dioxide elimination decrease with ageing (Figure 10.9). But the average percentage proportion of the two gases in the expired air changes differently; namely the proportion of oxygen increases while that of carbon dioxide decreases, indicating the balance between fat and carbohydrate utilization is shifted towards fat as a basal energy store (Table 10.4). These figures refer to cross-sectional sampling but longitudinal measurements have confirmed the overall trend. Apparently aged tissues cannot use inspired oxygen at the same rate at which it is used at young or adult ages; and at the same time the body produces and eliminates carbon dioxide at a lower rate. Although the average figures of basal metabolism with ageing form a comparatively smooth declining curve, the range of variations in each age group, particularly in old people, is usually wide, sometimes very wide indeed (Table 10.4). For example, in Figure 10.9 the highest rate of heat production in 80-year-olds is the same as the low points in the range of a 12-year-old. A similar high individual variation in basal metabolism is found in the ageing

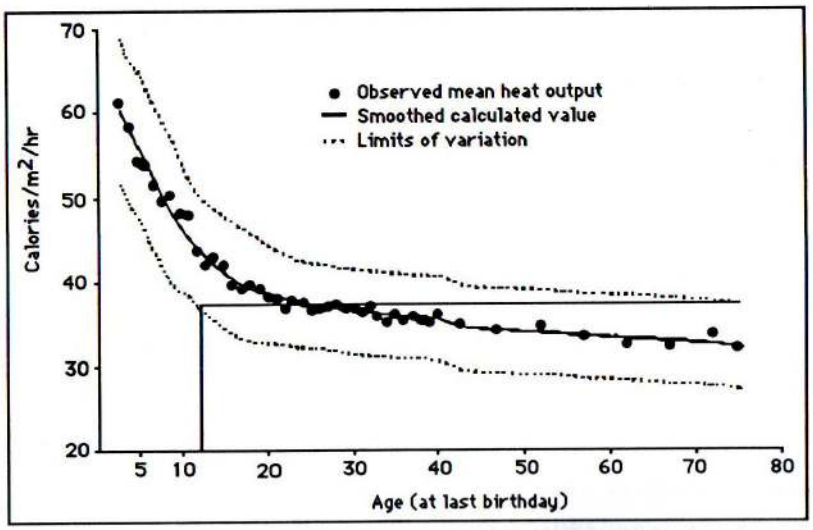


Figure 10.9. Basal heat production in males of different ages

Table 10.4. Basal metabolism in males of different ages

Age	ml/m²/min		% gases expired		Cal/m²/hr
	O^2 uptake	CO^2 output	oxygen	Cardon dioxide	heat production
12	154.5		16.99	3.49	45.03
14	149.7		16.84	3.58	43.46
16	141.8		16.35	4.01	41.13
18–27	125.9		16.48	3.80	36.57
40–49	123.5	99.7	17.89	2.58	35.73
50–59	121.9	98.9	18.04	2.52	34.50
60–69	112.9	93.4	18.12	2.44	33.00
70–79	113.1	90.5	18.28	2.26	32.60
80–89	104.7	84.6	18.43	2.14	30.05

curve of whole body respiration in laboratory rodents.

Because heat loss is related to surface area it is conventional to correct basal metabolism to surface area in order to make comparisons between individuals. Whilst this is a good method of making standard comprisons of respiratory metabolism within a particular age class it is not so useful when comparing young with old where ageing has resulted in a loss of cells. Because of age involution the ratio of surface area to total body cells is consistently higher in old subjects. Therefore, a very old individual having the same basal metabolic rate as a very young person probably has cells that are consuming oxygen at a much higher rate so increasing the bombardment of organelles and membranes with free radicals.

Dietary extension of lifespan is linked with high levels of cytoprotection. The charactistics of dietary restricted rodents have been shown by many researchers to be compatible with a lower level of free radical generation and an increase in antioxidant activity. Tissue lipofuscin, which is a major accumulation arising from lipid peroxidation is reduced by restriction of protein or total diet. Dietary restriction also reduces lipid peroxidation and increases the activity of catalase in homogenates. The antioxidants, glutathione, glutathione reductase, and catalase decrease with age and the rate of decrease is reduced by dietary restriction. There is also a lower level of membrane lipid hydroperoxide and a decrease in the production of superoxide and hydroxyl radicals. Cytosolic and mitochondrial superoxide dismutase activities are higher in diet-restricted animals. These findings take on importance when they are related to the effects of dietary restriction in extending lifespan and significantly reducing the amount of DNA damage in normal and dietary restricted animals at comparable chronological ages. However, it is important to note that the activity of the cytoprotection system as a whole does not decline with age in animals of either category, indicating that tissue degeneration is not due to an age-related failure of this system.

Free Radicals and Exercise-induced Muscle Damage

Using the urinary excretion of lipid peroxide as a measure of the rate of free radical production it has been concluded that exercise causes 'oxidative stress'. The view is that the greatly increased oxygen consumption in hardworking skeletal muscle (up to 100-fold stimulation) produces superoxide radicals which are associated with ultrastructural damage to mitochondria and myofibrils. There is an increase in SOD in skeletal and heart muscle of rats after exercise training and exhaustive exercise. Further evidence in support of the idea that free radicals play an important part in exercise-induced muscle damage in human subjects comes from the protective action of vitamin E which functions as a 'spin trap' for unpaired electrons. Exercise is also associated with the activation of a complex anti-inflammatory response which involves the short-term accumulation of neutrophils and the longer-term accumulation of monocytes in skeletal muscles, presumably to limit and repair the damage caused by oxidative stress. Paradoxically, both of these cell types can serve as a source of oxygen-free radicals.

Whilst is seems likely that muscular work is potentially damaging, there is no evidence that the tissue's antioxidant systems fails to take care of the increased production of free radicals. More work is needed to define the relationship between exercise and longevity, particularly in the light of findings that giving caged rats the opportunity to run increases their average lifespan. Reduced mortality of rats in exercise cages defines a physiological situation where the animals are able to maintain their normal growth rate with an increased level of food metabolism.

Lifespan Genes that Control the Level of Free Radicals

One of the most significant models in this area of research is the free living nematode, *Caenorhabditis elegans*. In particular, a mutant has been discovered with mean and maximum lifespans that are twice those of the wild type. The mutant type has a level of copper/zinc SOD that is 2–3 times that of the wild type. The lifespan of both types is inversely related to the oxygen tension of the environment. This can be clearly seen from measurements of the Gompertz coefficient of mortality curves which defines the rate at which age specific mortality accelerates. Compared with life at a normal tension of 21% oxygen ageing of both strains was accelerated under 60% oxygen and that of the mutant was retarded under 1% oxygen. Overall, lifespan of the mutant was much more sensitive to oxygen tension that the wildtype, the Gompertz coefficient almost doubling between 20 and 60% oxygen.

Effects of Oxidants on Development

In many organisms changes in oxidative metabolism seem to play a regulatory role in development. Oxygen tension influences developmental pathways, and oxidants appear to alter the expression of genes without interacting directly with DNA or chromatin. In some situations these effects of oxygen are clearly independent of direct actions on respiration. Effects on transcription and translation have both been observed and changes in the balance between the generation and removal of oxidants have been shown to alter the pattern of gene expression in a number of different types of cells. It has been postulated that all of these phenomena may be mediated by free radical reactions which have a directional role in regulating molecular and biochemical processes and thereby make a contribution to differentiation, ageing and malignant transformation of cells. The significance of this as an evolved system may be to tie the rate of development to the availability of resources of energy and carbon. Things can go wrong, and one developmental model of neoplasia with good experimental support involves oestrogen-induced cancer arising from the transformation and proliferation of an embryonic cell under the influence of oestrogen and reactive oxygen species.

These examples of an apparent directional role played by oxidants in development has been related to classical experiments in gerontology showing that the duration of both development and ageing can be varied by altering metabolic rate. Following this idea, some workers feel that free radical generation is the link between metabolic rate and the existence of a 'lifespan metabolic clock' which runs in relation to energy expenditure rather than time. In particular, oxidants are seen as effectors of oxygen-mediated changes in gene expression. Their relatively short lifespan and ability to mediate transient changes in the local intracellular redox environment makes them possible candidates for subcellular messengers. From this standpoint, changes in the levels of antioxidant enzymes, through regulating the level of short-lived messengers, precisely located in subcellular structures, could play a key role in changing patterns of gene expression. It has been suggested that a local upsurge in free radical formation, through local changes in redox state, could produce the point release of a sequestered metal. This could be the indirect link to activate the genome.

The difficulty is to reconcile evidence that byproducts of cellular respiration have a variety of deleterious outcomes on cellular function with a role for free radicals as regulators of species longevity as part of an evolutionary strategy. The only clear bridge to connect the two concepts is the idea that the concentration of free radicals appears to be regulated homeostatically by antioxidant enzymes. This implies that messages pass from free radicals to the DNA segments which control the level of these enzymes. The existence of this negative feedback system would be proof alone that DNA and free radicals can

interact in a non-destructive way. Indeed, it is likely that the normal steady state level of oxidants in cells has been integrated by evolution into the cell's systems for control and regulation.

There is even greater difficulty in relating free radicals to genes that are involved in the determination of lifespan. Relevant experimental work comes from *Neurospora* mutants where a single gene controls lifespan, development, and the organism's antioxidant defences. Age mutants have a lowered capacity to produce antioxidant enzymes in response to oxidative stress. They are also incapable of sexual differentiation unless their medium is supplemented with antioxidants. This model involves oxidants as directional signals which open up a sexual programme of development.

Evidence that Free Radicals are Involved in Degenerative Diseases

There is a great deal of evidence that a wide range of degenerative diseases have histological and biochemical characteristics indicative of free radical damage (Table 10.5). The problem is that they are invariably associated with late clinical manifestations. In diseases, such as Alzheimer's, Parkinson's, and diabetes, they may represent secondary responses, and there are often disagreements in diagnosis which are probably due to mismatches in age and tissue. The apparent susceptibility of the central nervous system to free radical

Table 10.5. Some indicators of free radical damage, one or more of which have been identified in human degenerative diseases as being above age-matched controls

Adduct accumulation
Increase in lipid peroxidases
Increased basal peroxidation
Increase in non-haem iron
Decrease in neurotransmitter receptors
Decreased membrane fluidity
Increased protein cross-linking
Reduced solubility of membrane proteins
Vacuolarization of neurons
Decreased glutathione content
Reduced activity of glutathione peroxidase
Reduced activity of catalase
Increased peroxidase activity
Reduced polyunsaturated fatty acids
Increased iron content
Defect in mitochondrial oxidation
Shifts to enzymes that produce hydrogen peroxide
Local inflammatory reactions with dietary-derived lipids

damage has been attributed to its high levels of non-haem iron to act as foci for free radical production, low levels of antioxidants, and relatively high levels of unsaturated fatty acids which are ideal substrates for peroxidation. Degenerative diseases may therefore develop against a background of a general age-related increase in vulnerability of the central nervous system to free radical attack. It is therefore difficult to make a water-tight case that local foci of, for example, increased basal peroxidase, are not local failures to combat damaging agents that are caused by the disease. With most diseases there are no experimental tests to confirm a fundamental role for free radical damage. For example, an experiment to transect tumour cells with genes for antioxidant enzymes is not a possibility in degenerative diseases of the human central nervous system which have no suitable laboratory model. Nevertheless there are optimists who cheerfully state 'a beautiful picture is emerging from studies on the origin of life, mutation, radiation biology, ageing, degenerative diseases, and free radical reactions in biological systems'.

CLOSED-LOOP MODELLING

Closed-loop Models

Even when causal models have a weighting placed upon each component, as mental models they can seriously limit thinking about a research problem. Their main fault is their static, one way approach to problems which are essentially dynamic with many lateral roots and branches. In particular, if a certain part of the body is exposed to change there is a reaction to counterbalance, or mimimize, the impact of the change. This involves the lateral mobilization of resources from elsewhere to meet a goal of restoring things to normal. This, in turn, triggers other responses to replenish the resources and/or set new priorities in physiological function which take account of the new demands on limited materials and energy. These interconnected loop functions are characteristic of all living things and have evolved as adaptations to meet the demands for resources to be mobilized along new pathways, and are designed to meet goals that have survival value.

Goalseeking is the continuous process of generating actions aimed at maintaining conditions in line with goals for these conditions. It requires operating three processes: a process for monitoring conditions; a process for comparing perceived conditions with goals; and a process for translating any resulting discrepancies into corrective actions. The sequential arrangement of these three kinds of processes to take the system towards its goal is called a closed-loop system. Closed-loop systems differ in how much freedom they have in implementing these three types of processes.

Making a Closed-loop Model

It is only worth making a model if it is possible to perceive a pattern of behaviour that characterizes, or captures, the essence of an issue or problem. The first step is therefore to clarify the most important issues or problems in the system under study that require an explanation. These issues form the basis for developing a pattern of behaviour, referred to as 'the model'. It is never possible to include everything about the real system in a model and developing this reference pattern of behaviour is an aggregation/simplification activity.

The sequence is as follows:

1. *Set time-scale and variables.* Decide on a time-scale for the model then select variables whose behaviour over this time interval best captures the phenomenon under investigation. Any variable whose time path has some peculiarity is a good candidate. Examples of peculiarities include such things a sharp reversal of a trend, an oscillation, or a relative expansion.
2. *Determine the structural elements.* Are there key actors or groups of key actors? 'Actors' are the identifiable elements whose goal-seeking activities are responsible for a variable. Each actor monitors certain conditions in order to determine its relationship with the environment. This perception is the basis for taking actions which change conditions in the desired direction. Any action requires resources to support it. Resource impediments to actors are major constraints in goal seeking.
3. *Is there a chain of events linking major resources?* A chain of accumulations that provides a sequence of stages in a flow of resources provides an infrastructure for a model. It can often highlight some important multistage flow process at the heart of the system.
4. *What are the important accumulations in the system?* Once an important accumulation is identified the flows into it and out of it can be attached to provide the basis for a model.

Defining Stocks and Flows

Structural diagramming requires a feeling for two important building units that are an essential part of the matrix, 'stocks' and 'flows'. 'Stocks' represent the condition of the system and 'flows' represent activities which change these conditions; they are respectively the nouns and verbs of the system's structure and its dynamics.

Stocks are used to represent accumulations which change, relative to the time-scale of the system and the level of aggregation of its elements, to give rise to pressures and action. They are variously known as inventories, reservoirs,

memory banks, buffers, vats, data bases, states of being, reserves, capacitors, resources and state variables. In each case stocks represent conditions within a system. By observing them you can infer how things have been going, how things are now, and make a guess at how things are likely to go in the future. They provide a motivation for taking action, as well as the resources for, and hence the constraints to, taking this action.

Flows in and out of stocks are the only way for the magnitude of a stock to change. Without flows stocks would be like bathtubs without taps and drains. Flows thus create 'dynamics', or changes in conditions over time. When the inflow to a stock exceeds the outflow, the stock builds up. When the outflow exceeds the inflow the stock depletes. Stocks can be either material or non-material (Table 10.6).

The real contribution of stocks and flows to systems modelling derives from their utility in representing goal-seeking processes. Goal seeking is the continuous process of generating actions aimed at maintaining the condition of a stock in line with goals for those conditions (Figure 10.10). It is essential to understand the closed-loop dynamics in order for research to resolve issues about what kind of behaviour pattern a particular set of assumptions will generate.

This simulation is an iterative process in which the dynamic pattern of behaviour generated by running the model again and again, is compared with the real behaviour of the system. If there is a discrepancy the assumptions are changed to remove the difference between the model and reality. Basically this process is an application of the scientific method of 'observing', 'hypothesising', 'testing', 'revising the hypothesis', and finally 'drawing conclusions'.

MODELS OF PHYSIOLOGICAL HOMEOSTASIS

The mammalian response to the uptake of glucose from the gut is a classic example of negative feedback control and regulation. Ageing results in a loss of tolerance to oral glucose and sometimes to a decrease in the accumulation of muscle glycogen. The latter has been attributed to experimentally observed

Table 10.6. Some commonly encountered stocks and flows

Stocks	Inflows	Outflows
Cash	Income	Spending
Charge	Build-up	Decay
Concentration	Solution	Dilution
Inventory	Production	Sales
Order backlog	Bookings	Sales
Population	Births	Deaths
Position	Positive	Negative velocity
Self-esteem	Increase	Decrease

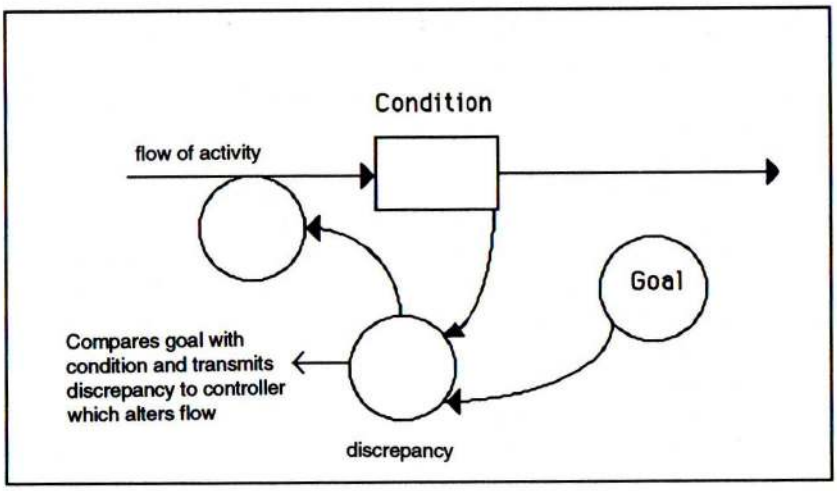


Figure 10.10. System of flows into and out of a stock with a closed loop structure to maintain a goal

failures in the pancreatic negative feedback loop to remove excess glucose from the blood, e.g. insulin binding and maximal insulin-stimulated glucose transport are reduced in adipocytes isolated from old rats.

A systems infrastructure for thinking about the age-related failure of glucose tolerance, particularly relevant to theories on diabetes, is set out in Figure 10.11. This model was built using 'Stella' software, by Barry Stevenson and Steve Peterson, based on the research into human diabetes by Richard Foster. It is set out using the screen icons designating the building blocks of systems thinking: the stock, the flow, the converter, and the connector. It was constructed to illustrate the power of user-friendly research/educational software that has been designed specifically to stimulate systems thinking. As it stands the system is capable of maintaining a steady state level of glucose and insulin in response to addition of glucose and 'removal' of insulin. It also simulates the theoretical proposal that diabetes results from a reduced basal secretory capacity of beta cells, and is caused by a reduced sensitivity of beta cells to glucose. The software allows the operator to challenge these propositions by running the model with pulses of glucose or insulin, and to reorganize the infrastructure as the operator's global view changes in response to the model's responses.

This model may be taken as a starting point for thinking about the complications which develop from diabetes and are a major threat to the quality and length of life. About half of people diagnosed as diabetic before the fourth decade of life die before the age of 50. To take in this wider Warthin viewpoint the glucose tolerance model has to be connected to a range of clinical disorders

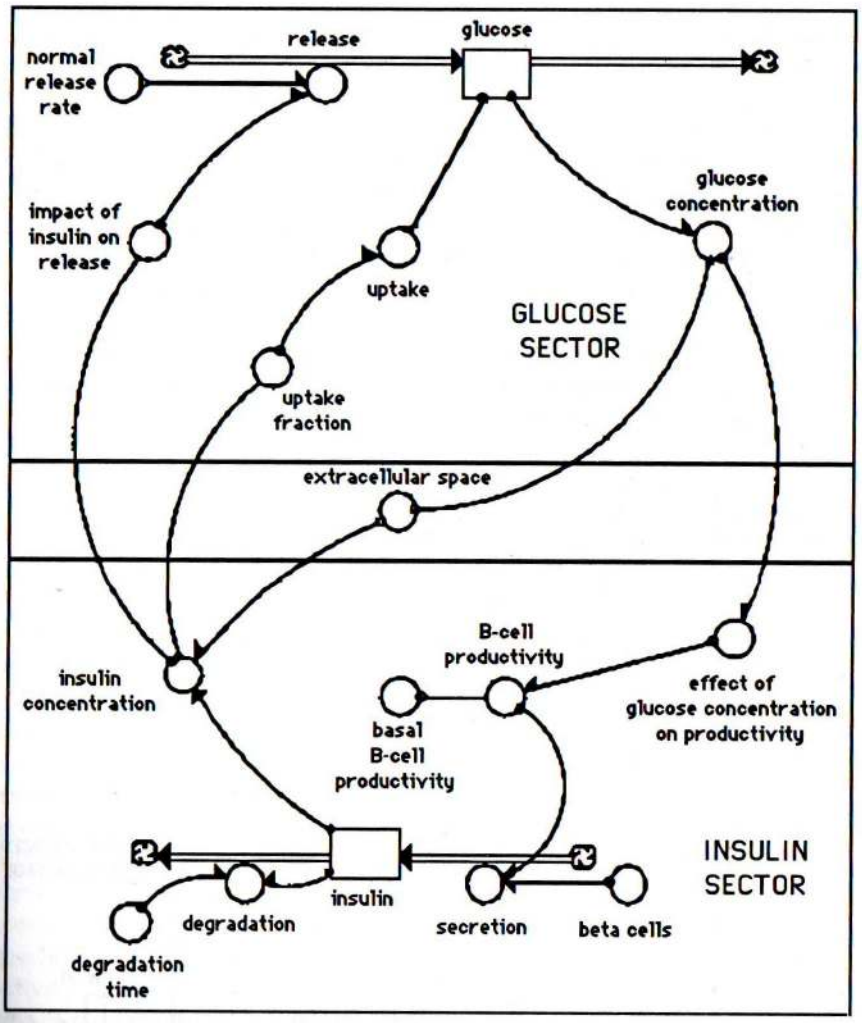


Figure 10.11. The Foster/Richmond/Peterson computer model of glucose-insulin homeostasis

which affect the vascular system, the eyes, the kidneys, the peripheral nerves and the skin. Most theories about the cause of these complications implicate chronically high levels of glucose in blood and tissues. A version of the model representing 'clinical ageing' has to explain how failure to regulate blood glucose can produce a 20-fold increase in the risk of blindness, renal failure, and gangrene of the extremities. This could begin with a version of the model representing 'molecular ageing' which incorporates the 'glycation' theory of

glucose as a non-enzymic cross-linking agent. This theoretical perspective has already been presented. It is based on the fact that the addition of glucose to protein is followed by rearrangements and dehydrations to form Schiff's bases, Amadori adducts, and ketoaldehydes. Ketoaldehydes react with protein to form many denatured products described as 'advanced glycation end-products' (AGE). A development of this theory is that free radical formation, involving catalysis by transition metals that have been translocated to denatured proteins, may be involved in damage of postmitotic tissues seen in diabetes (Figure 10.12).

Another classical research area concerned with closed-loop modelling is the mammalian response to cold stress. This area has also been the subject of gerontological research and some of the results have been described in an earlier chapter. The goal is the maintenance of a body temperature within narrowly defined limits in relation to changes in thermal demand of the environment. 'Thermal demand' is the total bodily heat loss through radiation, convection

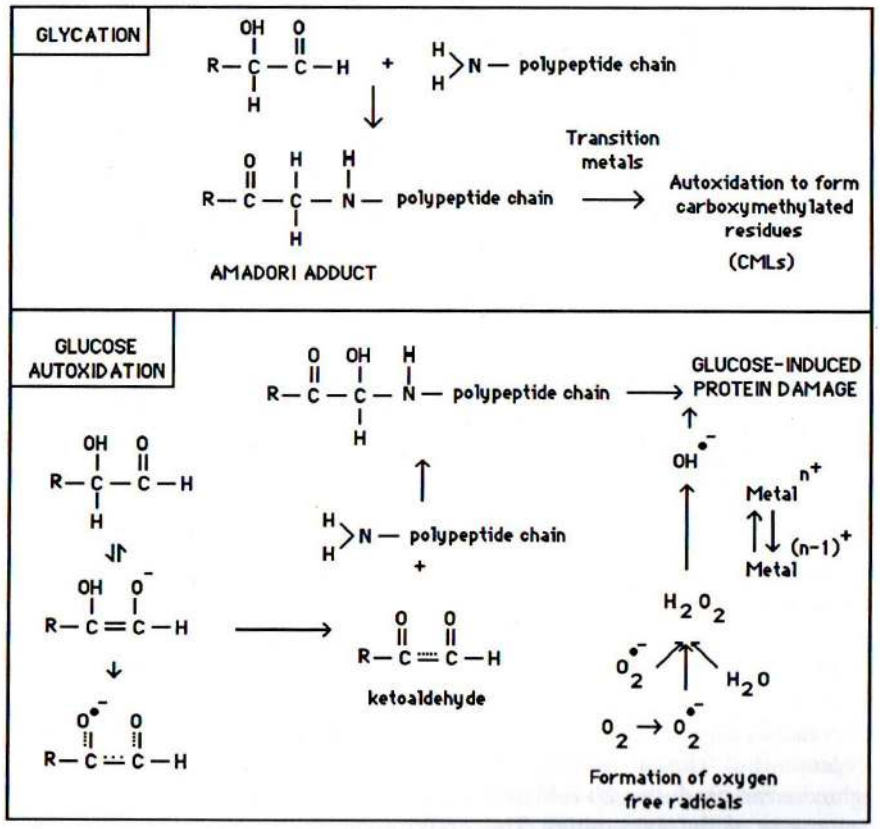


Figure 10.12. Glycation and glucose autoxidation as causal factors in protein damage

and conduction. Ageing is associated with a gradual failure to maintain body temperature against thermal demand.

A systems analysis of cold stress starts from a panoramic viewpoint that a brief exposure to cold requires only a temporary increase in either heat production, or thermal insulation; the latter may be brought about by behaviour such as nest-making. Chronic adaptation to long-lasting exposure entails structural changes as well, which can occur only if there is plenty of food. Usually, if there is a longstanding thermal demand of the environment which exceeds the temperature-maintaining ability of the organism, the animal dies. The outcome of a homeotherm's struggle against cold depends on the relation between three quantities: heat production, thermal insulation, and the temperature difference between the animal's core and the environment. The three main sorts of outcome of exposure to cold—metabolic, insulative and hypothermic—reflect these primary factors. Unfortunately, the study of ageing of this system has tended to concentrate only on the metabolic outcome.

When a mammal is first exposed to cold, if the cold is severe enough the animal shivers and so produces more heat. The blood supply to the periphery is also decreased. After some days the shivering and peripheral vasoconstriction decline, but heat production remains high. The important sites of non-shivering heat production are not yet fully known, but evidently include the viscera and still more the skeletal muscles and brown adipose tissue.

The extra 'non-shivering' heat produced by a cold-adapted homeotherm is an index of intracellular changes in catabolism. It might be expected that, once shivering had ceased, it would be possible to detect a changed but stable pattern of enzymic activity in, for instance, the liver of a cold-adapted animal. This is not the case. Substantial changes continue, even between 30 and 150 days of exposure, in the livers of laboratory rats kept at 4 °C. Hence, even in this one situation, there are evidently at least two phases of cold adaptation without shivering. For this, as for other aspects of adaptation to cold, the precise conditions of experiment, especially the duration of exposure, must be clearly specified. This requirement is illustrated, not only by changes in enzyme activity, but also by the rates of oxygen consumption of liver slices *in vitro*. The rate of oxygen uptake rises during the first 30 days of cold exposure, but after this it declines; by about 120 days there may be no decisive difference from the control figure. Yet, in some experiments, the rate of oxygen uptake by the whole animal remains indefinitely more than 2–5 times that of controls at about 23 °C. The discrepancy may be related to the enlargement of the liver and other heat-producing tissues, partly to a belated increase in heat production by other organs, especially muscles; or changes in the intact animal may not be fully reflected in the metabolism of isolated tissues. A quantitative expression of all these effects is needed, but is not yet available, even for laboratory rats.

Most of the scattered information about the chronic metabolic responses to cold concerns carbohydrate metabolism. After two days at 4 °C, the activity of

liver lactate dehydrogenase, measured by lactate formation, has risen by about 50%. This is only a temporary phenomenon, possibly related to the increased heat production by shivering. After 30 days' exposure, lactate dehydrogenase activity is back to normal, but other changes in enzyme activity have become evident.

The activity of glucokinase, which catalyses the phosphorylation of glucose, is increased by 20%. The activity of other enzymes involved in the Embden–Meyerhof pathway is also increased; these include phosphoglyceromutase, phosphopyruvate hydratase (enolase), and pyruvate kinase. Hence the rate of formation in the liver of pyruvate from dietary glucose is evidently increased at this stage of cold adaptation. At the same time, the activity of liver dehydrogenases that catalyse reactions involved in the tricarboxylic acid cycle is increased.

These observations concern the use of dietary carbohydrate as the source of energy. As for alternative sources, a few facts bear on the use of protein. During only two days' cold exposure, the activity of liver (and muscle) transaminases rises, and the new level is evidently maintained for many weeks. Hence, it is supposed, the rate at which amino acids can be used for heat production is increased. This is accompanied by a temporary cessation of growth or even by a loss of body weight.

The acute endocrine adjustments during cold exposure have been known for decades. Hormones are now seen as the agents which provoke the cellular changes. Lowered temperature of the skin and colon induce not only shivering but also, by way of the hypothalamus, increased secretions of pituitary and other hormones.

On exposure to cold there is an immediate increase in the secretion of catecholamines by the adrenal medulla. Of these, noradrenalin is especially important. This hormone activates liver phosphorylase reactions, and so increases the rate of production of glucose-l-phosphate from glycogen. This is evidently part of a mechanism for the prompt mobilization of reserves of energy. Noradrenalin probably continues to play an important part in cold-adapted animals, for—as we saw above—the responsiveness of the tissues of these animals to noradrenalin is increased.

The response of the thyroid is less rapid, for example, in laboratory rats exposed to a temperature of 6–12 °C, there was an observed increase in thyroid secretion within 8 hours. But more severe cold sometimes led to a decrease. The increased secretion of thyroid-stimulating hormone (TSH) on exposure to cold depends in turn on the hypothalamus: suitably placed injuries in the hypothalamus can result in diminished thyroid secretion, and electrical stimulation can increase it. If the pituitary stalk is cut, there is no thyroid response to cold. The action of thyroid hormone is usually said to be on catabolism: the effect of noradrenalin in increasing heat production is enhanced. The role of thyroid hormone is, however, not simple. In particular, cold can induce a rise in metabolic rate in the

absence of the thyroid. But thyroidectomized rats lose heat at a greater rate than controls, evidently owing to loss of the ability to constrict peripheral blood vessels.

Finally there are the steroid hormones of the adrenal cortex. Since adrenocorticotrophin (ACTH) secretion by the pituitary increases immediately on exposure to cold, so also does the secretion of adrenal cortical hormones. At least in adaptation to cold, their most important function probably concerns protein metabolism. The breakdown of keto acids for oxidation is accelerated by them. This is evidently a temporary measure to meet the demands for extra energy before food intake has increased. There is, however, even in a cold-adapted animal, a rise in protein turnover of the order of 50%. The additional deamination in the liver probably depends in part on the activation of enzymes. The breakdown of arginine to urea is evidently similarly regulated by adrenal steroids.

The recent rapid developments in knowledge of the chemistry of release of energy in cells, and its regulation by hormones, have made it possible to present in outline a causal chain from the first stimulus of cold to the final production of heat. Cold receptors, probably both peripheral and central, initiate reflex responses and also very rapid changes in the secretion of a number of hormones. The hormones in turn act on the cells which produce the extra energy. To what extent the various endocrine effects are confined to the period during which cold adaptation is taking place is not yet clear.

Other things equal, to produce more heat an animal must eat more. The need for extra food, on exposure to cold, is especially marked in small mammals, owing to their high rate of heat loss from a relatively large body surface. Hence their food intake must be assumed to fluctuate with season. Laboratory rats exposed to 2–5 °C increase their calorie intake, relative to body weight, of 40–50% over that in an ordinary laboratory temperature. Survivors of cold-exposed albino rats to 1–5 °C for 6–7 weeks, and fed on mixtures of constant protein content but with fat varying 5–44% by weight showed a constant raised calorie intake regardless of diet; growth was always less than at room temperature.

When a laboratory rat is transferred suddenly to a room at, say, 4 °C, it loses weight, much of it in the form of adipose tissue, as described below. Hence, during this initial phase, food consumption does not keep pace with heat production. However, laboratory rats exposed to seasonal cold in groups do not lose weight; and healthy wild rats have more adipose tissue during winter than during summer. This is evidence that a small mammal, unchanged by domestication, is able not only to eat and digest enough food in winter to maintain heat production, but can even 'over-eat' and so increase the amount of its food reserves and possibly its insulation.

It is sometimes assumed that there is a simple, fixed relationship between the amount of food eaten, on the one hand, and heat production plus growth and storing of reserves, on the other. This relationship holds only if the efficiency of food utilization is constant. In some situations, an appearance of superior

efficiency in a cold environment can be misleading. Male laboratory rats at about 21 °C, on a diet with only 4–3% protein stop growing and die within about 6 weeks. Other rats on this diet, kept at 5 °C, survive and grow; but when returned to the warm they stop growing and die within a few weeks. The primary effect of the cold environment was to double food intake. This incidentally increased the protein eaten to a level which permitted growth. Changes in enzyme activity in rats adapted to 7 °C for 3–4 weeks make possible growth on diets which, owing to an unbalanced amino acid content, will not support growth in rats kept in a warm environment. Here is authentic evidence of improved metabolic efficiency.

A laundry list approach to model cold stress has to take into account all of the above aspects and these are listed in Table 10.7.

Set against the above list what we actually know about ageing of the cold stress response is pitifully small, and for the most part defines the initial stages in the acute response in rats and mice. This partial paradigm models a drop in environmental temperature which increases heat loss through the skin, and triggers an increase in metabolic heat production from the oxidation of brown fat, and reduces heat loss by vasoconstriction (Figure 10.13). Ageing results in the failure to maintain deep body temperature against the drop in environmental temperature. A simple closed loop experimental model using mice involves measuring:

1. Deep body temperature with a thermocouple in the colon.
2. The central co-ordination response by assessing the flow of impulses down a sympathetic nerve to brown fat stores.
3. Whole body oxygen uptake.

Application of a standard cold stress, where old and young mice have the same rate of fall of colonic temperature, has shown that aged mice respond with a

Table 10.7. A tabular multifactorial model of the cold stress response

Temperature differential
Heat sensors
Central co-ordination
Sympathetic system
Shivering
Brown fat
Peripheral vasodilatation
Body insulation
Hypothalamic-trophic hormone system
Enzyme adaptation in liver and muscle
Food intake
Food composition
Behavioural insulation

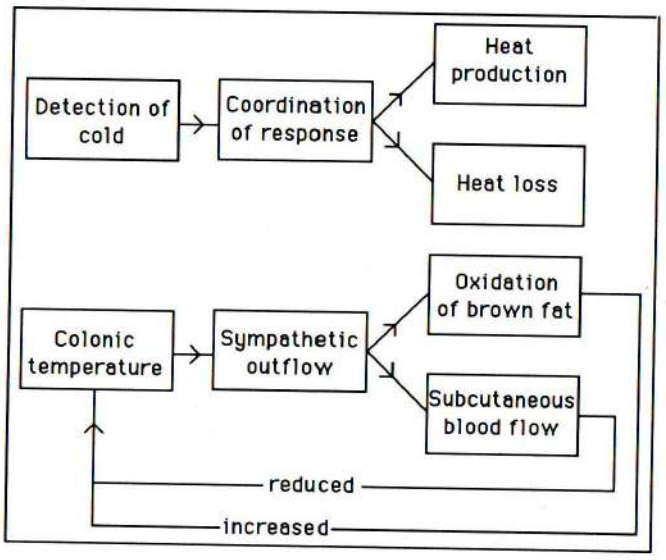


Figure 10.13. A closed loop model of the response to cold-stress

higher rate of sympathetic outflow. This indicates that the loss of ability to keep warm is not due to either a failure to detect cold or inefficiency in the nervous response. Compared with young mice, aged animals have a lower body weight and lower body temperature, with no difference in metabolic heat production. The search for the limiting factor therefore shifts to heat loss through the skin and possible failures in insulation and/or vasoconstriction.

When a mammal is exposed to cold, there are usually changes both in heat production and in insulation. Sometimes the response is almost wholly either one or the other. The effect on laboratory rats of exposure to cold in the laboratory, is a rise in metabolic rate; but wild rats, exposed to the more complex situation of winter out of doors, also improve their insulation. This is an example of chronic adaptation to cold. The acute response to cold must always be primarily metabolic. Even in the acute case, however, an insulative component enters in the form of changes in posture, pilo-erection and, if exposure to thermal neutrality preceded the cold, peripheral vasoconstriction as well. This means that a realistic systems model of the age-related failure to respond to cold stress has to combine all of these features.

The central concept of a comprehensive model is that of a thermally neutral environment. This is a situation in which an animal produces heat at the minimum rate. When the environment cools, a point comes at which the metabolic rate begins to rise; this is the critical temperature. The lower the critical temperature, the greater is the possible economy in energy expenditure. In some arctic species, this is realized to a remarkable degree. An extreme is the

very low critical temperature of −40 °C in the arctic fox; this is due to a thick coat of hair.

Peripheral vasoconstriction also contributes to insulation. It does not, however, always coincide with a rise in metabolic rate. The metabolic rate of fat men in a water bath rises when the bath temperature is lowered below 33 °C, although the maximum tissue insulation is not achieved until the temperature is much lower.

The reciprocal of the insulation conferred by superficial tissues, that is, the thermal conductance, is proportional to the skin blood flow. In a bare-skinned mammal subcutaneous fat enhances the control of insulation achieved by changes of peripheral blood flow. When the blood vessels of the skin are dilated, the subcutaneous fat can have little effect, since warm blood is moving through it to the surface, where heat exchange with the surroundings can take place. After vasoconstriction, this shunt no longer exists, and heat exchange must then take place through the fat.

While the white fox has a very low critical temperature, bare-skinned mammals such as naked man, the young pig and the shorn sheep have high critical temperatures. Hence critical temperature, thermal insulation and deep body temperature are intimately related. This comprehensive causal model is presented graphically in Figure 10.14).

As the animal's insulation increases, the slope of metabolism against temperature in Figure 10.14 moves from BC to B'C' pivoting on the animal's deep body temperature, T. The critical temperature falls at the same time from that opposite B to B', and the cold limit falls from L1 to L2, at the same maximum

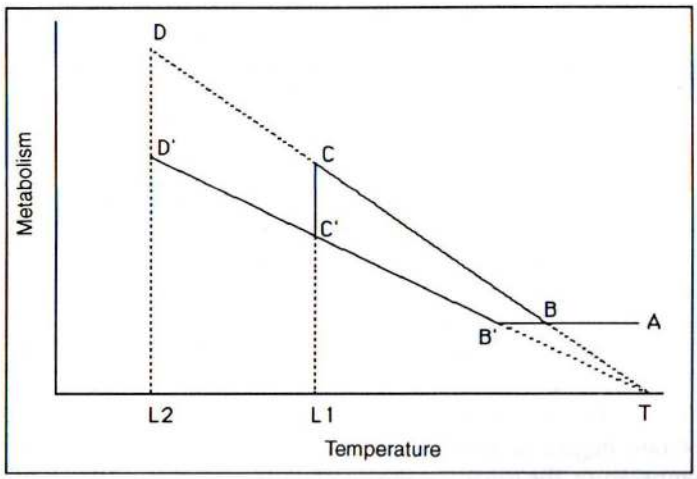


Figure 10.14. The 'Hart' model of the metabolic response to cold stress

metabolism: this is 'insulative adaptation' to cold. In 'metabolic adaptation', the curve BC is extended to BCD; at this point, the cold limit is extended from L1 to L2 by a rise in metabolic rate from C = D' to D. Variation in the level of minimal metabolism A would move B relative to the temperature scale, and thus change the critical temperature.

To sum up, the effective environment comprises a number of factors, the combined effects of which cannot be stated for all mammals by any single universally applicable quantity. Although the influence of each factor must be assessed separately, the factors have to be combined into a general system of the whole animal in its environment. In this respect the Hart model outlined above remains but an ideal for a future integrated attack on the ageing of thermoregulation. The aim would be to produce a family of graphs for cohorts of increasing age, each of which defines the slopes and thresholds set against the risk of death from hypothermia.

The fact that the discussion of cold stress and glucose tolerance have involved the description of partial models is indicative of the piecemeal approach to research. The loose ends are characteristic of the absence of a holistic systems approach. Success in modelling depends critically on the view taken of the system which is generating the behaviour in question. All of us filter our experience through biased lenses. These lenses consist of an implicit set of assumptions about what is important to attend to, and how what is being attended to, works. As individuals, our decisions to devote time and resources to one line of research rather than another are determined by our limited theoretical and technical know-how. Increasingly, because of the high cost of tooling-up a laboratory for biochemical research, there is also a tendency to go, willy nilly, with the momentum of a particular approach and leave other experts to sort out the loose ends.

A SYSTEMS MODEL OF 'DAMAGE'

Because of the many legitimate levels of approach and short-lived fashions, ageing probably offers the worst odds of any facet of biology for an individual breakthrough. Wherever research into ageing will lead in the future, the central ideas behind laboratory strategies will be directed at the origins, progression, and persistance of structural damage to tissues, cells, organelles and macromolecules. The main question will be 'Why do damaged parts accumulate when it is evident that evolution is capable of producing systems for their replacement?'. In relation to this question, the prizes of research will come from a greater understanding of the role of DNA in old tissues. The loss of structural and functional integrity would be expected to result from a decline in gene expression. In particular, if the molecular structure of DNA was altered by random physicochemical damage it would lose its capacity to act as a coded

template, a serious risk for a somatic cell that only has two copies of the code for its self-maintenance.

The importance of DNA damage in ageing is implied from the observed loss of DNA from tissues and organelles, and the diminished expression of genes. There is also direct evidence from measurements on the accumulation of multiple deletions in the DNA sequences of postmitotic tissues.

A nucleic acid can be damaged through any physicochemical impact that produces one or more of the following changes:

1. An apurinic/apyrimidinic site.
2. A cytosine deamination.
3. An abnormal alkylation.
4. A pyrimidine dimer.
5. An adduct formed by an electrophilic agent.
6. A single-strand break.
7. A double-strand break.
8. A interstrand cross-link.

A response to DNA damage can take place at the cellular level when the malfunctioning cell dies or is destroyed. In stem cell systems the dead cell would be be replaced by the daughter of a cell with intact DNA. In postmitotic tissue the only strategy to maintain cellular integrity is either to periodically replace all DNA or to activate an intracellular repair system when damage occurs. Such a system is illustrated in Figure 10.15 in terms of the impact of a free radical on a thymine base. This activates a complex and precise repair system which operates at the site of impact to remove the damaged part and replace it.

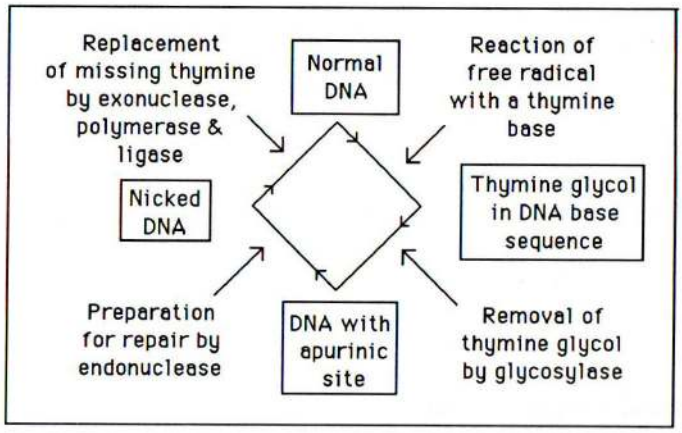


Figure 10.15. A DNA repair system

DNA turnover occurs in all cells and takes place with a range of turnover times spanning hours to days depending on the tissue. The relationship of repair to turnover has not been defined. Although DNA repair systems have been characterized, the blueprints under which they operated are obscure. Vital questions remain unanswered such as: Do they operate in all cells; what fraction of damage are they instructed to repair; are they directed to the preferential repair of their own DNA templates; are there systems that periodically replace strands? Is ageing a failure to produce repair enzymes? Research bearing on the last question comes from direct correlations between slow repair of DNA and short lifespan genotypes. Experimentally, it has been concluded that the reduced lifespan of the vast majority of mutagen sensitive strains of *Drosophila* are due to a loss of genes for DNA repair.

The diagram in Figure 10.15 is not an operational structure. Repair may be considered as a last resort and has to be considered in relation to the operation of systems that produce agents of damage, the responses of those that protect against damage, and the level of damage that can be tolerated. To establish a systems model of damage, links have to be established between these four parameters which define the principal building blocks of an operational model in terms of stocks, flows, signals, and feedback loops. This takes an approach which led to the derivation of the infrastructure of a closed-loop feedback system defined in Figure 10.10. A basic component is the idea of a stock which is an 'amount' of something, i.e. a balance between its 'buildup' by a production system and its 'loss'. This is presented diagrammatically in Figure 10.16A.

A system that ages is limited at any time by the amount of damage that has accumulated. Production of damage at any time is related to the level of 'agents

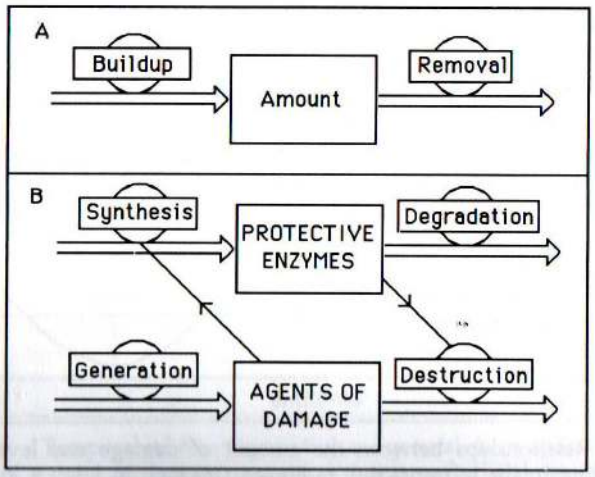


Figure 10.16. Derivation of an infrastructure for a model of damage repair

of damage'. The latter are a balance between their rate of generation and the rate at which they are destroyed by 'protective enzymes' (Figure 10.16B).

The protective system is driven by the presence of agents of damage. For the protective system to operate there has to be a flow of information about the concentration of the agents of damage and whether or not it exceeds a threshold for degradation. If the threshold is exceeded, enzymes are activated or synthesized to remove the excess.

The protective enzymes are themselves the stock of an infrastructure which controls their synthesis in relation to the rate of their destruction. This is a special case of protein turnover. The dynamic relationship between agents of damage and protective enzymes is presented in Figure 10.16B. From a modelling perspective it is a simple infrastructure which is at the heart of a wide range of oscillatory behaviours in biological systems. It is the simplest example of coflow regulation in which two stocks are required, each one serving as the stimulus to the other stock's flow. A rise in the amount of damage stimulates the activation, or production, of enzymes for removing and repairing the damage. In the model the production of agents of damage and the destruction of protective enzymes are treated as constants. This infrastructure generates a sustained oscillation of the kind plotted in Figure 10.17.

The plot shows that repair and damage will cycle out of phase with one another. One stock will achieve its most rapid rate of change in each cycle at the point where the other stock's inflow equals its outflow. Since each stock serves as the basis for generating the other stock's flow, the system will never come to rest. When the level of protection is minimum the rate of build up of agents of damage is proceeding at its fastest, and vice versa. The characteristics of the cycle in terms of period and magnitude of the oscillations, and the mean level of agents of damage to aim for will depend on the instructions given to the repair system.

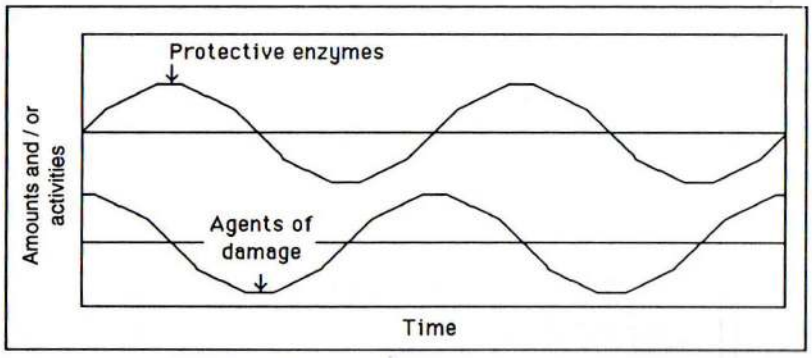


Figure 10.17. Relationships between the amount of damage and level of repair enzymes according to the infrastructure in Figure 10.12

Starting from this simplest of infrastructures the operational map can be expanded to include the factors governing the actual impact of the agents of damage, and components of the system which repairs the subsequent damage. Impact depends on the general cellular level of agents of damage and the accessibility of a potential site of damage. The coincidence of these two features is likely to be random. A signal has to pass from the area of point-damage for the repair system to be activated. A graphical model of the temporal relationship between damage and repair in a particular cell is represented in Figure 10.18.

The diagram defines some matters of principle when DNA damage results from oxidative stress. Local point damage occurs at some critical level of the agent of damage which is then repaired after a lag period for activation of repair enzymes. The burst in damage agents is brought under control by protective enzymes. With regard to DNA damage the two infrastructures of 'protection' and 'repair' are thus connected by a functional link between the level of damaging agents and their rate of impact (Figure 10.19).

At this point is is worth comparing Figures 10.6 and 10.11 with Figure 10.19. Both are attempts to define a mental model of some of the most important features of the biochemistry of ageing. There are large differences in principal between them in the way they have been constructed. Figures 10.6 and 10.11 are idosyncratic, causal, concrete models, based on specific objects and events that have been observed experimentally in isolation, and then put together with arrows indicating their sequential relationships. In contrast, Figure 10.19 is much more abstract and all-embracing and would have inbuilt 'guestimate

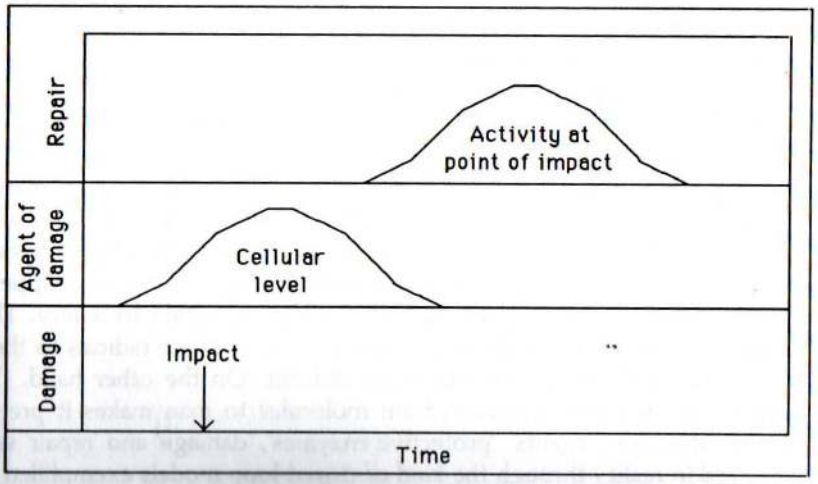


Figure 10.18. Temporal relationships between a general rise in agents of damage, the occurrence of a point of damage, and the activation of a local repair system

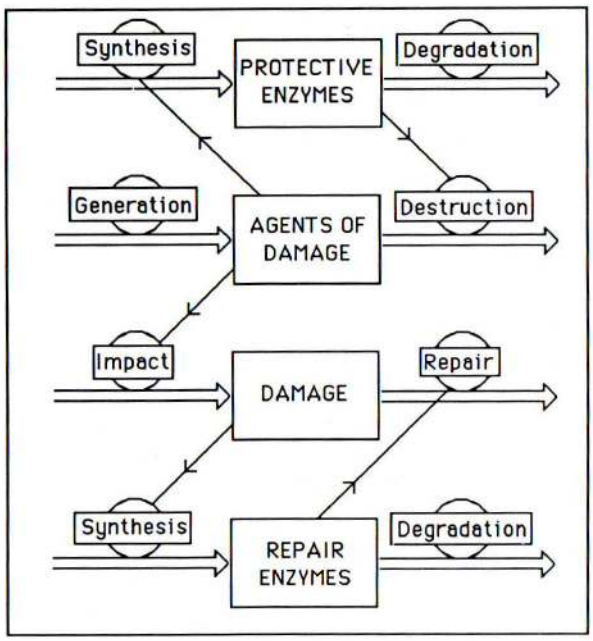


Figure 10.19. Closed-loop model of a DNA repair and protection system

equations' to generate patterns of behaviour that can be tested against reality. Also, the model in Figure 10.14 is expressed in a simpler standard format with the aggregation of detail, into stocks and flows.

Up until now models in gerontology have been mostly in the causal category, close to the bench, and therefore highly detailed and event focused. We are still some way from testing closed-loop models of the second category but they are important to consider because they not only tell us what to look for, but also sharpen the theoretical perspectives. For example, it is likely to be many years before experimentation can produce results to plot the temporal relationships between free radicals and antioxidant enzymes. Until this happens we will not know whether Figure 10.19 is an approximation to reality. The gathering of experimental evidence on the movement of free radicals to their point of impact is likely to be even more difficult. On the other hand, the universality of feedback regulation from molecules to man makes it pretty certain that 'damaging agents', 'protective enzymes', 'damage' and 'repair' will be expressed in reality through the kind of closed-loop models exemplified in Figures 10.14 and 10.15.

It could be fruitful to examine current evidence about protection and repair for clues to see how such a model could be developed now. The main matter of

principle in the model as it stands is that it postulates that in every tissue, at any time, there will always be a certain level of agents which cause damage to DNA, and a certain level of damaged DNA. Experimental work has shown free radicals and damaged DNA are detectable at all ages. More importantly, damaged DNA increases with age, which suggests that postmitotic cells have strategy, developed in Kirkwood's disposable soma theory of the evolution of ageing, to let a certain level of damage accumulate. It could be more fruitful to develop a closed loop model in the direction of a system for minimizing the level of damage, rather than one to maximize damage repair. Systems thinking in this direction raises theoretical questions about the efficiency of regulating damage at the level of antioxidants compared with investing in the removal and replacement of damaged bases at the level of nucleic acids. Probably enough has now been said to demonstrate that systems thinking with closed-loop paradigms tends to encompass a broader and richer set of conceptual inter-relationships than simple causal conceptual mapping. It should also be evident that the study of ageing is a fertile field for systems thinking, and is likely to remain so for a long time.

> *The vantage point from which you operate—and you must adopt one in order to operate!—serves as the first filter on what you experience. What you experience in turn, provides the substrate for all of the mental modelling processes which constitute thinking activity. It therefore makes good sense to become aware of 'where you stand' and the implications of that position for the development of your thinking capabilities.*
>
> *The second filter on what we experience is the set of assumptions that we all carry around in our heads about how the world works. These assumptions are so deeply engrained that we rarely, if ever, become aware of them. However, just like 'where we stand', 'how we look' exerts a huge influence on both what we see and how we make sense of what we see.* (Richmond 1992)

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Index compiled by Campbell Purton