Environmental Health Criteria 144

Principles for evaluating the effects of chemicals on the aged population

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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

ENVIRONMENTAL HEALTH CRITERIA 144

PRINCIPLES OF EVALUATING CHEMICAL EFFECT ON THE AGED POPULATION

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organisation, or the World Health Organization.

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The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are kindly requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

INTRODUCTION
The aged population and the number of chemicals in the environment have been increasing and will undoubtedly continue to increase. It is estimated that there will be 612 million people aged 60 years and over by the year 2000, and of these 61% will live in developing countries. The numerous physiological and biochemical changes occurring during aging can modify the pharmacokinetics and pharmacodynamics of chemicals in the elderly, resulting in either higher or lower levels of toxicity. It is expected that the adverse effects of chemical exposure on the elderly will increase in importance as a health care issue. IPCS has been active in the development and validation of methodology for the assessment of risks from exposure to chemicals. One area of concern has been the evaluation of methodology appropriate for the assessment of risks in "high-risk" groups. The fifth meeting of the IPCS Programme Advisory Committee endorsed the need for an Environmental Health Criteria monograph dealing with the effects of chemicals on the aged population and the aging processes. This monograph integrates relevant studies of toxicology and gerontology; toxicology examines the potential health effects of exposure to chemicals, while gerontology focuses on the scientific explanations for the phenomena and mechanism of aging.

A planning meeting was held in St Petersburg from 5 to 9 September 1988 and was organized locally by the N.N. Petrov Research Institute of Oncology, Ministry of Health, Russian Federation. Financial support through the UNEP Country Projects was provided by the Centre for International Projects (CIF), State Committee for the Protection of the Environment, Moscow, Russian Federation. Dr M.I. Gounar, CIF, formally opened the meeting, and Dr V. Anisimov, on behalf of Dr N.P. Napalkov, former Director of the Petrov Research Institute of Oncology, welcomed the participants. Dr G.C. Becking welcomed the participants on behalf of the Executive Heads of the three IPCS cooperating organizations (UNEP/ILO/WHO). Dr V. Anisimov and Dr L. Birnbaum were Joint Chairmen and Dr P.K. Ray and Dr A. Lkhachev were Joint Rapporteurs.

After discussing the scientific issues relevant to both the aged population and aging processes, the committee considered that there was sufficient epidemiological, clinical and experimental data to support the preparation of an Environmental Health Criteria monograph to evaluate chemical effects on the aged population. However, the differing views on the mechanisms of aging and how chemical exposure might alter such mechanisms preclude at present the preparation of an evaluation of the chemical effects on the aging process. It was decided to prepare a monograph on principles for evaluating chemical effects on the aged population, with only a brief discussion of the present concept of aging. An outline of the monograph together with a list of possible authors was produced.

Drs L. Birnbaum and V. Anisimov prepared the first draft of this monograph based on 13 background papers written by various authors (Appendix 1). Dr V. Anisimov prepared the second draft incorporating comments received following the circulation of the first draft to IPCS Contact Points for Environmental Health Criteria monographs and to IPCS Participating Institutions. Dr L. Birnbaum made a considerable contribution to the preparation of the final text.

A WHO Task Group Meeting met from 9 to 13 December 1991 in Geneva. Dr B.H. Chen, IPCS, opened the meeting and welcomed the participants on behalf of the Director, IPCS, and the three IPCS cooperating organizations. Dr J. Vijg and Dr K. Kitani were Chairman and Vice-Chairman, respectively, and Drs L. Birnbaum and V. Anisimov were Joint Rapporteurs.
The Task Group considered it likely that the aged population is more susceptible to the harmful effects of environmental chemicals. However, very few environmental chemicals have been tested for toxicity in the elderly. Some age-associated diseases may lead to an increased susceptibility to the harmful action of specific environmental chemicals. The effects of environmental chemicals on the process of aging remain to be evaluated. It was suggested that a special scientific workshop be devoted to this topic.

Drs B.H. Chen (IPCS Central Unit) and G.C. Becking (Interregional Research Unit) were responsible for the overall scientific content, and Dr P.G. Jenkins (IPCS Central Unit) was responsible for the technical editing.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

ABBREVIATIONS

ACTH  adrenocorticotrophic hormone
BMAA  beta- N-methylamino-L-alanine
BOAA  beta- N-oxalylamino-L-alanine
cDNA  complementary DNA
CNS   central nervous system
GABA  gamma-aminobutyric acid
GH    growth hormone
GI    gastrointestinal
HDL   high density lipoprotein
hnRNA heterogeneous nuclear RNA
LDL   low density lipoprotein
LH    luteinizing hormone
mRNA  messenger RNA
SDAT  senile dementia of Alzheimer type
T₃    triiodothyronine
T₄    thyroxine
TSH   thyroid-stimulating hormone
UDP   uridine diphosphate
UDPGA UDP-glucuronic acid

1. SCOPE OF THE PROBLEM

1.1 Objectives

The main objective of the Group involved in the preparation of this report was to review present knowledge concerning the effects
of environmental chemicals on the aged population and to evaluate available models for the assessment of these effects and the consequent risk to human health in the aged population.

About ten million natural and synthetic chemicals have been identified by the Chemical Abstract Service Registry and some eighty to one hundred thousand have been identified as important to commerce. The restricted knowledge of the toxicological properties of the natural substances makes it difficult to give clear evidence of whether the elderly population is at risk for this category of compounds, but the impact of these natural toxins might be even larger than that of most man-made toxicants. As the requirements for more toxicological data on natural toxicants become more important internationally, the possible effects on the elderly population should also be included in the assessment.

The following relationships need to be considered: a) the special response of the aged as compared to that of the young following exposure to environmental chemicals; and b) the impact of exposure to environmental chemicals on the processes of aging. This report focuses on the first relationship, i.e. the elderly as a population at special risk. The elderly are heterogeneous with respect to aging processes, life-style and diseases. Indeed, in most instances the deficit in the majority of the elderly relates more to life-style and diseases than to the aging processes per se. The Group recommended that the evaluation of the effects of environmental chemicals on the process(es) of aging should be the focus of a separate scientific workshop. This monograph will focus on environmental chemicals as opposed to pharmaceuticals and food additives, although information on these latter chemicals will be used when necessary to support the issues.

The study of the effects of chemicals on the aged population requires the integration of two disciplines, toxicology and gerontology. Toxicology examines the potential health effects of exposure to chemicals, while gerontology focuses on the scientific explanations for the phenomena and mechanisms of aging. The lack of a unified theory of aging, together with the inability at present to distinguish intrinsic aging from natural disease and toxic response, creates difficulties which make the objective of the Group only partially attainable.

1.2 Definitions

1.2.1 Aging versus senescing

Plant biologists often sharply differentiate between these terms (Leopold, 1975). They may use the word aging to refer to all of the changes in structure and function in an organism throughout the life course, including the period of development. They reserve the term senescence for the deteriorative alterations in structure and function that are the immediate precursors of tissue and organismal death. Mammalian gerontologists, however, typically use the terms aging and senescence (or, more properly, senescing) interchangeably to describe the constellation of changes that occur after the attainment of sexual maturity and the young adult stage of life. This is not to deny the critical importance of developmental events in setting the stage for subsequent patterns of senescence. For example, a specific chemical, physical or infectious agent, acting during a crucial period of ontogeny, could conceivably deplete, but not ablate, a subset of stem cells or their partially differentiated progeny without any phenotypic consequences until additional depletion, related to some normative aging process, reaches a clinically significant threshold.
1.2.2 Aging of individuals and populations

At the organismal level, endogenously and exogenously induced injuries are more likely to occur as the organism ages. There is a decreasing probability, as a function of chronological time, that the organism will survive. Thus, there is an exponential increase in the death rate over time. Although subject to important environmental influences, the ages at which such exponential increments of death rates begin and the kinetics of their progression are subject to strong genetic influences in that they are species-specific. The basic observations have been summarized by the following equation (Gompertz, 1825):

\[ R_m = R_0 \cdot e^{\alpha t} \]

where \( R_m \) indicates the mortality rate at time \( t \), \( R_0 \) is a parameter empirically determined by extrapolating an exponential curve back to zero time (sometimes referred to as the "initial vulnerability"), \( e \) is the natural logarithm, \( t \) is time and \( \alpha \) is a slope constant. Better fits to empirical data are obtained if a second constant is added to the right hand side of the above equation (the Gompertz-Makeham equation).

The Gompertz-Makeham equation is a satisfactory approximation to the kinetics of specific mortality in human populations in the age range 20-80 years. Correspondingly, the value of \( \alpha \) characterizes the rate of aging only within this interval. Although some deviations of \( \alpha \) within this interval in human populations have been noted (Pakin & Hrisanov, 1984), the analysis of the parameters of the Gompertz-Makeham equation permit one to make objective estimations of the changes in the mortality in populations (Sacher, 1977; Hirsch, 1982). It is important to note that the use of this method for measuring the rate of aging in populations of experimental animals is especially reliable when the external conditions (e.g., the housing of animals) remain constant throughout the whole period of a study.

1.2.3 Chemicals of concern

The first class of agents of concern would be those with a special potential to injure elderly subjects because of their unusual susceptibility. This sensitivity might be the result of intrinsic biological aging, chronic exposure to deleterious environmental agents, a high prevalence of various age-related diseases, or a combination of all of these. A rational approach to this problem requires detailed knowledge of the altered physiology, biochemistry and special pathologies of older people (those over age 65) and, especially, of the very old (those over age 85). Examples include the special vulnerability of many elderly subjects to air pollutants, to certain pharmaceuticals and combinations of pharmaceuticals, and even to injury from methane gas explosions. The latter results from a high prevalence of atrophic change in the olfactory network, with consequent marked reduction in the ability of many older people to detect the low concentration of sulfide contaminants that are deliberately added to household gas to warn of leakage. It is apparent that, with respect to such classes of agents, public health actions can be of immediate benefit to older people.

The second class of chemicals of concern would be those that might modulate the processes of aging. These could either accelerate ("gerontogens") or retard ("geroprotectors") the aging processes.
1.2.4 Time and dose of exposure

As indicated above, chemical agents that have the potential to accelerate aspects of aging could act at any time during the life course, from before birth to death. Strictly speaking, however, agents that are most likely to closely mimic natural aging processes are slow, insidious and progressive. Moreover, since the phenotypic consequences of aging are often subtle and, for the human species, develop over a period of decades, it would be difficult indeed to establish a minimum effective dose for such putative chemicals. The task is somewhat less difficult in the case of agents to which the elderly have some special vulnerability, since acute and sub-acute end-points are often involved. For example, the prevalence of cardiopulmonary morbidity can be related to ambient concentrations of specific urban pollutants.

1.3 Chemical exposure

The world is irrevocably dependent on man-made chemicals, modern technology bringing a dramatic increase in their production and consumption. More than 750 000 chemicals are known to be in our environment and between 1000 and 2000 new ones enter the market each year. A major proportion of these chemicals find use as components of various consumer products, or they enter the environment as industrial waste, posing health risks as well as benefits.

In present-day society, we use chemicals to boost our food production, make our lives easier and protect our health. Many of these chemicals are hazardous and great care must be taken during their usage, storage and disposal. Their releases into the environment, whether intentional or not, can have severe consequences.

Billions of tons of hazardous industrial waste materials, produced every year, may enter the environment through complex and interrelated pathways (air, water, food, etc.), and could affect humans. Pesticides, fertilizers and herbicides enter the environment as a result of direct application; nitrogen oxides, sulfur oxides and polycyclic aromatic hydrocarbons result from combustion processes. Many manufacturing processes liberate unwanted by-products and waterborne and airborne wastes, which are sometimes more toxic than the raw materials. Incidents such as the contamination of water by mercury, the widespread distribution of industrial oils (e.g., polychlorinated biphenyls), and the destruction of the ozone layer in the stratosphere due to the release of aerosol propellants (chlorofluorocarbons) have made the public aware of the ability of some chemicals to cause unexpected results at some point far removed from where they were originally introduced. Chemicals undergo transformation once they enter the environment, and a relatively harmless chemical may become a toxic by-product. It may further enter the food chain and accumulate in living organisms, eventually reaching humans.

In both developed and developing countries there has been a great impact of life-styles upon the quality of life and upon the life span of the population concerned. Of particular interest are the aged individuals, who, having lived longer in an environment containing toxic agents, may suffer from their cumulative effects even when exposure levels are relatively low. The multiple life-long, though low-level, human exposure to chemicals is difficult to assess adequately in terms of associated health risks (Pines et al., 1987). It has been reported that some chronic low level exposures to chemical or physical stressors have beneficial
effects on longevity (hormesis) (Sacher, 1977; Neafsey, 1990).

Among the chronic health effects of chemicals, cancer is of major concern. Many substances have found in recent years to be carcinogenic in one or more species of laboratory animals (WHO, 1983). In humans, cancer is seldom manifest until 10-40 years after exposure to the carcinogenic agent (IARC, 1990). Thus, cancers caused by chemicals are most often observed in the aged population. However, it is not easy to identify the hazards unless past exposure is known. Similar comments may be made about atherosclerosis, which may also be related to chemical exposure (Penn et al., 1981).

In many cases, especially with respect to long-term effects, the response to a chemical may vary, quantitatively or qualitatively, in different groups of individuals depending on predisposing conditions, such as nutritional status, disease status, current infection, climatic extremes, and genetic features, sex and age of the individuals. Understanding the response of such specific risk groups is an important area of toxicology research today.

There is no biological basis for classifying substances according to their environmental source (e.g., industry or agricultural use), or use patterns (e.g., food additives). Chemical substances with neurotoxic potential, for example, are found as natural metabolites (e.g., quinolinate), biological poisons in plants (e.g., gossypol) and animals (e.g., batrachotoxin), natural components of food (e.g., beta-oxalylamino-L-alanine (BOAA)) and beverages (e.g., ethanol), food contaminants (e.g., ergot), synthetic food additives (e.g., aspartame), flavours and fragrances (e.g., dinitromethoxybutyl toluene), pollutants of air (e.g., lead), water (e.g., zinc pyridinethione) and industrial processes (e.g., carbon disulfide), and therapeutic drugs (e.g., phenothiazines). In addition, numerous chemicals, if they have damaging actions, may contribute to the aging phenotype.

Chemicals influencing the processes of aging and/or affecting the aged population may be classified into several groups according to their chemical properties and metabolic behaviours. Chemicals that are poorly metabolized fall into two groups. The first are absorbed and distributed into certain tissues according to their partitioning behaviour, based on their physical/chemical properties. For example, organochlorine compounds concentrate in adipose tissue. During fasting, adipose tissue is mobilized and accumulated chemicals are liberated into body fluids. Organo-chlorine compounds are detectable in adipose tissue, blood and breast milk long after cessation of exposure. The second group comprises chemicals that are poorly excreted and accumulate in the body. Some of these chemicals are detoxified by binding to specific proteins, resulting in long-term storage. For example, cadmium, lead and mercury induce specific proteins such as metallothionein which help in the detoxification of heavy metals (Oh et al., 1978; Onasaka & Cherian, 1981). The appearance of cadmium toxicity among the population over 50 years of age may be related to the decreased capacity of metallothionein synthesis with advancing age (Hunziker & Kagi, 1985).

Chemically and biologically active chemicals are readily metabolized. Thus, they do not accumulate in the body. Continuous exposure to these chemicals is of concern because these may be metabolized to reactive intermediates that can interact and damage cellular macromolecules. Such damage may be cumulative, resulting in the aged population being more vulnerable. Other types of chemicals which belong to this group (e.g., NOx, SO2) can also cause more...
severe adverse effects on the aged whose defensive mechanisms are weakened. It should be stressed that several chemicals can enter the body at the same time, causing a more complex problem.

1.4 Aged population

1.4.1 Demographic consideration

The United Nations has defined people of 60 years and over as the aged. In 1988, it was estimated that there were about 488 million people in the world fitting this criterion. The number is expected to rise to 612 million by the year 2000, 61% of whom (i.e. 376 million) will be living in developing countries (Fig. 1) (WHO, 1990).

Many countries are, however, using 65 years and over as the definition of the elderly. The corresponding numbers for this age group are 327 million in the world in 1990 and 423 million by the year 2000, of whom 250 million will be in developing countries (WHO, 1990). The increase in the elderly population will be particularly marked in Asia, primarily as a result of the rapid growth expected in the numbers of the aged in China and India. This trend is illustrated in Fig. 2, which indicates the 20 countries with the largest aged population in 1980 and the expected growth of the aged population. By the year 2020, there will be an increase of 270 million elderly citizens in China and India. The size of the aged population is expected to rise by more than 20 million in both Brazil and Indonesia, and by roughly half that number in Mexico, Nigeria and Pakistan (WHO, 1989).

On the other hand, a much smaller absolute increase in the elderly population is anticipated for the European countries, where population aging began much earlier. As a result, the developing countries will gradually account for the largest elderly population in the world. Indonesia, for example, is expected to move from tenth place in 1980 to fifth in 2020 (Fig. 2), and Mexico is expected to have the eighth largest elderly population, ahead of Italy, France, and the United Kingdom (WHO, 1989).

The elderly population of the USA is growing much more rapidly than the population as a whole. In the 1970s, the population aged 65 and over increased by 28% and the population aged 85 and over increased by 59%, whereas the total population increased only by 11%. The population aged 85 and over is expected to triple between 1980 and 2020 and is the fastest growing of the four older age groups (55-64, 65-74, 75-84, and 85 and over). Census projections for 2050 indicate that the proportion of the population aged 65 and over (22%) will be almost twice as great as it is today (12%). In the last two decades alone, the 65-plus population has grown by 54% while the under-65 population has increased by only 24%. At the beginning of this century, less than one in eight Americans was age 55 and over. The increase in the numbers of elderly people is expected to occur in two stages. Until the year 2000, the proportion of the population age 55 and over is expected to remain relatively stable at 22%. By 2010, because of the maturation of the post World War II baby boom, more than a quarter of the total population of the USA is expected to be at least 55 years old, and one in seven of the population will be at least 65 years old. By 2050, one in three persons is expected to be 55 years or older and one in five will be over 65 (US Senate Special Committee on Aging, 1986).
It is commonly assumed that today's large percentage of elderly people in the population is a result of increased longevity and...
decreased birth rate. For example, in Japan the proportion of people aged 65 or more had increased to 10.3% in 1985. At the same time, the values were 15.1%, 14.5%, 12.4% and 16.9% in the United Kingdom, Federal Republic of Germany, France and Sweden, respectively. Japan is a new "aged-type" country with the greatest rate of increase in the elderly in the world. By the year 2025, the proportion of the Japanese population aged 65-plus will rise to 23.4% (Hosomi, 1990).

In China, the proportion of the population aged 60 and over was 7.3% of the total population in 1953. By 1984 it had increased to 9% of the total population, and by the year 2000 the proportion of the population 60 and over will be as high as 10.6%. The proportion is predicted to increase to 26.2% by 2025 (Xiong, 1990).

At present, the aged population is growing more rapidly in China than in countries of Europe and North America. According to data from the US Bureau of the Census, it took 115 years (1865-1980) for the proportion of people aged 65 or more in France to increase from 7% to 14%, 85 years (1890-1975) in Sweden, 66 years (1944-2010) in the USA, 45 years (1930-1975) in the United Kingdom, and 26 years (1970-1996) in Japan. For China, the pattern of the growing number of elderly is similar to Japan, i.e. the proportion of people aged 65 or more will be 7.4% in 2000, and by 2025 it will have increased to 12.8% (Hosomi, 1990; Yao, 1990; Xiong, 1990).

1.4.2 Life expectancy

Life expectancy at birth is a statistical index calculated by the use of a life table from the age-specific death rates of the population. This illustrates the overall level of health in a country or a region. Throughout this century, it has been evident that, as a result of improvements in many aspects of health status, an individual can expect to live longer. From 1960 to 1990, life expectancy at birth for the total population had increased by 13.5 years. The life expectancy at birth for the period 1985-1990 was estimated to be 63.9 years for the world as a whole, 74.0 years for the more developed regions, and 61.4 years for the less developed regions. The longest life expectancies are in Japan (78.3), Iceland (77.5), Sweden (77.1), Switzerland (77.1), and the Netherlands (76.9) (World Population Prospects, 1991).

The trend for life expectancy at birth in the USA showed an increase from 1900 (46.4 for males and 49.4 for females) to 1950 (65.6 and 71, respectively) and in the year 2000 is expected to be 72.1 and 79.5 (for males and females). By 2050, it may have increased to 73.6 for males and 81 for females (US Senate Special Committee on Aging, 1986).

In China, the life expectancy at birth before 1949 was about 35 years of age, being one of the lowest in the world at that time. By 1957 it had increased to 57 years, from 1973 to 1975 it was 63.6 years for males and 66.3 years for females, and in 1981 it was 68 years. It is expected that the continued increase in the average life expectancy of the people of China will be slow due to a high death rate from cardiovascular diseases (Gu, 1986).

1.4.3 Life-style in aged populations

A basic issue in planning for the consequences of demographic aging is whether elderly people should be considered a specific target group for the development of services, or whether their needs should be catered for within the context of planning for the population as a whole. One approach to a rational policy for this issue is to consider the nature of human aging. For this it is
necessary to view the physical, psychological and sociological
dimensions of aging as a whole.

Life-style influences the effects of chemicals on human health,
including that of the elderly, both quantitatively and
qualitatively. Environmental chemicals and their uses are diverse.
Specialized nutritional elements of the diet have become popular,
while in certain countries many people prefer predominantly
vegetarian diet. Others take supplements and additives which contain
pure preparations of vitamins, minerals, amino-acids and other
substances. Whether such substances have either adverse or
beneficial effects on the elderly and aging processes, has not
generally been fully evaluated. Another important source of human
exposure to chemicals comes from the intake of different kinds of
cosmetic agents and fragrances, such as shampoos, creams, perfumes,
oral deodorants, sunscreen and suntan lotions, and insect
repellents. These are often chemical mixtures whose components have
not been evaluated or tested beyond acute toxic potential.

Occupational status, indoor air quality, recreational
activities, exercise, eating and drinking habits, alcohol
consumption, and tobacco smoking can all affect the elderly and
aging processes to a certain degree. Elements of life-style can
strengthen or reduce the risk of developing aged-related
degenerative diseases. They can also accelerate or delay
physiological and anatomical changes. Typical examples are the
various age-related diseases caused by toxic chemicals in tobacco
smoke and the reduction of the risk of cardiovascular diseases
produced by regular exercise (Committee on Chemical Toxicity and
Aging, 1987).

As far as the possible influence of life-style factors on the
manifestations of aging is concerned, many studies have shown that
loneliness and physical and intellectual inactivity are common among
the elderly, especially widowed people. Several studies have
revealed that living conditions have an influence on health and
well-being, resulting in an increase in the demand for social care
and medical service. Marital status and living arrangements have
important significance for the unique life-style of the elderly.
There are striking differences between the proportions of elderly
males and females who are married: in many countries the proportion
of widows is very high and that of widowers relatively
low (WHO, 1984).

Migration is also one of the life-style variables of the
elderly. In rural areas of Asia, many older women move to cities to
join their children after they have been widowed. Another common
type of move is the migration of the recently widowed or chronically
ill elderly from urban areas to their home towns or villages. For
many countries in Africa and Asia, the urban-rural migration is most
apparent among males, who return from urban to rural areas when they
are old. Worldwide, only a minority of elderly people live in urban
areas (WHO, 1984).

1.5 Theories of aging

During the last century, more than 100 various hypotheses
concerning the origin and mechanism of aging have been put forward.
All of them could be grouped generally into two broad categories:
those that invoke deterministic, or "programmed", alterations in
gene expression or gene structure; and those that invoke a variety
of stochastic, or "random", alterations in the structure and
function of macromolecules, cells, and organ systems. This distinction, however, has some limitations, because stochastic alterations in individual cells can lead to predictable phenomena in the large populations of cells. The use of terminal differentiation to explain the limited replicative life span of somatic cells (Martin et al., 1974) could be an example of the blurring of the stochastic and non-stochastic categories. For each individual cell, differentiation is a random event; however, for a population of cells, the process appears deterministic.

The mechanisms of aging are likely to be coupled to the reproductive strategy of the organism. One example is the synchronous, rapid physiological declines and mortalities that are characteristic of species with single massive episodes of reproduction (e.g., migrating Pacific salmon or soybean plants). Placental mammals, however, have ample opportunity for a variety of stochastic processes to take place during their long reproductive and postreproductive phases. The associated patterns of structural and functional decline can vary substantially, both qualitatively and quantitatively, among individuals within a species and among different related species. Evolutionary biologists in fact present compelling arguments that aging did not evolve because of any adaptive value to the individual or to the species, as would be assumed by strictly programmed theories (reviewed in Rose, 1991). Aging is thought to occur simply because of the decline in the force of natural selection for gene action that is postreproductive. Such gene action could be related to accumulations of late-acting mutations in the constitutional genome or to selection for forms of genes that have positive effects on reproductive fitness early in the lifespan, but whose effects may be negative late in the lifespan (the "antagonistic pleiotropy" theory of aging) (Rose, 1991).

It is beyond the scope of this monograph, however, to consider the potentially large numbers of specific mechanisms that may be modulated by such accumulated constitutional mutations or pleiotropic genes. The reader is referred to recent reviews of the many postulated theories of aging (Warner et al., 1987; Committee on Chemical Toxicity and Aging, 1987; Finch, 1991; Cutler, 1991). These can be classified in a variety of ways (Dilman, 1987; Medvedev, 1990).

2. STRUCTURAL AND PHYSIOLOGICAL CHANGES IN THE AGED

2.1 Changes in gene structure and function in aging

Changes in gene expression are of critical importance to an organism. Aging can potentially alter not only the structure of genes, but the way in which they function. Changes in the DNA are often thought to be integral to aging. It is clear that not only mutations, but chromosomal rearrangements accumulate with age (Vijg, 1990). Repetitive sequence families may play a crucial role in the processes of aging. In addition, the organization of DNA and protein in chromatin is important structurally and functionally. Therefore, changes in chromatin could play a major role in the age-related change in the regulation of gene expression (Richardson et al., 1983; Medvedev, 1984; Thakur, 1984; Richardson et al., 1985).

2.1.1 Chromatin structure

Chromatin changes may involve either proteins that interact with DNA or the chemical structure of the DNA molecule itself. Although no change in the stoichiometry of the major histones has been observed with increasing age (Richardson et al., 1983;
Medvedev, 1984), several investigators have reported changes in the subspecies of histone H1 (Medvedev, 1984; Mitsui et al., 1980; Niedzwiecki et al., 1985). The acetylation of histones, which has been proposed to alter histone-DNA interactions thereby making DNA more accessible, decreases by 30% to 70% with increasing age (O'Meara & Pochron, 1979).

With respect to age-related changes in DNA chemical structure, there is now conclusive evidence for the "spontaneous" induction of a variety of DNA lesions in different organs and tissues of both humans and experimental animals (for a review, see Mullaart et al., 1990). Most of these lesions seem to be repaired (see below), but not all. For example, Cathcart et al. (1984) and Fraga et al. (1990) estimated that in rats about 10^5 oxidative DNA lesions occur per cell per day. Since the rate of repair does not entirely equal the rate of induction of damage, there is a net increase of spontaneous DNA lesions with age. Fraga et al. (1990) calculated for one specific lesion, 8-hydroxy-deoxyguanosine, that about 80 residues accumulate per rat cell per day.

Although some DNA lesions are repaired quickly, this is not the case for all lesions. Indeed, after treating rats with low doses of 2-acetylaminofluorene (AAF), Mullaart et al. (1989) were still able to detect about 30% of the major lesions induced as late as 21 days after treatment. Such incomplete repair could be responsible for accumulation of DNA lesions during continuous or frequent exposure to genotoxic agents.

2.1.2 DNA repair

To preserve the DNA chemical structure, cells are equipped with a battery of repair systems to remove damage. As yet the various mechanisms of action of these DNA repair systems and their interrelationships are incompletely understood (for a recent review, see Lehmann et al., 1992). In general, repair systems can be divided into three categories, i.e. direct repair, excision repair and post-replication repair. In direct repair, the lesion itself is removed without any further (transient) changes in the DNA structure. Direct repair includes the enzymatic photo-reactivation of UV-induced pyrimidine dimers and the removal of O6-alkyl adducts by specific alkyl transferases.

DNA excision repair is brought about by a complex multi-enzyme system, the components of which are involved in the various steps in this repair process (Vijg & Knook, 1987). The third type of repair, post-replication repair, does not actually remove the damage but allows the replication system to bypass the damage. It is this latter process especially that is considered to be associated with nucleotide misincorporation (mutation).

Accurate assessment of an organism's capacity to repair specific lesions is difficult and subject to error. In general, the most reliable data can be obtained when the induction and disappearance of the relevant lesions themselves are monitored in the different organs and tissues of an experimental animal. Unfortunately, in most studies on the possible existence of a decline in DNA repair activities with age, assays were used which measured the DNA synthesis phase of excision repair. The general conclusion from these data, mostly obtained with cultured cells, is that there is no age-related decline in the efficiency of DNA repair systems (Tice & Setlow, 1985; Likhachev, 1985; Hanawalt, 1987). It cannot be ruled out, however, that during aging DNA repair systems become more error prone, leading to an accelerated induction of mutations (Vijg & Knook, 1987). In any case, a certain degree of
imperfection is a general characteristic of DNA repair systems as indicated by the actual accumulation of both DNA lesions and DNA sequence changes (see above). The question that should be addressed is what type of DNA alterations occur, how many exist, and at what rate do they accumulate with age. Finally, their relevance in terms of actual physiological decrements or the initiation of disease should be assessed.

2.1.3 Transcription

Several review articles have been published in the past decade that discuss the effect of age on transcription (Rothstein & Seifert, 1981; Richardson et al., 1983; Richardson et al., 1985; Richardson & Semsei, 1987; Slagboom & Vijg, 1989). A major problem in this area has been the difficulty in accurately measuring the rates of synthesis of specific RNA species and their intracellular levels. With the major advances in recombinant DNA technology, this problem has now been virtually eliminated and our knowledge of how aging affects the expression of specific genes is rapidly growing.

At present, it appears that the overall transcriptional activity of a cell declines as an organism ages. However, the level of total RNA tends to remain constant suggesting a decline in the rate of RNA turnover (Horbach et al., 1986).

The levels of some specific mRNA species using cDNA probes for specific genes have been measured recently (Richardson & Semsei, 1987). In general, no consistent trend has emerged. The levels of some mRNA species decrease with age; however, other mRNA species do not change with age, and others actually increase (Slagboom & Vijg, 1989).

In most of the studies, a good correlation has been found between the age-related changes in the level of an mRNA species and the level of protein (or enzyme activity) specified by the mRNA species. This has been demonstrated in rat liver for albumin (Horbach et al., 1984), alpha2u-globulin (Richardson et al., 1987), and superoxide dismutase and catalase (Semsei et al., 1989), and in rat kidney and small intestine for calbindin-D (Armbrecht et al., 1989). The age-related decline in mitogen-induction of interleukin 2 (IL-2) (Wu et al., 1986; Nagel et al., 1988; Pahlavani et al., 1988) and IL-3 (Li et al., 1988) mRNA in lymphocytes from rodents and humans corresponded to the age-related decline in the biological activities of these two interleukins. In contrast, Strong et al. (1990) reported an uncoupling of tyrosine hydroxylase transcription and translation in the adrenal glands of old rats.

Investigators usually assume that age-related changes in the levels of a particular mRNA species arise from a change in transcription. However, only a few studies have actually measured the transcription of a specific gene as a function of age using nuclear run-off assays. While an age-related decrease occurs in the nuclear transcription of the alpha2u-globulin (Richardson et al., 1987; Murty et al., 1988a), cytochrome P450(b+e) (Rath & Kanungo, 1989), and superoxide dismutase and catalase (Semsei et al., 1989) genes, the nuclear transcription of tyrosine amino-transferase and tryptophan oxygenase (Welling & Guigoz, 1986), albumin (Horbach et al., 1988b) and the c-myc (Buckler et al., 1988) genes was similar in young and old rodents. Studies are now underway to explore in more detail age-changes in specified mRNA species in terms of the transcription factors involved (Post et al. 1991).

One exciting development in the area of transcription and aging
has been the observation that dietary restriction, which enhances the longevity of rodents, alters the expression of some genes at the level of transcription (Richardson et al., 1987; Semsei et al., 1989). However, the expression of all genes is not affected by dietary restriction (Waggoner et al., 1990).

In addition to nuclear synthesis, post-transcriptional processing of hnRNA plays an important role in the regulation of gene expression. Müller et al. (1989) recently discussed various views of how the post-transcriptional processing of hnRNA might alter with age. At present, there is little evidence that major changes occur with age in the size of the poly(A)-segment of mRNA (Birchenall-Sparks et al., 1985). Interestingly, in the many studies in which mRNA species have been analysed by Northern blot analysis, there has been not a single report of a significant change in the size of the mRNA species examined with increasing age (Richardson & Semsei, 1987). Thus, there is very little direct evidence at present to support the view that the processing and/or nuclear transport of hnRNA is altered with age.

2.1.4 Translation

Increasing age generally results in a decrease in total protein synthesis in plants, invertebrates, rodents and cultured cells (Richardson & Birchenall-Sparks, 1983; Ward & Richardson, 1991). Recent studies have focused on the influence of age on the translation of mRNA into specific proteins and on the ability to modulate age changes in protein synthesis. There is no evidence that a decrease in the fidelity of protein synthesis occurs with advancing age but technical limitations do not permit a definitive conclusion (Rosenberger & Kirkwood, 1986). The influence of age on protein synthesis differs from protein to protein and much more work must be done in assessing the effect on key individual proteins. Attempts to modulate protein synthesis have recently begun. The rate of protein synthesis in the liver is higher after maturity for dietary restricted than for ad libitum fed rats (Ward, 1988). In an in vitro system, growth hormone increases protein synthesis in muscles of old rats to the level found in muscles of young rats (Sonntag et al., 1985). Much more study is required, focusing on individual proteins, different tissues and different organisms.

2.2 Changes in tissues, organs and systems in aging

The progressive modification of body functions with age involves alterations not only at the genetic, molecular and cellular levels, but at the level of the tissues, organs, systems and entire organism. It is important to attempt to differentiate between age-related pathology and true physiological aging. This is often difficult because the majority of age-related changes increase the vulnerability of the aging organism to disease and ultimately death.

In the following, each organ or system will be discussed in reference to age-related changes in its structure which might predispose to alterations in function, not only inherently as part of aging, but in response to environmental agents. The focus will be on the healthy aged as opposed to the diseased.

2.2.1 Nervous system

The brain may undergo a progressive deterioration with age at all levels of organization - structural, biochemical and functional. CNS disorders, including Parkinson's and Alzheimer's diseases, are common in the elderly.
2.2.1.1 Structural changes

Brain weight decreases slightly with aging. This is due to atrophy of both grey and white matter (Creasey & Rapoport, 1985). At the cellular level, the major age-associated modification is in the number of neurons, which are significantly diminished in discrete areas of the brain (Brizzee, 1985), particularly in the basal ganglia, cerebellum (probably related to decreased motor control), locus ceruleus (associated with alterations in sleep patterns), nucleus basalis of Meynert (associated with senile dementia of Alzheimer type (SDAT) (Bondareff, 1986), and the spinal cord. Neuronal loss, which is associated with an increase in the number of glial cells, is relatively mild in the healthy aged, but is much more severe in SDAT, Parkinson's disease and in the early aging associated with Down's syndrome.

In addition to a reduced number of neurons, the aged brain is characterized by a reduction in the number of dendrites and dendritic spines, probably due to a slowing renewal process (Scheibel & Tomiyasu, 1978). Synapse density declines in discrete areas of the brain, but this is partially compensated by enlargement of the remaining synapses (Bertoni-Freddari et al., 1990). Intracellular changes include dilation and fragmentation of the Golgi apparatus (Mervis, 1981), distortion of membranes and the nucleus, and accumulation of lipofuscin, in both neurons and glial cells within discrete brain areas. With advancing age, there is an increase in neurofibrillary tangles (intracellular tangled masses of paired helical filaments) (Terry, 1963), extracellular neuritic plaques (a core of amyloid surrounded by material derived from dystrophic neurites), and reactive glial and microglial cell accumulation (Master et al., 1985). Again, these changes occur in normal aging at a moderate level, but are much more frequent in SDAT (Iqbal et al., 1982) and other dementias.

There are also age-related changes in the morphology of the peripheral and autonomic nervous systems. These include reductions in the number of sensory and motor neurons, increases in demyelination, increases in connective tissue, and a mild loss of myelinated fibres (Tomlinson & Irving, 1977; Spencer & Ochoa, 1981). The central processes of dorsal root ganglion cells typically undergo distal dystrophic and degenerative changes. Regressive changes have been reported in the terminals of motor axons.

2.2.1.2 Biochemical changes

Besides the pathological changes, there are many age-related alterations in brain chemistry required for cell-to-cell communications (Rogers & Bloom, 1985; Finch, 1991). These include changes in the concentration and/or turnover of the amines (e.g., acetylcholine, norepinephrine, epinephrine, dopamine, serotonin), amino acids (e.g., glycine, glutamate and GABA) and peptides (e.g., enkephalin, substance P, thyrotropin-releasing hormone, cholecystokinin, somatostatin). There are numerous studies showing impairments of adrenergic, dopaminergic and serotonergic activity in the senescent animal (Zhou et al., 1984; Roth & Joseph, 1988; Telford et al., 1988). One of the underlying causes of these alterations seems to be an overall loss of receptors (Weiss et al., 1984; Roth & Joseph, 1988).

Synapses may utilize one or more neuromodulator (e.g., norepinephrine and neuropeptide). The multiple levels of control and the regional diversification of different synapses in discrete brain regions make it difficult to define the age-related alterations in
neurotransmitter/neuropeptide function. In fact, rather than a uniform drop in the level of a specific neurotransmitter throughout the nervous system, a "desynchronization" of signals may occur. For example, while the brain content of norepinephrine and dopamine are decreased in old age, that of serotonin is unchanged or even increased, depending on specific brain areas. In some cases, the greater the concentration of a neurotransmitter in a discrete brain region, the higher the decrement with aging and vice versa (Timiras et al., 1984). In fact, age-dependent alterations in different neurotransmitter/neuropeptide concentrations do not always occur simultaneously. Each neurotransmitter has its own timetable: dopamine levels decrease in the cerebral hemispheres of rats from the age of one year, whereas in the same areas serotonin levels remain unaffected until three years of age (Timiras et al., 1984). Aged-related changes in neurotransmitter receptor number and function have also been reported (Greenberg & Weiss, 1983; Roth & Joseph, 1988). Changes in binding affinity have not been frequently detected. Beta-adrenergic receptor responsiveness is decreased in the elderly (Vestal et al., 1979; Lakatta, 1980). This appears to be due to uncoupling of the beta-receptor from the adenylate cyclase complex which transmits the signal (Wood, 1985). In the rat pineal gland, corpus striatum and cerebellum, a reduced responsiveness to catecholamines is present due to a decrease in the affinity of beta-adrenergic receptors to their ligand. However, there is no change in receptor number (Greenberg & Weiss, 1978). This may be due in part to a reduced ability to increase the number of beta-adrenergic receptors after decreased noradrenergic input (Greenberg & Weiss, 1979).

Changes in general biochemical properties of the cells occur in the nervous system as they do elsewhere in the aging organism. Lipid composition may change, resulting in altered membrane viscosity. Protein synthesis decreases in discrete brain regions. Lipofuscin accumulates, although the functional significance is unclear, and there are alterations in electrolytes and trace elements (Brizzee, 1985). For example, aluminium levels may increase sharply in elderly people (Bjorksten et al., 1989). Decreases in zinc may be important in the light of the zinc requirement of various enzymes and growth factors, including nerve growth factor (NGF) (Dunn et al., 1980). In addition, aging is accompanied by a decreased brain water content (Meisami, 1988). Alterations in vascular flow have also been reported (Katzman & Terry, 1983).

2.2.1.3 Functional changes

Despite the morphological and biochemical changes observed in the aging brain, the functional efficiency of the nervous system seems to be well maintained in most elderly people. However, CNS disorders do occur in some individuals, though it may be difficult to discriminate age-related pathology from physiological aging phenomena. Perhaps the most ubiquitous and significant change observed in the older organism is slowness of behaviour (Birren et al., 1979). The slowing of behaviour with age not only appears in motor responses and perceptual processing, but is also apparent for the more complex processing of information associated with short-term memory (Smith et al., 1980). Related cross-sectional studies using global measures of intellectual function such as the Wechsler Adult Intelligence Scale (WAIS) show evidence that some performance abilities decline by the late 60s and early 70s, while others (e.g., verbal abilities) appear to be maintained throughout life in healthy individuals (Gallagher et al., 1980). The slowing of reaction time may be associated with the age-associated slowing and loss of coordination in motor tasks, such as those involved in...
handwriting and other purposeful movements.

The age-related modification of biorhythms is exemplified by the alterations of the sleep/wakefulness cycle, which is largely dependent on the reticular system. Alterations of sleep patterns with aging are qualitative rather than quantitative (Dement et al., 1985) and affect primarily the "deep sleep" phases, as confirmed by the alterations observed in the brain electrical activity (Müller & Schwartz, 1978). Among neurotransmitters, serotonin seems to be implicated.

Alterations in posture and locomotion in the elderly (Klawans & Tanner, 1984) also depend on CNS impairment. Peripheral modifications such as decreased nerve conduction velocity, reduced muscle mass and increased rigidity occur. Autonomic system dysfunction is also implicated in many pathophysiological changes of age including hypotension, thermoregulation, gastrointestinal function and urinary incontinence (Finch & Landfield, 1985). Other changes in the autonomic system include changes in vascular and cardiac reflexes, galvanic skin responses, and potency (Katzman & Terry, 1983). Sympathetic hyperactivity is commonly present in the aged and could interfere with cognitive functioning.

2.2.2  Sensory organs

All of the sensory organs are affected by aging, both those in which the cells are continuously renewed (such as cutaneous sense tissues) and those in which the cells are terminally differentiated early in life (vision and hearing).

2.2.2.1  Vision

Both neural (retina) and optical (cornea, lens, pupil, aqueous and vitreous humours) components of vision are affected by age. The changes in the optical compartment are probably the primary cause of visual impairment in the elderly (Sekuler et al., 1982). The most common alterations are in the lens with increased hardness and decreased transparency (Graham, 1985). The former results in reduced refractive power (Marsh, 1980). The loss of transparency relates to the following chemical changes in the lens: protein oxidation, racemization, glycation, aggregation, polymerization and precipitation (Taylor, 1989). These alterations are associated with presbyopia and cataracts, respectively.

In the neuronal compartment, the retina undergoes progressive loss of rods, while cones may be augmented. Morphometric analysis of the retina demonstrates an increase in electron-dense plaques and a decrease in the ground substance during aging. Such retinopathies result in decreased light sensitivity and reduced colour vision (Marsh, 1980).

Vision declines as a function of age (Weale, 1986) and can be measured in several tests, such as the Humphrey Field Analyser (Iwase et al., 1988), and retinal potentials (Trick, 1987). Visual acuity is substantially decreased. The ability to detect light gradually decreases (Sample et al., 1988) and light adaptation declines (Katz & Robinson, 1987).

2.2.2.2  Hearing

Decrements in hearing are frequently observed in the elderly. There is also a progressive loss of hearing in animals with age (Willott, 1986). Both auditory structures and neuronal components are involved. While the outer and middle ear show few modifications,
degenerative changes occur in the hair cells, which are the auditory receptors, and in the mechano-electrical transducing organs resulting in otosclerosis. This accounts for the preferential loss of hearing of high frequency sounds (presbycusis) (Marsh, 1980). The degree of hearing loss may affect the two ears differentially, thus causing defects in sound localization. Presbycusis has a great impact upon speech perception, since consonants, which make speech intelligible, are generated by high frequency sounds, whereas vowels, responsible for audibility, are produced by low frequency sounds.

Hearing defects may also result from changes in the neural components (Allison et al., 1984) of hearing, and in particular in the nerves connecting the cochlea with the auditory centres in the brain, specifically in the superior temporal gyrus.

2.2.2.3 Olfaction

The age-related alteration in the sense of smell is generally underestimated. The reduction in olfactory sensitivity is mainly due to the progressive loss of olfactory neurons, which protrude through cilia from the superior nasal cavity and represent the receptor sites for odour and the chemo-electrical transducing mechanism (Naessen, 1971). Loss of neurons have also been demonstrated in the olfactory bulbs of the brain (Bhatnagar et al., 1987).

2.2.2.4 Taste

Taste thresholds are known to increase with age. The taste of salt is preferentially altered in the elderly. The loss appears due both to a decline in the number of taste buds and papillae (Bradley, 1979) in the tongue, as well as to the loss of neurons in the cerebral centers of the gustatory system.

2.2.2.5 Somatic sensations

The somatic sensory system (touch, pressure, vibration, proprioception, heat, cold and pain) is variably affected by age. Tactoperceptual ability and vibrotactile sensations are decreased in the elderly due to the loss of Meissner end-organs and Pacinian corpuscles present in the skin (Bruce, 1980). For more complex somatesthetic abilities (stereognosis, body part recognition) as well as for pain and thermal sensitivity, the biological causes of their alterations with age involve not only the sensory end-organs, but also affective and cognitive factors (Marsh, 1980).

2.2.3 Endocrine system

Hormones play an important, often critical, role in the regulation of a large number of physiological and behavioural processes, and their influence can be demonstrated throughout the lifespan. Some hormones have a role in differentiation in that their presence or absence during certain developmental periods will affect the way in which physiological and behavioural processes proceed or are expressed in adulthood. Throughout each period of the life span, the maintenance of an appropriate endocrine milieu is essential to the numerous homeostatic processes required for survival. With advancing age, there are several, well-documented changes in the ability of the organism to synthesize and secrete a number of hormones. It is, therefore, likely that the typical age-related change in an organism's endocrine balance would result in, or at least contribute to, the impairment of homeostasis frequently observed in the elderly. Such impairments can be noted in the decreased rate of recovery of the elderly from the insults of injury.
or disease.

Hormones may also play a significant role in the aging process. For example, age-related changes in several physiological functions appear to be closely linked to the level and pattern of hormonal stimulation present during adulthood. As such, different patterns of exposure to a hormonal environment may alter the "rate of aging" within a specific neuroendocrine system and, in turn, affect the susceptibility of the organism to environmental insults at different segments of the life span. There are a number of different ways in which endocrine systems and the hormonal signalling operations that they use may undergo alterations with age and toxicant exposure. These can be categorized as changes in: (a) the availability of hormones for binding to the target tissues, (b) the reception of the pertinent transmitter or hormonal signal by the target cells, and (c) the nature of the hormonal message.

At any point in time, the concentration of a hormone in the blood is a consequence of both its metabolism and secretion. Such changes in the size of the available signal pool may have corresponding effects on the magnitude of the response by the target tissue. Other changes may reflect declines with age in the homeostatic controls, which rely heavily on endocrine feedback relationships within organ systems.

Serum hormonal levels, as a rule, are not maintained at constant levels. They tend to fluctuate, sometimes markedly, throughout a 24-h period. In the young adult man, peak morning testosterone values can fall by one-third to an early evening nadir, before rising again through the late evening and early morning hours (Bremner et al., 1983). A similar circadian rhythm in circulating levels of testosterone is prevalent in the rat (e.g., Kinon & Liu, 1973; Ellis & Desjardins, 1982). Human cortisol (Bilchert-Toft, 1978) and rat corticosterone (Moberg et al., 1975; Kato et al., 1980) concentrations also exhibit well-known rhythmic fluctuations, as do those of thyrotropin (Vanhaelst et al., 1972; Leppaluoto et al., 1974) and growth hormone (Millard et al., 1985). Reported attenuations with age in the rhythms of human and rat serum testosterone (Bremner et al., 1983; Steiner et al., 1984), luteinizing hormone (LH) (Vermeulen et al., 1989), and growth hormone (Sonntag et al., 1980; Frinz et al., 1983), among other hormones, can present differences in young-versus-old comparisons, depending on when such sampling is performed.

While observable changes in hormonal rhythms or significant differences in circulating hormone concentrations may reflect disturbances in the overall functional integrity of the associated organ system, the absence of such changes should not be necessarily assumed to indicate a corresponding absence of a functional alteration. The notion of a "system at risk" presupposes an increase in the susceptibility to disruption of the homeostatic controls. An aging system that may be undergoing a subtle erosion in its endocrine balance could be more likely to exhibit alterations in its response to a stressor or toxic insult. In this respect the stimulation of growth hormone release by clonidine, L-dopa and insulin is substantially depressed (Riegel & Miller, 1981), while arginine-stimulated growth hormone (GH) secretion after arginine infusion is preserved (Aschoff, 1979). Secretion stimulated by GHRH (GH releasing hormone) is only partially reduced (Coiro et al., 1991).

Regardless of these alterations, it remains established that the 24 h production of GH is significantly reduced in elderly humans Aged Population, principles for evaluating the effects of chemicals (EHC 144, 1992)
(Prinz et al., 1983), whereas that of prolactin is increased
(McGinty et al., 1988; Blackman, 1987). These data have been
confirmed in animals (Ceda et al., 1986; Sonntag & Gough, 1988),
although measurements of hormonal profiles may have involved
different procedures in animals and man, thus giving rise to
slightly different interpretations.

Similar difficulties are encountered in studies of age-related
alterations in pineal hormone secretion, including melatonin, whose
circadian rhythmicity is certainly changed with age (Reiter, 1986;
Anisman & Reiter, 1990).

In order to illustrate age-related alterations in hormone
control, it is useful to focus on the integrated systems which
involve more than one gland or hormone. Although three such systems
are reviewed below, this discussion is by no means intended to be
comprehensive. One theme common to studies of age-related changes in
endocrine function is that such alterations are often hormone and
species specific. Finally, the extent to which any of these changes
relate to potential adverse health outcomes in the older organism
remains to be demonstrated.

2.2.3.1 The pituitary-thyroid axis and the basal metabolism

Thyroid hormones are required during development for growth and
in adult life for regulating oxygen consumption. Maintenance of
thyroid function is generally assured even in old age, although
following repeated stress and demands the reserve function may
become exhausted and a dysthyroid state may follow (Ingbar, 1978).

Changes with aging in the levels of both thyroid stimulating
hormone (TSH) and thyroid hormones (thyroxine (T4) and
triiodothyronine (T3)) are controversial, because concomitant health
disturbances may cause significant fluctuations in the levels of
these hormones (Gregerman & Solomon, 1967; Utiger, 1980). Both hypo-
and hyperthyroidism are not uncommon in the elderly. In general, the
size of the thyroid decreases with age (Gambert & Tsitouras, 1985).

Older people show a normal response to decreased thyroid
function by increased secretion of TSH (Eden, 1987). TSH levels
undergo few changes (Miller, 1989), suggesting that the hypothalamic
control of TSH release has not been altered. However, structural
modifications of TSH have been reported (Klug & Adelman, 1977). T4
levels remain unchanged with age, even though the rate of synthesis
is reduced. However, the blood levels of T3 are reduced with
advanced age (Chopra et al., 1978), while levels of reverse T3 are
unchanged. It should be noted that severe and chronic illnesses, not
directly involving the thyroid, can lower the levels of T3 and
T4.

The alterations observed in thyroid hormone levels are
inadequate to explain the age-associated decline in various
functions that are dependent on thyroid hormone. One possible
explanation is that peripheral sensitivity to thyroid hormone action
is modified by aging. However, with advancing age, the basal
metabolic rate remains unchanged if based on lean body mass, but
decreases if expressed based on body surface area (Masoro, 1985).

2.2.3.2 The pituitary-adrenal axis

The major function of this axis, which is largely based on
pituitary hormones (ACTH) and adrenal hormones (corticosteroids), is
to provide an adaptive response to environmental stress (Selye,
1950; Sapolsky et al., 1986). Any harmful agent, in addition to inducing a specific reaction in the body (anaesthesia, emotion, fever, etc.), activates a specific and common response, the so-called "General Adaptation Syndrome" (Selye, 1950), characterized by increased adrenocortical secretion, thymic involution, lymphopenia and eosinopenia. With advancing age this axis may undergo desynchronization, thus resulting in a failure of homeostasis and adaptation (Anisimov & Reiter, 1990).

ACTH secretion, which shows a circadian rhythm based on melatonin fluctuations, is generally preserved in advanced age (Halberg, 1982), although minor modifications of blood levels may occur due to variations in renal clearance or alterations in sleep patterns. However, there is evidence of diminished sensitivity of the hypothalamic/pituitary axis feedback inhibition by glucocorticoids (Greden et al., 1986; Blackman, 1987; Dilman, 1987; Sapolsky et al., 1987). The elderly suffering from Alzheimer's disease are extremely resistant to glucocorticoid negative feedback (Sapolsky et al., 1986).

Finally, certain cell populations (e.g., CA3 neurons in the hippocampus) are particularly susceptible to glucocorticoids. Long-term stress may result in their dysfunction and death (Sapolsky et al., 1987).

2.2.3.3 The endocrine pancreas and carbohydrate metabolism

It is well documented that with advancing age the ability to maintain glucose homeostasis is impaired, but the underlying mechanisms are still not well defined. Several hormones contribute to the regulation of glucose homeostasis: above all, insulin and glucagon, secreted by the endocrine pancreas, and somatostatins and the pancreatic polypeptide, which modulate the secretion of insulin and glucagon, respectively. In addition, glucose metabolism may be affected by other hormones, including T3 and T4, growth hormone, glucocorticoids and epinephrine (Minaker et al., 1985).

Only modest morphological alterations are observed in the endocrine pancreas with advancing age. In spite of this fact, blood sugar levels after fasting are elevated and glucose tolerance is lowered in the elderly (Magal et al., 1986; Eden, 1987; Ammon et al., 1987; Wang et al., 1988; Groop, 1989). Plasma insulin concentration increases and insulin sensitivity decreases. Alterations in insulin turnover are detectable in the elderly after glucose load, such as reduced insulin secretion and increased secretion of the inactive prohormone, proinsulin (Marx, 1987), but these changes are too modest to account for the observed glucose intolerance. One alternative explanation is an increase in peripheral resistance to insulin. In fact, peripheral uptake of glucose is indeed reduced in the elderly, due to a reduction in insulin receptors (Fagano et al., 1981) as well as alterations in the post-receptor signalling process (Rowe et al., 1983). No evidence exists regarding the possible involvement of age-related changes in glucagon affecting glucose intolerance in the elderly.

Other factors, however, may contribute to glucose intolerance. These include: (a) reduced liver sensitivity to insulin, resulting in reduced glycogenesis; (b) changes in diet and physical exercise; and (c) increased body fat with reduced muscle mass. This last point seems to merit particular consideration in view of the observation that insulin resistance is certainly increased in obese humans (Runcie, 1985). In fact, intracellular fat accumulation leads to a reduced concentration of insulin receptors (Bolinder et al., 1983).
2.2.4 Reproductive system

The age-related modifications of the reproductive system are primarily based on alterations in the central nervous system, pituitary gland and gonads. While menopause is a time-fixed event involving cessation of ovarian function, the decline of testicular function is a slow and gradual process, involving limited hormonal alterations. Older persons show the normal response to deficient gonadal function by increased synthesis of gonadotropins (Piva et al., 1987). This occurs in both sexes. In fact, alterations in serum levels of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) have been reported (Blackman, 1987). The reduced presence of sex steroids in women may have an influence on the function of other endocrine glands. Estrogens have well-documented effects on salt and water balance and on plasma proteins, which in turn have effects on the level of thyroid hormones through a suppression of TSH secretion. Estrogen also stimulates the production of growth hormone and prolactin. Thus, the decline in gonadal function during age could have far-reaching consequences on the individual's physiological function.

2.2.4.1 Female aging

In females, cessation of ovarian function consists of the transfer from regular menstrual cycles to amenorrhea, usually preceded by a period of cycle irregularity. The initial changes have been reported to occur in hypothalamic-pituitary control of the ovaries. For example, the age-related decline in reproductive function is associated with a decreased sensitivity of the hypothalamic-pituitary complex to feed-back regulation by estrogens (Dilman, 1971, 1987). This leads to an age-related enhancement of pituitary gonadotropins (FSH, LH) (Chakravarty et al., 1976), leading in turn to hyperstimulation of the ovaries. However, despite the compensatory increase in ovarian hormone production, the level of estrogens is insufficient to induce ovulation because of hypothalamic insensitivity, possibly due to an age-related decrease in the level of biogenic amines and/or peptide hormone receptors (Dilman & Anisimov, 1979). In addition, the progressive loss of oocytes plays an important role in the decline in reproductive function since the reduction of maturating oocytes may induce desynchronization of pituitary-ovary hormonal interactions (Aschheim, 1976).

The most common consequences of menopause include imbalances of the autonomic nervous system, psychological modifications, and physiological alterations of target organs due to metabolic changes. Alterations of estrogen target organs are among the most evident effects of menopause. Vulvar skin and vaginal epithelium may undergo atrophy. Glycogen content is generally reduced, with a consequent decrease of lactobacilli, rise of vaginal pH, and increased growth of pathogenic microbes. The uterus and oviducts atrophy due to the decreases in estrogen levels. In ovaries, follicular cysts and atresia result in response to the altered hormonal status. Hyperplasia of the theca cells occurs. Fibrosis also occurs in these tissues but, in addition, can affect the bladder and urethra, resulting in an increased incidence of cystitis, dysuria and non-infectious urethritis. The reduced thickness of the skin is also a result of the decrease in estrogen (Schiff & Wilson, 1979).

Menopause has major health consequences for the cardiovascular and skeletal systems. The reduction in estrogen secretion removes the protection offered by these hormones against coronary heart disease, development of atherosclerosis, and accompanying
alterations of lipid metabolism. Osteoporosis, resulting from increased bone reabsorption relative to bone formation, is a common problem in postmenopausal women (Riggs, 1987). Two types of osteoporosis may be identified. Type I is associated with estrogen withdrawal and may begin in middle age (Riggs & Melton, 1983). The biological effects are linked to disruption of the complex relationship between calcium intake and loss, and the secretion of calcitonin, parathyroid hormone and 1,25-dihydroxy-vitamin D. Estrogens prevent the transfer of calcium from bone to blood and its loss through urine. This induces parathyroid hormone secretion, which stimulates the formation of 1,25-dihydroxycholecalciferol, the active metabolite of vitamin D. The function of the parathyroid is affected by increasing age (Eden, 1987). The relevance of estrogen for bone loss is further supported by the effectiveness of estrogen therapy in delaying the osteoporotic process in post-menopausal women (Edman, 1983). With advancing age type II osteoporosis (senile osteoporosis) may occur, which is probably due to the poor intestinal absorption of calcium (Riggs, 1987).

2.2.4.2 Male aging

The reproductive system is less affected by aging in males than in females. It is generally accepted that testosterone levels are maintained within the physiological range throughout life, although a decrease in testosterone production in response to gonadotropin action may occur in old age due to a reduction in Leydig cell number and function (Harman et al., 1982). Testis and accessory sex organs do not show substantial modifications with age, and sperm is found in the ejaculate of very elderly men. The volume of seminal fluid is generally decreased.

While the prostate undergoes involution in the majority of old men, in about one-third of males it undergoes hypertrophy with consequent obstruction of the urethra and urinary flow from the bladder. The cause of the hypertrophy is still unclear (Mawhinney, 1985). The prostatic enlargement results in compensatory hypertrophy of the bladder. When such compensation is no longer sufficient, retrograde filling of the renal pelvis and ureters may occur, resulting in hydronephrosis and eventually renal failure.

2.2.5 Immune system

With advancing age a progressive increase occurs in the incidence of various infectious diseases, autoimmune processes and tumours. These may be in part based on age-related defects in the immune system. The association of so many age-related pathologies with defects in the immune system has led to the suggestion that aging of the immune system may be rate limiting for life span (Walford, 1969). However, while there are numerous experimental and clinical studies demonstrating an age-related deterioration in immune efficiency, this decline is not sufficient to account for all manifestations of aging.

There are several recent reviews on aging and the immune system (Revskoy et al., 1985; Lipschitz, 1987; Segre et al., 1989; Miller, 1991). However, it is still difficult to draw a comprehensive picture, because of the many cellular and humoral components involved in immune reactions and the many modulating extra-immunological factors which may also be compromised in the elderly. The immune and haematopoietic systems are intimately related, being derived from a common pluripotent stem cell. Both play central roles in host defense, prevention of neoplasia, and response to infectious agents (Lipschitz, 1987). However, basal haematopoiesis in both animal models and man seems to be either
unchanged or minimally altered with age (Dybker et al., 1981; Lipschitz, 1987). The reserve capacity may be reduced resulting in a decreased ability to respond to stress.

2.2.5.1 Aging of lymphoid organs

Peripheral lymphoid organs, such as the spleen and lymph nodes do not show consistent modifications in size with aging. Bone marrow is not consistently affected by age. Stem cell production is generally well preserved in old age (Harrison et al., 1978), although a slight change in the replication rate of stem cells has been reported by some authors (Schneider et al., 1979). Thymic involution has been considered to account for the major age-related changes in the immune system, beginning at puberty. Such an involution consists of a progressive loss of cellularity with lymphoid cell depletion in the cortical areas and cystic changes in the epithelial cells. These are the source of various peptides involved in differentiating thymic lymphocytes (T-cells) from lymphoid cells of earlier lineage. The export of newly differentiated T-cells is reduced with advancing age (Globerson et al., 1989). The synthesis and the secretion of polypeptide thymic hormones, such as thymosin (McClure et al., 1982), thymopoietin (Lewis et al., 1978) and thymulin (Bach et al., 1972), are progressively diminished. In all cases, the reduction of thymic endocrine activity seems to have a pathogenic role in age-related immune dysfunctions, since replacement by exogenous administration of the hormones is capable of restoring various immune functions in old age (Zatz & Goldstein, 1985). The turnover of zinc, which is essential for immunocompetence (Iwata et al., 1979; Chandra, 1985), decreases in old age. Zinc supplementation can restore immune functions (Fabris et al., 1990).

2.2.5.2 Aging of cellular constituents

Mature T-cells, bone marrow lymphocytes (B-cells) and natural killer cells (NK-cells) can be detected in blood and in lymphoid organs by specific monoclonal antibodies. With this type of analysis, no major modifications in the proportion of the various lymphoid cell subpopulations have been observed in humans. However, the major alteration in the immune system appears to arise in the functioning of T-cells (Thompson et al., 1987). While the total number of T-cells in the peripheral blood does not change appreciably with age, there are clear-cut differences in the relative proportion of T-cell subtypes (Wagner et al., 1983; Fernandes, 1984; Revskoy et al., 1985; Thompson et al., 1987; Lipschitz, 1987).

The number of immature lymphocytes of the T-lineage increases with age, as does the percentage of apparently activated T-lymphocytes bearing immature thymic phenotypic markers. There is a relative increase in cytotoxic/suppressor T-cells, and a decrease in the number of helper/inducer T-cells (Lipschitz, 1987; Thompson et al., 1987). Correlated with the decrease in the helper/inducer population, is a functional defect in cell-mediated immunity (Lipschitz, 1987; Thompson et al., 1987). Cells from aged humans or experimental animals are less capable of responding to allogeneic lymphocytes, phytohaemagglutinin, concanavalin A and soluble antigens. Lymphocytes from older mice are less able to elicit graft-versus-host reactions than those from younger mice of the same inbred strain (Thompson et al., 1987). Fifty percent of healthy people over age 50 have impaired cutaneous hypersensitivity (Lipschitz, 1987; Dilman, 1987). Accompanying the decrease in helper/inducer T-cells and cell-mediated immune functions is a rise
in autoantibodies and autoimmunity (Thompson et al., 1987). Changes in humoral immunity (B-cell function) with aging are more subtle (Lipschitz, 1987; Senda et al., 1989). Studies on the effects of age on antibody production have yielded conflicting results, perhaps because of the wide range of experimental values generally observed in older individuals. It has, however, been well established that aging is significantly associated with the presence of various autoantibodies, in particular, antibodies against nuclear antigens. There is also evidence that aging effects the rate of antibody production by activated B-cells (Lipschitz, 1987).

From a functional point of view, defects have been observed at various levels. Firstly, the proliferative capacity of T-cells from old individuals is generally reduced, regardless of the stimuli used (antigens, mitogens), and the defect consists both in a reduced number of cells responding to stimulus and in a precocious exhaustion of the cloning capacity (Fabris et al., 1983) of responding cells. Secondly, the response to interleukins, which physiologically mediate the modulation of the proliferative reaction, is depressed and this phenomenon has been documented not only for T-cells but also for NK cells, which are less sensitive in old age to the boosting action of IL-2 or interferons (Provinciali & Fabris, 1990).

With respect to accessory cells (phagocytic cells, macrophages), their number and function are not altered by age, and, in certain circumstances, their activity seems to be enhanced.

2.2.5.3 Neuroendocrine-immune interactions

The immune system, although regulated to a large extent by intrinsic cellular and humoral events, is also sensitive to signals generated from the nervous and endocrine systems. Communication between nervous and immune networks is mediated by hormones and neurotransmitters which reach lymphoid organs and cells via blood or direct autonomic nervous system connections (Bullock, 1985; Felten et al., 1985). The neuroendocrine immune interactions are mediated by circulating humoral factors from the pineal-hypothalamic-pituitary axis, either directly via neuropeptides and hormones, or indirectly by the effects of this axis on the hormonal secretion of peripheral endocrine glands, which also exert immunomodulating actions (for review, see Fabris, 1991).

The nervous and neuroendocrine systems not only act as modulators of the immune network, but also as targets for signals generated within the immune system, such as those exerted by thymic factors (Hall et al., 1989) and interleukins, (Besedovsky et al., 1985), and by pituitary-like factors (ACTH, TSH, GH, PRL, gonadotropins, endorphins), which are produced by mature lymphocytes upon antigenic stimulation (Weigent et al., 1990).

The sharing of humoral signals, as well as of the specific receptors between neuroendocrine and immune cells (for reviews, see Fabris & Provinciali, 1989; Weigent et al., 1990), implies that biological response modifiers of neuroendocrine-immune origin might be developed in the near future for therapeutical purposes. On the other hand, potentially harmful agents for one of these homeostatic systems may also cause alterations in others.

From an experimental point of view, it has been demonstrated that treatments of old animals with thyroid hormones (Fabris et al., 1989), GH (Kelley et al., 1986) and analogues of LH releasing hormone (Greenstein et al., 1987) are able to induce regrowth of the thymus.
and reacquisition of its endocrine activity (for review, see Fabris 1991). Analogous treatments, such as with melatonin (Pierpaoli et al., 1991), GH (Davila et al., 1987), TSH and thyroid hormones (Provinciali & Fabris, 1990), are also able to recover various age-related peripheral immune deficiencies, such as T-cell functioning and NK cytotoxicity. In humans, little work has been done in this area, although indirect evidence, obtained primarily from studies on endocrinopathies in the elderly (Fabris et al., 1989; Travaglini et al., 1990), suggest that recovery of both thymic and peripheral immune function can be achieved by a neurohumoral approach.

Little is known on the potential effect of thymosins, interleukins and lymphocyte-derived pituitary-like cytokines on age-related alterations of the nervous and of the neuroendocrine system. Experimental information from old animals showing recovery of the hormonal and metabolic profile following immune manipulation (for review, see Fabris et al., 1988) is undoubtedly opening a new research approach for human investigations.

Receptor sites for many hormones are present on the membrane of lymphoid cells (Fabris & Provinciali, 1989). The number of glucocorticoid receptors in spleen cells decreases in old animals (Roth, 1979a). Hormones that modify the turnover of cyclic nucleotides result in consequent activation or inhibition of immune functions (Hadden, 1983). Hormones influence the production of several lymphokines and monokines (Kelso & Munck, 1984).

The neuro-endocrine system seems to act not only as a modulator of the immune network but also as a target for signals generated within the immune system. Examples of such interactions are the alterations that can be induced in the neuroendocrine balance, either by removal of relevant lymphoid organs such as the thymus or by dysfunction of the immune system itself as a result of reactions to immunogenic or tolerogenic doses of antigen (Besedovsky et al., 1975). In addition, mature lymphoid cells, when stimulated by antigens, produce humoral factors similar, if not identical, to classical hormones and neurotransmitters (such as ACTH, TSH, GH, PRL, gamma-endorphins) (Blalock et al., 1985). These reciprocal influences between the neuroendocrine and the immune systems (Fabris, 1981; Fabris et al., 1988) occur throughout life, but have particular relevance during aging (Fabris & Piantanelli, 1982).

2.2.6 Cardiovascular system

The frequency of cardiovascular diseases, which are the major cause of death in industrialized countries, increases with age. Diseases such as hypertension and atherosclerosis occur most commonly in the elderly. In addition, degenerative changes of the cardiovascular system, involving the myocardial cells as well as cells of the pacing-conduction system, that arise during the aging process lead to impaired cardiac function and arrhythmia even in people without any clinical evidence of hypertension or coronary artery diseases. Inadequate function of the cardiovascular system induces effects in peripheral tissues and organs. Changes in peripheral organs resulting in hyperlipidaemia, hypercholesterolaemia, and hypo- and hyperglycaemia can also affect the cardiovascular system in the elderly.

2.2.6.1 Heart

The heart itself can be considered to be made up of two parts: a) the conduction system responsible for electrically controlling
the heart rhythm; and b) the myocardium performing the contractile function of the heart and composed of a system of trabeculae.

The biophysical and biochemical mechanisms that govern cardiac muscle change with age, resulting in characteristic alterations in muscle function (Lakatta, 1987a,b). Many of the steps in the excitation-contraction system in cardiac muscle are altered by aging. In an isometric contraction, the transmembrane action potential (TAP) excites the cell and the contractions that ensue are longer in duration. The magnitude of the prolongation of depolarization of the TAP in senescent muscle is striking (i.e. about twofold). The action potential amplitude is also greater in senescent than in adult muscle in both high and low calcium-loading conditions. These deficits of the senescent muscle may be related in part to the diminished Ca²⁺ pumping rate by sarcoplasmic reticulum. The duration of the elevated myoplasmic Ca²⁺ level is prolonged in senescent muscle.

Sagiv et al. (1988) found that left ventricular contractility increases less on stimulation in elderly subjects than in younger people. Although the aging process is associated with normal resting contractile function, diastolic properties are altered, resulting in reduced and delayed early left ventricular filling and enhanced atrial contribution to diastolic volume. Exercise cardiac output is maintained in healthy elderly individuals, but there is a shift from reliance on an increase in heart rate and a decrease in end systolic volume to use of the Frank-Starling mechanism to increase stroke volume. This age difference in the cardiovascular response to exercise is probably mediated by an age-associated decreased responsiveness to beta-adrenergic stimulation.

In muscle tissue of the aging heart, some morphological changes are observed both in animal and human studies (Koobs et al., 1978; Speijers, 1983). The most common change in the aging heart is hypertrophy (Lakatta, 1985). Other alterations consist of the appearance of slight focal necrosis and fibrosis in the myocardium, amyloidosis (Finch & Hayflick, 1977) and the appearance of lipofuscin (Hendley et al., 1963; Koobs et al., 1978). Peroxidative damage to the myocardium is cumulative and irreversible (Koobs et al., 1978).

2.2.6.2 Blood vessels

Both physiological and morphological changes are observed in the vascular system, especially in the small and large arteries. The morphological changes in the arteries seen in the elderly vary considerably both in appearance and in localization (Goyal, 1982; Hazzard, 1985). The thickness of the aorta increases significantly with age, while the number of nuclei in the cells of the arterial media decreases in humans as well as in mice. The majority of these changes in humans are categorized as atherosclerotic. These changes can progress and result in complicated coronary atherosclerosis and ischemic heart disease, but other factors may also cause clinical effects such as angina pectoris, arterial spasms, and myocardial infarcts (Speijers, 1989a).

Morphological changes in the veins are less pronounced than in the arteries.

The physiological changes observed with aging are often a result of changes both in heart function and in the arteries. These changes are reflected in haemodynamic parameters such as an increase in diastolic and systolic blood pressure, mean arterial blood
pressure and vascular resistance, and a decrease in responsiveness, and in contraction and relaxation responses (Lakatta, 1986, 1987b; Duckles, 1987; Mazzeo & Horvath, 1987; Zemel & Sowers, 1988; O'Malley et al., 1988; Cleroux et al., 1988).

Aging is often accompanied by increases in the incidence and prevalence of hypertension. Geriatric hypertension is generally of a salt-sensitive nature with a disproportionate frequency of isolated systolic hypertension. The age-related increase in salt sensitivity is due to a decline in renal function (Zemel & Sowers, 1988) and deregulation of vascular tone. Age-associated declines in the activity of membrane sodium/potassium-ATPase may also contribute to geriatric hypertension because this results in increased intracellular sodium loading, causing reduced sodium/calcium exchange and thus increased intracellular calcium and vascular resistance.

It is commonly accepted that atherosclerotic changes take place to a certain extent in every individual. Multiple factors determine the extent and velocity of the atherosclerotic process (Hazzard, 1985). The incidence of clinically observed atherosclerotic effects is higher in elderly subjects than in younger individuals. Atherosclerotic damages result in impaired cardiovascular function.

Atherosclerosis is defined as a multifactorial disease with variable effects in the intima followed by changes in the media of arteries. These changes consist of focal accumulation of lipids, proliferation of smooth muscle cells, and accumulation of complex carbohydrates (i.e. glycosaminoglycans, proteoglycans), blood and blood products, collagen and calcium compounds (Campbell & Chamley-Campbell, 1981; Velican, 1981; Speijers, 1989a). The resultant modifications of arterial wall integrity can lead to the following: erosion of the wall with consequent reduced resistance to blood pressure, rupture, and haemorrhage; progressive thickening of the wall due to reactive proliferation of tissues with consequent reduction of blood flow; and clotting of the blood at the level of the injured wall with consequent sudden obstruction. The basic lesion seems to develop in the first decade of life (Lee, 1985).

In the etiology of the lesion two localized cofactors should be taken into account: the blood supply of the arterial wall; and blood turbulence, since lesions are more frequently found around the orifices of arteries branching off major arteries or at bifurcations (Patel & Vaishnaw, 1980). Hypertension, diabetes, autoimmunity and stress are also risk factors contributing to atherosclerosis.

2.2.6.3 Characteristics of atherosclerotic lesions

The first event in the formation of the lesion is still debated. Both an initial thickening of intima, due to an accumulation of blood-born amorphous material (lipids, protein and sulfated proteoglycans) and a proliferation of muscle cells (due to still undefined stimuli), with consequent degeneration and reactive macrophage and connective tissue accumulation, have been proposed as major initiating phenomena (Benditt, 1977; Ross, 1981). The subsequent phase of the lesion involves repair mechanisms that cause further thickening of the intima. Following this, lipid increases both in the cells and in the intercellular spaces. Lipid accumulation is progressive, leading to an increased number of foam (lipid-containing) cells which disintegrate and form the gruel-like substance that has given the name of atheroma to the lesion. The accumulation of such material acts as an irritant, inducing a
proliferative reaction (encapsulation) which leads to the development of a plaque. Calcification follows, making the arterial wall more rigid. Alternatively, when the capsule breaks, an ulcer can occur, leading to loss of tissue in the arterial wall, blood clots, haemorrhage, and consequent thrombosis and/or rupture of the arterial wall.

2.2.6.4 Theories of atherosclerosis

Atherosclerosis is undoubtedly a multifactorial process, which is reflected by the many theories proposed (Baker & Rogul, 1987). The lipid accumulation theory is based on the progressive accumulation of oxidized lipids (mainly low density lipoproteins) not only in smooth muscle cells but also in migrating monocytes (Avogaro et al., 1983). The link between lipid deposition and consequent alterations remains unclear (McCaffrey et al., 1988).

Theories on monoclonal proliferation of smooth muscles cells are based on a mutagenic event in these cells, due to a physico-chemical or viral insult (Benditt, 1977; McCaffrey et al., 1988), leading to the formation of a kind of benign tumour. The thrombogenic theory is based on the early adherence of platelets to small alterations in the endothelium, leading to thrombus formation and release of growth factors by platelets which induce smooth muscle cell proliferation (Ross, 1981; Bang et al., 1982). Other risk factors that should be taken into consideration are hypertension, cigarette smoking, increased body weight, high serum uric acids and consumption of saturated fats.

The immune system in the elderly is weakened. Consequently there is increased susceptibility to chronic infections, autoimmunity, and elevation of circulating immune complexes. New data in the literature indicate that some viral agents may cause cardiac and arterial cell lesions and subsequent inflammation. Thus, viruses and altered immune cells may cooperate and play a role in arterial wall lipid accumulation, possibly acting as initiating factors for atherosclerosis (Butenko, 1985).

2.2.7 Respiratory function

Respiratory function is based on gas exchange (oxygen absorption and carbon dioxide elimination), gas transport (red blood cells), and on internal metabolic processes that utilize oxygen at the cellular level. Aging may affect all of these processes (Masoro, 1981), but it is the first that is usually most compromised in advanced age. The decline in the gas-exchange system may involve the lungs, the thoracic cage, the respiratory muscles and the respiratory centres in the CNS. The deterioration of the lungs is largely dependent on environmental factors, in particular on the contamination of air with toxic substances, dust and microbiological agents. Therefore, age-related lung alterations may vary according to life styles (smoking, physical exercise), environmental conditions (urban/rural), and intercurrent diseases (infections, work-related diseases) (Davies, 1985).

2.2.7.1 Gas-exchange organs

The most evident lung alterations with advancing age are represented by enlargement of alveolar ducts with flattening of alveoli and loss of septal tissue, reduction of elastic fibres, and increased fibrosis of the capillary system (Liebow, 1964). Functional consequences are a reduction in the surface area for gas exchange with an increase in the physiological dead space, reduction
of the ventilatory flow rate, and irregular distribution of blood flow (Mauderly, 1978). Age-related alterations also occur in the chest as a consequence of calcification of costal cartilage, increased stiffness of costovertebral and vertebral joints, and general rigidity of the chest. Both lung and chest alterations contribute to the changes in lung volume and pressure: vital capacity decreases, residual volume increases, and flow rates (particularly expiratory flow rate) decline (Morris et al., 1971). The alteration in the gas-exchange capacity causes reductions in oxygen uptake and pressure and lower arterial pO₂, whereas pCO₂ remains constant even in very old age (Morris et al., 1971). The control of ventilation by brain centres is also altered in old age. It is still unknown whether such alterations are due to intrinsic damage of the neural component or to a reduced responsiveness of neuromuscular activity in the chest (Peterson et al., 1981).

All these alterations, while not necessarily life-threatening for the elderly, may favour pathologies such as chronic bronchitis, pneumonia and emphysema (Peterson et al., 1981). The concomitant hypoxia (low oxygen levels) may cause increased production of red blood cells with consequent polycythaemia. This may contribute to hypertension and cardiac failure.

2.2.7.2 Erythropoietic activity

Although specific age-related alterations in the life cycle of erythrocytes have been reported (Danon, 1969), the overall function of the erythropoietic system seems to be well preserved. The regenerative potential following hypoxia seems nearly inexhaustible. In addition, haemoglobin turnover does not seem to be affected by age (Lipschitz, 1987).

2.2.8. Kidney and body fluid distribution

The urinary tract is affected by aging both in its renal functions of excretion and ionic control of body fluids, and in its control of bladder and urethral activity.

2.2.8.1 Renal function

Kidney function decreases both due to anatomical and physiological alterations with age (Wesson, 1969; Kaysen & Myers, 1985; Brown et al., 1986; Corman & Michel, 1986; Owen & Heywood, 1986; Meyer & Bellucci, 1986; Anderson & Brenner, 1986, 1987; Goldstein et al., 1988; Euans, 1988; Rudman, 1988). These alterations have been observed in both experimental animals and in humans.

The weight and volume of the kidney decrease by 20 to 30% between the ages of 30 and 90 years. The atrophy is primarily cortical and seems to be related to intrarenal vascular changes. The number of surviving nephrons is reduced and these remaining nephrons tend to be enlarged (Kaysen & Myers, 1985; Brown et al., 1986; Lindeman, 1986; Rudman, 1988). The number of glomeruli decreases by 30 to 50%, and there is an increasing percentage of sclerotic and/or abnormal glomeruli. The glomerular filtration rate (GFR) decreases with age resulting in an adaptive increase in glomerular perfusion pressure (Kaysen & Myers, 1985; Lindeman, 1986; Meyer & Bellucci, 1986; Anderson & Brenner, 1986, 1987; Blum et al., 1989). This decline in GFR is due in large part to the progressive reduction in blood flow to the kidneys (Brown et al. 1986; Lindeman, 1986). Glomerular mesangial volume increases by 50% and 1 out of 10 glomeruli is sclerotic at the age of 80 years compared with 1 out of 100 in the young adult (Brown et al., 1986). The renal tubules
decrease in number, proximal tubule volume and length decrease, and distal tubules develop increased diverticula. The renal arterioles develop intimal thickening, reduplication of the lamina elastica interna, and mild hyalinization (Brown et al., 1986; Lindeman, 1986; Rudman, 1988).

An age-related reduction in secretory and resorptive capacity is seen in the tubules, which is explained by a progressive loss of functioning nephrons (Lindeman, 1986). Tubular function, which regulates water and salt balance, is also affected. A decrease in the ability to concentrate urine with age has been well documented in humans. This appears to result from a decreased medullary tonicity caused mainly by an inability to respond normally to antidiuretic hormones (ADH) (Kaysen & Myers, 1985; Brown et al., 1986; Meyer & Bellucci, 1986; Lindeman, 1986; Euans, 1988). Despite age-related decreased renal function, the blood pH, partial pressure of carbon dioxide, and serum hydrogen carbonate concentration of the geriatric population without renal disease do not differ significantly from those of the young under basal conditions (Lindeman, 1986). Both the ability to maximally dilute the urine and to maximally concentrate it, are controlled by serum ADH and by the action of that hormone on the collecting ducts (Kaysen & Myers, 1985; Os et al., 1987). Increased arginine-vasopressin (AVP) secretion per unit of plasma reflects a decrease in collecting tubule sensitivity to AVP. This change in sensitivity is not completely offset by increased ADH release (Davis & Davis, 1987). The suppression of ADH secretion is not maximal when serum osmolality is reduced.

The renin-angiotensin-aldosterone system is also poorly responsive to volume depletion in aging subjects. As a result, the elderly cannot maximally retain sodium under conditions of plasma volume contraction (Kaysen & Myers, 1985; Lindeman, 1986; Euans, 1988). The activity of the renin-angiotensin system is progressively reduced with age. It has been suggested that angiotensin II does not play an important role in the maintenance of blood pressure and kidney hemodynamics in normal senescence (Corman & Michel, 1986). The mean blood pressure is correlated with age and the decline in renal function (Lindeman et al., 1987).

The kidney is also the site of vitamin D1 hydroxylation, which is dramatically reduced during aging (Kaysen & Myers, 1985).

2.2.8.2 Lower urinary tract

The most frequent age-related alterations in the lower urinary tract result in urine incontinence in both sexes and urine retention in males. Incontinence occurs at high incidence although it is not an inevitable consequence of aging. The high number of physiological requirements for urinary continence may account for such frequent failure (Williams & Pannill, 1982). In women, the reduction of estrogen levels after menopause may decrease the tone of the smooth muscle around the pelvic floor and the bladder outlet, thus favouring urinary incontinence. In men, hypertrophy of the prostate represents the major cause for involuntary loss of urine, because of the associated frequent instability of the detrusor muscle. Other causes for urinary incontinence are represented by delirium, infections, restricted mobility and polyuria.

2.2.9 Gastrointestinal function

Changes in the gastrointestinal tract during aging consist mainly of a reduced cell turnover, leading to mucosal hypoplasia.
Accessory organs, such as the exocrine pancreas and the liver, are affected by aging independently.

2.2.9.1 Gastrointestinal tract

In contrast to the cardiovascular or excretory system, the gastrointestinal (GI) tract does not exhibit marked structural or functional changes with age (Penzes, 1984). Aging may affect all regions of the GI tube. The first signs of aging are generally observed in the mouth. Teeth undergo discoloration, pulp recedes from the crown, dentine is often poorly renewed, and the gingiva frequently recede (Walker, 1985). These alterations favour bacterial growth which can lead to chronic periodontal inflammation and tooth loss.

In the stomach, reduced secretion of hydrochloric acid and pepsin is often found in the elderly. These changes result from alterations in enzyme-secreting cells or from altered hormonal and neural regulation. However, in some cases, increased acid secretion may occur with advancing age, leading to gastritis, erosions and, ultimately, ulcers (Kumpuris, 1983).

No major morphological alterations are observed in the intestine. The relatively mild modification of villi, the increased collagen content and the reduced mucosal cell proliferation (Webster, 1985) cannot account for the impaired absorption of nutrients, including minerals (calcium, iron and zinc) and vitamins often found in the elderly. Other factors such as reduced motility and inadequate intestinal blood supply may play a more important role than the slightly altered anatomical integrity.

In the lower GI tract the rectal tone is generally decreased in the elderly and the sphincter is weakened, leading to incontinence. Neurological alterations (dementia), muscle atrophy, diarrhoea and constipation can all contribute to anal incontinence.

2.2.9.2 Pancreas

Pancreatic function is not seriously compromised during normal aging. Structural changes mainly involve a reduction in the number of secreting cells, with a consequent decrease in size of the pancreas and a moderate increase in collagen content. Reduction in the levels of trypsin, hydrogen carbonate, amylase and lipase in the pancreatic juice occurs commonly in the elderly (Vellas et al., 1988). Overall, however, the functional efficiency of the pancreas is not lost during aging.

2.2.9.3 Liver

Structural changes in the liver with age are relatively minor. However, the most significant change in human liver is a decrease in volume (Wynne et al., 1989). The life-span of the hepatocyte is long, with cells only dividing once or twice during the lifetime in the absence of a growth stimulus (Popper, 1986). Whether the function of the aging hepatocyte is impaired is unclear. Kupffer cell function may be impaired as demonstrated by a reduction in phagocytic activity. Endocytosis is reduced in Kupffer, but not endothelial, cells. An increase in collagen also characterizes aging liver.

The function of aging liver also undergoes a number of alterations. In humans, cholesterol synthesis is reduced in the elderly, while biliary secretion is increased (Popper, 1986).
Hepatic blood flow is reduced by as much as 50% (Sherlock et al., 1955). Age-related changes in liver enzyme expression have been studied in more detail in rodents (Fishbein, 1991). The efficiency of carbohydrate and intermediary metabolism is decreased in senescent rats or mice of both sexes, due in part to decreased insulin-binding and the impaired regulation of enzymes such as pyruvate kinase. Hepatic protein synthesis also decreases with age, and the sex-specific expression of drug and steroid-metabolizing enzymes in male rats appears to be feminized in senescence (Kitani, 1991).

2.2.10 Musculo-skeletal system

Aging dramatically affects bone, joints and skeletal muscles. The basic phenomena responsible for the aging of the musculo-skeletal system are not completely understood because of the involvement of many factors, e.g., hormones, nutrition and physical exercise, in addition to specific age-related alterations at the tissue level, resulting in general deterioration of the system.

2.2.10.1 Bones

Bone should not be considered as metabolically quiescent since it is constantly remodelled according to mechanical demand and continuous turnover due to new bone formation (by osteoblasts) and bone resorption (by osteoclasts) (Exton-Smith, 1985).

With aging, the balance between bone formation and bone resorption is altered in favour of resorption, resulting in a reduction of bone mass. This starts from the medullary region with enlargement of the cavity and moves towards the epiphysis of the bone and then to the outer surface with a final reduction of cortex thickness. As a consequence, the bone strength is reduced (Smith et al., 1981). The prolonged period where bone resorption exceeds bone formation may result in osteoporosis (Dawson-Hughes et al., 1987; Eastell & Riggs, 1987; Riggs, 1987; Croucher et al., 1989).

The factors responsible for bone resorption and remodelling are not well known. More knowledge is available concerning two other factors which profoundly affect bone metabolism: calcium turnover and hormonal profile. Calcium is absorbed through the intestinal mucosa and is excreted from the kidney, the blood level remaining remarkably constant throughout life. Calcium is required for many essential functions in the body, such as cell division, cell intermediary metabolism, and cell functions (excitability, secretion and movement). In the case of a negative balance, calcium is mobilized from the bone with a consequent reduction in bone mass.

Calcium metabolism is under hormonal control. Parathyroid hormone increases the plasma calcium level by promoting bone resorption and mobilization, whereas calcitonin lowers blood calcium by inhibiting bone resorption. Calcitriol (the active derivative of vitamin D) increases intestinal absorption and decreases excretion of calcium, thus acting to increase the body burden of calcium. The age-associated decrease in intestinal calcium absorption lowers the plasma calcium level, leading to an increase in parathyroid hormone. Calcitriol levels may also be deficient in the elderly, as a result, perhaps in part, of the decrease in vitamin D synthesis in old skin and alterations in renal metabolism.

Bone metabolism also depends on other hormonal factors. Estrogens have a strong positive effect on bone density. After menopause, bone resorption exceeds bone formation resulting in bone
mass loss. However, the occurrence of osteoporosis greatly depends on the adult bone peak mass, physical activity, and calcium intake (Eastell & Riggs, 1987; Riggs, 1987; Bornor et al., 1988; Stevenson et al., 1988; Lindsay, 1989).

Glucocorticoids lower plasma calcium levels, which can increase bone resorption by stimulating parathormone secretion. Thyroid hormones may also cause bone loss, although the mechanism is still unclear. Growth hormone, somatomedins and insulin all promote bone formation, and therefore defective secretion (as in diabetes) may cause osteoporosis.

2.2.10.2 Joints

Articular disorders are practically universal in elderly people, and are associated with pain and disability. The major alteration consists of the loss of the smooth surface of cartilage, which becomes thicker and less elastic. The cartilage develops rough surfaces and mechanical irregularities, which represent the early steps in osteoarthritis (Evens & Hawkins, 1984).

2.2.10.3 Skeletal muscles

With advancing age muscles become smaller in size, due to reduction in the number of fibres, and less elastic, due to an increase in collagen content. The fat content of muscle tissue also increases with age. The causes for such defects are still controversial: alteration of sarcoplasmic reticulum, decrease of contractile protein synthesis and a reduction in the number of mitochondria are all found in aged muscle cells.

In addition to muscle cell alterations, the neuromuscular junction is modified in aged muscle. The acetylcholine content of nerve terminals is consistently reduced and acetylcholine receptors are irregularly distributed. Motor nerve conduction velocity is also impaired. All these defects contribute to the progressive failure of muscular efficiency, although some muscles, such as the diaphragm, do not seem to suffer age-related effects (Finch & Hayflick, 1977). Thus, the additive results of the loss of bone mass, disorder of joints, decline in skeletal muscle power and nervous system discoordination may lead to loss of body stability, falls and bone fracture in old people.

2.2.11 Skin

The age-related modification of the skin is the most dramatic, common and constant event in life, so that it may constitute one of the best external markers of aging. Only recently has it been possible to distinguish between intrinsic aging of the skin and photo-aging (Gilchrest, 1984). Cutaneous neoplasia is one of the best documented interactions between aging of the organism and environmental effects. A variety of environmental factors, the most notable being ultraviolet radiation, has been shown to cause cancer of the skin in humans and animal models (Rogers & Gilchrest, 1990).

The structure of skin changes throughout life (Behl et al., 1987), although many of the alterations are due to exposure to sunlight rather than intrinsic aging (Gilchrest, 1984). Skin thickness increases during maturation and then gradually declines (Vogel, 1983). However, recent studies in mice and rats by Monteiro-Riviere et al. (1991) have not reported any significant changes in skin thickness or blood flow from maturity throughout the life span. Hydration, which can affect chemical solubility, decreases while keratinization increases.
Skin aging is linked to alterations affecting both the epidermal and the dermal components. The epidermis suffers from a reduction in the turnover of epidermal cells, due both to a reduction of basal cell renewal and to a slower maturation toward the stratum corneum. The dermo-epidermal interface is flattened so that the total external body surface area is reduced (Selmanovitz et al., 1977). The reduction in epidermal cell turnover is responsible for the slowing of wound healing and possibly for the dryness and/or roughness of aged skin. The dermis is reduced in thickness in the elderly and is characterized by a reduced collagen content with a biochemical modification of the collagen itself, which makes it more strong and less elastic. Vascularization in humans is also reduced.

Aging also results in modifications in the skin appendages. Sweat gland function is decreased resulting in impaired thermoregulation. Sebaceous glands produce less oil and wax (dryness of skin), and hair usually loses pigment. The numbers of skin sensory organs (Pacinian and Meissner's corpuscles) decrease with age and sensation is consequently modified.

Collagen aging occurs in the skin, as in other tissues, leading to increased rigidity. Collagen is composed of several related proteins, which are produced by fibroblasts and extruded into the extracellular space. Here they can be chemically deposited in a nearly pure form, as in the tendons, or immersed in an extracellular matrix, also produced by fibroblasts, as in the skin. The collagen fibres undergo maturation in the extracellular ground substance by parallel arrangements of tropocollagen fibres which became assembled together through cross-linking. This phenomenon is an index of maturational change. With age, however, the cross-linking increases (Houck et al., 1967), leading to a reduction of tensile strength and plasticity (Verzar, 1968). The extracellular matrix also shows age-related changes, since alterations in its physicochemical composition lead to increased density, reduced permeability and impaired transport of nutrients (Imayama & Braverman, 1989).

3. BASIS OF ALTERED SENSITIVITY TO ENVIRONMENTAL CHEMICALS

The perception of altered sensitivity of the elderly to environmental insults is based in large part on the enhanced incidence of adverse or idiosyncratic drug reactions in this segment of the population (Vestal et al., 1985). The clinical pharmacology of the aged has been extensively reviewed in the last two decades (Triggs & Nation, 1975; Crooks et al., 1976; Reidenberg, 1980; Vestal et al., 1985), and evidence indicates that the response to drugs changes with age, as does the frequency of adverse drug reactions (Krupka & Vener, 1979). This may be in part related to issues of polypharmacy and compliance (Weber & Griffin, 1986). Morbidity and malnutrition, which are often associated with aging, could also contribute to altered pharmacokinetics in the elderly (Kitani, 1988). However, it is clear that age-related differences in drug/chemical disposition (pharmacokinetics/toxicokinetics) or sensitivity (pharmacodynamics/toxicodynamics) also play a role in altered responses to chemicals in the elderly. For sake of simplicity, the terms "pharmacokinetics" and "pharmacodynamics" will be used for both drugs and environmental chemicals.

3.1 Pharmacokinetics

Pharmacokinetics describes the processes of the absorption, distribution, metabolism and excretion of drugs or other chemicals
in the body. The numerous physiological and biochemical changes that occur during aging can modulate any of these processes of disposition. Changes in chemical disposition (pharmacokinetics being the mathematical description of these processes) can lead to an altered dose or dose-rate to the target tissue resulting in changes in response. This topic has been the subject of several recent reviews (Sellers et al., 1983; Van Bezooijen, 1984; Stevenson & Hosie, 1985; Blumberg, 1985; Birnbaum, 1987, 1989, 1991; Ritschel, 1988; Loi & Vestal, 1988; McMahon & Birnbaum, 1990a).

The highlights in this field will be covered as well as the more recent data, especially that focusing on age-related alterations in the pharmacokinetic behaviour of xenobiotics, as opposed to drugs.

3.1.1 Absorption

The absorption of drugs and environmental chemicals, defined as uptake into the blood, occurs primarily via the skin, lungs and gastrointestinal (GI) tract. Alterations in the structure and function of these three organs clearly occur with age. In contrast to the skin and GI tract, little is known about the effects of aging on pulmonary absorption of xenobiotics. Few age-related differences have been reported in lung structure (Stiles & Tyler, 1988), although the lung volume is greater in old rats. An age-related decrease in respiratory function has been reported in several species (Mauderly, 1979a,b, 1982). In addition, there appears to be a decrease in the rate of alveolar-capillary gas exchange in older organisms (Mauderly, 1979b).

Dermal exposure represents a major portal of entry for environmental chemicals. Several studies have indicated a decline in human skin permeability with aging (Christophers & Kligman, 1965). In experimental animals, the scarce data suggest that percutaneous absorption is decreased in senescent rodents as compared to young adults. The chemical absorption of 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) and related chemicals is greatest at weaning (Jackson et al., 1990), decreases at maturity and then undergoes a further decline during aging (Banks et al., 1990).

Age-related alterations in GI absorption have been studied in more depth. A decrease in gastric acid secretion is commonly seen in the elderly (Bender, 1968). The resulting increase in pH can alter the ionization of compounds, enhancing or retarding their ability to diffuse passively across cellular membranes. Decreases in gastric motility (Lin & Hayton, 1983) can prolong the transit time of chemicals in the gut, thus enhancing their potential for absorption. In rats, splanchnic blood flow declines in the first year of life but stays unchanged in the second year (Yates & Hiley, 1979; Kitani, 1988). In contrast, splanchnic blood flow declines progressively with age in humans (Sherlock et al., 1955). An increase with age in mucosal weight (Holt et al., 1984; Hebert & Birnbaum, 1987), as well as intestinal epithelial cell proliferation (Holt et al., 1988), has been reported in rats.

GI absorption can be active, passive or involve phagocytosis. Xenobiotics are primarily absorbed by passive diffusion. Age-related changes in the oral absorption of a variety of drugs in people have not been observed (Castleden et al., 1977b; Stevenson et al., 1979; Greenblatt et al., 1988). The passive absorption of TCDD in the small intestine does not change with age in rodents (Hebert & Birnbaum, 1987), nor does that of many small endogenous molecules,
such as glucose (Eastin & Birnbaum, 1987), vitamin A (Hollander et al., 1986), vitamin D, vitamin B₁₂ and niacin (Fleming & Barrows, 1982a,b) and many amino acids (Penzes, 1974).

Active transport appears to decrease with age (Eastin & Birnbaum, 1987). Doubek & Armbrecht (1987) have demonstrated that the decrease in the carrier-mediated component of glucose transport in rats occurs in the brush-border membrane of the small intestine. Their suggestion that the decrease is due to a reduction in the number of sodium-linked glucose carriers has been supported by recent studies in human intestinal tissue (Vincenzini et al., 1989).

The active transport of other small molecules such as calcium and phosphorus (Armbrecht, 1986), and galactose and iron (Reidenberg, 1980) has also been reported to decrease with age. As with the active transport of glucose, the marked decrease in calcium active transport of calcium occurs between young adulthood and middle age (Mooradian & Song, 1989) and results from changes in the number of calcium transporters in the intestinal basal lateral membranes (Armbrecht et al., 1988).

3.1.2 Distribution

The distribution of chemicals throughout the body is governed by the fact that the physicochemical properties of the compound affect its transport within the body and localization to various tissues. Lipophilic molecules readily pass across cellular membranes and accumulate in lipid-rich tissues. Binding to proteins also modulates distribution, since few compounds are transported free in the blood. While there is no evidence of alterations in relative blood volume with age, changes in body composition, blood flow and macromolecular binding have all been documented.

The decrease in lean body mass in both animals (Lesser et al., 1973) and humans (Novak, 1972) has been well documented. Loss of body water with age (Edelman & Leibman, 1959) results in a decrease in the volume of distribution for water-soluble compounds, leading to enhanced toxicity of ethanol (YORK, 1982) and ethylenediamine (Tang et al., 1984). Body fat can account for approximately 15-40% of the total body weight in humans (Ritschel, 1983) and rats (Bertrand et al., 1980; Birnbaum, 1983). The increased size of the fat compartment in older, sedentary animals would be expected to increase the body burden of lipid-soluble substances and reduce the overall rate of elimination from older animals. Following an inhalation exposure of old rats to methylchloroform (Schumann et al., 1982a,b), a better fit to a physiologically based pharmacokinetic (PbPkr) model was obtained by increasing the volume of the fat compartment from 7 to 18% of the body weight for rats and from 4 to 18% for mice (Reitz et al., 1988). Similar improvements were noted by Lutz et al. (1977) in their PbPkr model of polybrominated biphenyl compounds in rats.

Not only does cardiac output decrease with age (Bender, 1965), but regional blood flow can change differentially (Yates & Hiley, 1979). Since adipose tissue volume increases but blood flow decreases with age, lipophilic compounds tend to show greater retention in the elderly. This has been shown for polychlorinated biphenyls (Birnbaum, 1983) and halogenated solvents (Schumann et al., 1982a,b). A clinical pharmacokinetic study (Klotz et al., 1975) which demonstrated a 5-fold increase in the distribution volume of diazepam in the elderly is in agreement with the data on experimental animals.

Binding to blood components can also change with age. Although
the total plasma protein content does not change dramatically with age, there is a small but significant reduction in albumin in both animals (Rodgers & Gass, 1983) and humans (Bender et al., 1975). For drugs or xenobiotics that can be bound, such a decrease in albumin enables a higher concentration of free drug to reach the target site. A decrease in binding of drugs to red blood cells has also been reported to occur during aging (Chan et al., 1975), again leading to a higher level of free drug.

3.1.3 Metabolism

As with absorption and distribution, there are physiological changes that occur during aging which can influence biotransformation reactions, both in the liver and in extrahepatic tissues. Although the liver is the main site for the metabolism of drugs and environmental chemicals, significant metabolic reactions occur in all the portals of entry tissues (skin, respiratory tract, GI tract) as well as the kidneys, gonads, adrenals, etc. Metabolism is often considered to be divided into two types of reactions: phase I (functionalization reactions) and phase II (conjugation reactions). Phase I reactions tend to increase the polarity of chemicals, and the corresponding enzymes include the cytochrome-P450- and FAD-containing monooxygenases as well as the alcohol and aldehyde dehydrogenases, monoamine oxidases, nitro- and azo-reductases, esterases and amidases. Phase II reactions involve the conjugation of functional group (either already present on the chemical or as a result of phase I activity) with an endogenous cofactor such as an amino acid (glycine, glutamate), peptide (glutathione), sugar (glucuronic acid) or a small molecule such as a sulfate, acetate or methyl group. These reactions may occur on cellular membranes or in the cytosol, and are regulated in a tissue-specific manner.

The only generalization that can be made concerning age-related changes in biotransformation is that few patterns exist. Metabolic changes with aging appear to be substrate, sex, strain and species dependent (Schmucker & Wang, 1989; Kitani, 1988; Birnbaum, 1989; McMahon & Birnbaum, 1990b). In addition to intrinsic changes related to altered physiology, the study of the influence of age on metabolism is further complicated by the effects of diet, alcohol, drugs and pollutants, which can induce or inhibit enzyme activities (Ritschel, 1988).

Most of the research on the effects of aging on metabolism has focused on the microsomal mixed-function oxidases. The protein components of this system have been reported to decline or remain unchanged with age. In general, the decline in total cytochrome P450 levels reported in rats appears to be due to the age-related decrease in the amount of the male-specific forms (Kamataki et al., 1985a,b; Sun et al., 1986). This results from the age-related decrease in circulating testosterone levels (Kitani, 1985) or to alterations in the secretory profiles of growth hormone (Fishbein, 1991). This pattern is most pronounced in rats, few changes being observed in other rodents or sub-human primates (Birnbaum, 1987). However, recent human studies have suggested that some degree of sexual dimorphism does exist in hepatic drug metabolism. Plasma antipyrine half-lives were prolonged in elderly males, but not elderly females, as compared to young adults (Greenblatt et al., 1988). This was caused by an age-related reduction in clearance, which has been interpreted as being the result of an impairment of antipyrine metabolism in elderly men. Similar gender-specific results were observed for the kinetics of chlordiazepoxide, another low-clearance oxidatively metabolized drug (Loi & Vestal, 1988).
A study by Chengelis (1988a) has supported the earlier report of Rikans (1984) that measuring components of the monooxygenase system cannot lead to predictions of toxicity. Sex differences in rats were only significant up to one year of age. However, Rikans (1989a) has recently reported that metabolism of specific substrates (aniline, benzphetamine and nitroanisole) does decline with age in the female as well as in the male rat, but the magnitude of change in the female is smaller. This agrees with a study by Bitar & Shapiro (1987), who suggested that an age-related increase in haem degradation plays a role in the decreased metabolism of hexobarbital and aniline. This observation, that microsomal drug metabolism activities do not have to be sexually dimorphic to be altered in old age, implies that factors other than loss of the male-specific cytochromes P450 contribute to the age-associated alterations in some rat strains. Decreases in NADPH cytochrome P450 reductase in rat liver with age have been reported by Blanco et al. (1987), Chengelis (1988a) and Rikans (1989a).

Despite a large body of data obtained from experimental animal studies, there is no clear evidence that monooxygenase activities decline in the livers of healthy elderly humans. All the data derived so far from human liver biopsy specimens have shown no correlation between enzyme activities expressed per mg protein and age of subjects (Boobis & Davies, 1984; Schmucker et al., 1990). The often reported decreases in clearance values of drugs metabolized primarily by the liver in the elderly may in large part be accounted for by the decrease in liver volume with age (Kitani, 1988). Further evidence is needed to determine whether hepatic drug-metabolizing enzyme activities in humans decline with age, as is observed in some rodents, but the extent is unlikely to be as drastic as that observed in male rat liver.

A change in the composition of cytochrome P450 enzymes, suggested by differential changes in enzyme activity, has been demonstrated in male rats (Kamataki et al., 1985a; Sun & Strobel, 1986). Leakey et al. (1989a) showed that dietary restriction could also alter the profile of cytochrome P450 isoforms, possibly by delaying the age-related demasculinization of the liver. Friedman et al. (1989) showed that aging affected the composition of testosterone-binding cytochromes P450 in the liver of male rats. Changes in P450 isoforms in old rats were also reported by Paramonova & Dovgi (1987). Studies with humans have demonstrated that the effect of age on metabolism even varies for different metabolites of the same parent compound (Posner et al., 1987). This supports the observation of changes in cytochrome P450 isoform composition with age, as has been reported for male rat liver.

Hepatic xenobiotic metabolism can be modulated by many factors. In humans, smoking often confounds studies of age-related changes in pharmacokinetics. However, the age-related decrease in the metabolism of antipyrine (Loft et al., 1988), theophylline and cortisol (Crowley et al., 1988) may be independent of smoking status. In fact, the inductive properties of smoking on hepatic metabolism do not appear to diminish with age. Phenytoin also increased theophylline metabolism to an equal extent in both young and old healthy men (Crowley et al., 1988). In contrast, Rath & Kanungo (1989) demonstrated that the rate of transcription of the phenobarbital-specific isozymes was nearly two-fold higher in young rat liver than in old. However, these differential effects may reflect the specific isoforms involved, since smoking and phenobarbital preferentially induce different forms. The recent studies of Rikans (1989b), which demonstrate that induction of
hepatic microsomal drug metabolism by ethanol or acetone is unaffected by the aging process, lend support to this concept.

Age-related changes in phase I hepatic drug-metabolizing enzymes other than the mixed-function oxidases have been subjected to much less examination. Rikans & Moore (1987) demonstrated an age-related increase in liver alcohol dehydrogenase in male rats. However, no age differences have been observed in the activity of this enzyme in female rats. Hydrolytic reactions may decrease with age. For example, liver esterase activity, using diethylhexyl phthalate as a substrate, declines in old rats (Gollamudi et al., 1983). Using aspirin as a substrate, no correlation was found between age and esterase activity in human liver (Yelland et al., 1991). These results provide further evidence that age is not a major determinant of hepatic drug metabolism in the elderly (Schmucker et al., 1990).

Alterations with age in extrahepatic phase I metabolism also appear to be substrate, sex, strain and species specific. Pulmonary metabolism of benzo[a]pyrene was found to increase in old rats (Sun & Strobel, 1986) in agreement with earlier studies of Rabovsky et al. (1984). In contrast, oxidation of 2-aminofluorene, which is catalysed by a different isoform of cytochrome P450, decreased (Robertson & Birnbaum, 1982). Renal metabolism of acetaminophen has been reported to decrease with age (Beierschmitt & Weiner, 1986), while salicylate oxidation by kidney extracts did not change in rats (Kyle & Kocsis, 1985). Age-related alterations in intestinal Phase I metabolism appear to be site specific. Sun & Strobel (1986) reported that oxidation of benzo[a]pyrene in the colon increased throughout the life of rats, while McMahon et al. (1987) observed no age-related change in the metabolism of this substrate in the small intestine. Newaz et al. (1983) observed higher metabolic rates of dimethylyhydrazine in colonic tissue from aging humans as compared to younger individuals. McMahon et al. (1989) suggested that plasma esterase hydrolysis of benzyl acetate may decline with age in both rats and mice. Alcohol dehydrogenase activity was found to remain unchanged in the aging colon (McMahon et al., 1987).

Changes in phase II enzymes also appear greatly variable in rodent liver, although Loi & Vestal (1988) conclude that age has little effect on phase II reactions in humans. The major conjugation reactions involve sulfation, glucuronidation or reaction with glutathione leading to mercapturic acid formation. All these reactions are catalysed by multiple isoforms showing varying degrees of substrate and sex specificity, as is the case for the phase I enzymes. Iwasaki et al. (1986) demonstrated differential age effects on two distinct sulfotransferases using male and female rats and various alcohols and amines. Sulfation of phenolic substrates appears to decline with age (Galinsky et al., 1986; Sweeny & Weiner, 1986) while conjugation of bile salts increases in old male rats (Galinsky et al., 1986). In contrast, changes in female rats were not seen (Galinsky et al., 1990). Chengelis (1988b) observed no significant changes in sulfotransferase activity with age in either male or female rats using beta-naphthol as the substrate. Similar results were seen by Leakey et al. (1989b) for the conjugation of both estrone and naphthol, whereas the sulfation of androsterone and corticosterone increased with age in male rats.

Hepatic glucuronidation also shows substrate specificity for alterations with age. Depending on the substrate, increases with estrone and acetaminophen (Galinsky et al., 1986), decreases with the rubber antioxidant 4,4'-thiobis-(6- t-butyl- m-cresol) (Borghoff et al., 1988), or no effect with naphthol,
p-nitrophenol, morphine and testosterone have been observed (Galinsky et al., 1986; Sweeny & Weiner, 1986) in male rats. In contrast, Chengelis (1988b) observed a marked decrease in senescent male and female rats using both p-nitrophenol and chloramphenicol. To complicate matters further, Leakey et al. (1989b) also saw a decrease in naphthol, testosteron, androsterone and tetrahydrocortisone conjugation with glucuronic acid, but observed no effects of aging on conjugation with 2-aminophenol, 5-hydroxytryptamine, billirubin or estrone. Tarloff et al. (1989b) saw no change in acetaminophen glucuronidation with advancing age. Although the activities of the UDP-glucuronosyltransferases appear highly variable, an age-related decrease in hepatic levels of the cofactor, UDP-glucuronic acid (UDPGA) (Borghoff et al., 1988), suggests that glucuronidation could be limited in older animals.

The concentration of hepatic glutathione, the cofactor involved in the third major class of conjugation reactions, has been reported to increase (Borghoff & Birnbaum, 1986), decrease (Stohs et al., 1982) or remain unchanged (Chengelis, 1988b; Rikans & Moore, 1988) in old rodents. Variability in age effects has also been reported in the activity of the glutathione-S-transferases, which exist as a family of dimeric proteins having broad and overlapping substrate specificity. The isozymes are composed of two subunits from at least six different peptides, including both homo- and heterodimers. Thus reports of an increase (Leakey et al., 1989b), decrease (Fujita et al., 1985; Blanco et al., 1987; Leakey et al., 1989b), or no change (Birnbaum & Baird, 1979; Sweeny & Weiner, 1985; Borghoff & Birnbaum, 1986; Chengelis, 1988b; Leakey et al., 1989b; Carrillo et al., 1991) in hepatic glutathione-S-transferase activity may, as in the cases of cytochrome P450 mixed-function oxidases, sulfo transferases and UDP-glucuronosyltransferases, reflect age-dependant changes in isozyme ratios (Spearsman & Leibman, 1984; Carrillo et al., 1991). Restriction in dietary protein decreases the activity of the glutathione-S-transferases more in old than young rodents (Carrillo et al., 1989, 1990).

Much less has been reported on the effects of aging on other phase II metabolic reactions in the liver. Hydrolysis of epoxides to form diols or dihydrodiols is catalysed by the epoxide hydrolases. Epoxide hydrolase activity has been reported both to increase (Birnbaum & Baird, 1979) and decrease (Ali et al., 1985; Kaur & Gill, 1985; Leakey et al., 1989b) with age. Again, this may reflect differential age effects on multiple enzymatic species. However, some of the difference may be due to the ages of animals used for comparison, since Chengelis (1988b) reported a gradual increase in epoxide hydrolase activity throughout much of the life span, followed by an abrupt decrease in senescence.

Human studies have suggested that the alterations observed in salicylate pharmacokinetics (Cuny et al., 1979) with age might be due to a decrease in glycine conjugation (Kyle & Kocsis, 1985). However, elevated levels of salicyluric acid, the glycine conjugate of salicylate, have been observed in elderly humans undergoing chronic salicylate treatment (Montgomery & Sitar, 1981). Recent studies in rodents have not demonstrated any age-related decline in glycine conjugation with non-nephrotoxic doses of salicylate (McMahon et al., 1990a) or in the formation of hippuric acid from benzoate (McMahon et al., 1989). Acetylation, however, has been reported to decline both in man (Bauer et al., 1989) and rats (Leakey et al., 1989b).

Changes in extrahepatic phase II reactions have been investigated in even less depth than extrahepatic phase I reactions. In the lung and small intestine, glucuronidation of p-nitrophenol
appeared not to change with age (Borghoff & Birnbaum, 1985), whereas in the colon an age-related decrease was reported by McMahon et al. (1987). In contrast, colonic glucuronidation of 4-methylumbelliferone rose significantly in older rats (McMahon et al., 1990b). A decrease in UDP-glucuronosyl transferase activity in the kidney (Borghoff & Birnbaum, 1985; Tarloff et al., 1989a) was accompanied by a decrease in the renal concentration of UDPGA (Borghoff et al., 1988).

Glutathione content has been examined in a variety of tissues (lung, kidney, brain, testes and blood) and, except for an age-related decrease in the lens, has been found to remain constant (Rikans & Moore, 1988). Unchanged glutathione levels in the colon occurred although the activity of glutathione-S-transferase decreased in this tissue (McMahon et al., 1987). Spearman & Leibman (1983, 1984) observed differential age- and sex-related changes in this activity in the lung depending on the substrate examined. The renal activity of glutathione-S-transferase declines significantly in old rats (Beierschmitt & Weiner, 1986). In contrast, elevated glutathione-S-transferase levels were observed in the brain of old rats (Blanco et al., 1987), while no changes were observed in the heart.

Epoxide hydrolase activity has been examined in the lungs, small intestine and kidneys by Kaur & Gill (1985). They observed a decrease in lung and small intestine activity in old rats which was substrate dependent. In the rat kidney, epoxide hydrolase activity decreased using trans-stilbene oxide as the substrate but did not change with cis-stilbene oxide, supporting again the differential effects of aging on different isozymic forms of drug-metabolizing enzymes. In addition, deacetylation of acetaminophen in the kidney appears to be either unchanged with age (Beierschmitt & Weiner, 1986) or slightly decreased (Tarloff et al., 1989b). Acetylation in the kidney also decreases with age (Wabner & Chen, 1984).

One additional enzyme which can be considered to fall into the phase II class is beta-glucuronidase. This enzyme hydrolyses glucuronic acid from conjugated xenobiotics. The activity of this enzyme has been reported to increase with age in rat liver (Schmucker & Wang, 1979; Van Manen et al., 1983) and kidney (Borghoff & Birnbaum, 1985), but remain unchanged in the colon (McMahon et al., 1987). However, beta-glucuronidase was found to decrease with age in fecal contents (McMahon, 1988). The balance between glucuronidation and deglucuronidation reactions may play a role in determining the level of reactive compounds in the organism.

Depending on the chemical, aging can result in either an increase or a decrease in the metabolizing capacity of different organs and tissues (liver, kidney, gastrointestinal tract, lungs and skin). The elevation or reduction in metabolism can both lead to higher and lower toxicity, depending on the relative reactivity of the metabolic intermediates and end-products. Thus studies on the effect of aging on metabolism should be considered case by case.

3.1.4 Excretion

Excretion leads to the elimination of a chemical and/or its metabolites from the body. The kidney is the major excretory organ, with the liver and lung also playing important roles in the elimination process. In addition, sweat, saliva and sex-linked processes such as lactation can serve as routes of excretion.

The effects of age on renal function appear to play a major
role in altered pharmacokinetics in the elderly (Vestal, 1978; Koch-Weser et al., 1982). The fact that changes in the physiology of the kidney occur has been known for many years (Schmucker, 1979). Renal blood flow decreases with age leading to a decrease in glomerular filtration rate. Tubular secretion and resorption are also reduced in the elderly. The number of functional nephrons declines to a similar extent as the decline in glomerular filtration rate and active secretion, suggesting that the nephron loses its function as a unit (Friedman et al., 1972). Decreases in renal function can result in a decreased rate of renal clearance, leading to a greater potential for elevated and/or persistent levels of chemicals in the body which could lead to toxicity (Sellars et al., 1983). In humans, decreased renal clearance in the elderly has been demonstrated for many drugs, including the aminoglycosides, tetracyclines, lithium, digoxin, procainamide, methotrexate, and phenobarbital (Kampmann & Hansen, 1979).

Aging rodents are extremely susceptible to chronic glomerulonephropathy (Goldstein et al., 1988), much of which may be attributable to diet (Masoro & Yu, 1989). Altered glomerular morphology, characterized by thickening of the basement membrane and sclerosis, is progressive and increases in severity with advancing age, eventually resulting in scarring and loss. Renal tubules are also subject to degenerative changes, which are accompanied by proteinuria, especially albuminuria (Neuhaus & Flory, 1978). This appears to result from increases in glomerular permeability and a loss of fixed glomerular polyanion (Baylis et al., 1988). The high percentage of urinary protein represented by albumin in the aging rat may be due to non-selective protein leakage into the urine resulting from increases in glomerular permeability to large proteins, since the protein percentage in urine approaches that in the plasma of senescent rats (Horbach et al., 1988a). Similar albuminuria has been observed in aging mice (Yumura et al., 1989), and was correlated with glomerular sclerosis. Such changes, however, should not be simply extrapolated to humans, since there is no evidence for an increase in protein loss in the urine of the healthy elderly.

Additional tubular changes occur in the aging kidney, resulting in hyperplastic and degenerative changes. Some of these changes resemble responses to specific environmental chemicals (Konishi & Ward, 1989). A decrease in renal transport of organic acids has been observed (Wabner & Chen, 1984). Aging appears to diminish the turnover of sodium/potassium ATPase in the proximal tubules (Marin et al., 1985), which could play a role in the observed age-related decrease in tubular secretion.

Hepatic elimination may also be compromised by aging. Kitani (1985) has suggested that the excretory capacity of the liver decreases with age due to some functional alteration in the hepatocytes. In contrast to rats, where blood flow does not change after maturity (Kitani, 1988), blood flow to the liver declines in humans (Sherlock et al., 1955). Bile flow rate has been reported to be reduced (Borghoff et al., 1988) or remain unchanged (Kitani et al., 1985a) in rats. Biliary transport declines, especially in the case of polar compounds (Kitani et al., 1985a). The elimination of sulfobromophthalein, a model compound for the study of biliary excretion of organic anions, is also decreased in old rats (Kanai et al., 1985). Sato et al. (1987) demonstrated that the biliary excretion of the neutral glycoside ouabain decreases with age in both males and females. This may be due to an age-related decrease in hepatic uptake, resulting in less biliary elimination (Ohta et al., 1988). In addition, the biliary canalicul transport system
declines steadily during aging (Kanai et al., 1988). The decreases in both hepatic uptake and biliary excretion may reflect changes in the hepatocyte plasma membrane (Zs-Nagy et al., 1986).

3.2 Pharmacodynamics

Age-related changes in chemical sensitivity cannot all be explained on the basis of altered pharmacokinetics in the elderly. Pharmacodynamic changes occur at the target site and may involve changes in cell populations, cellular receptors, cellular responsiveness or in the regulation of the amount or activity of drugs, including the cardiac glycosides, benzodiazepines, tricyclic anti-depressants, and the non-steroidal anti-inflammatory agents (Bender, 1979), which have demonstrated altered receptor sensitivity in the aged (Wilson & Hanson, 1980).

3.2.1 Central nervous system

Numerous studies have shown that, with advancing age, there is a decrease in the ability of the nervous system to synthesize and/or release neurotransmitters and neuropeptides (for review, see Rogers & Bloom, 1985). This decline may reflect the fact that there are fewer neurons present to synthesize the chemical messenger or that the enzymes involved in their synthesis are altered. This would imply that xenobiotics which lead to neuronal death or interfere with neurotransmitter or neuropeptide synthesis and release could have a greater adverse effect on the elderly than on the young organism. An example of such an interaction has been noted within the dopaminergic extrapyramidal circuits controlling motor movements (nigrostriatal pathway). This pathway has been the subject of many experimental and clinical studies and provides one of the best functional units in which to study neurotoxicity in the aged. Nigrostriatal neurons are lost as a normal correlate of aging, and while these losses may not be expressed in the majority of individuals as movement disorders, there is a clear reduction in the functional reserve of this dopaminergic system, making it more vulnerable to neurotoxicants. This has been demonstrated by the observed acceleration or simulation of "age-associated" movement disorders by neurotoxicants such as 1-methyl-4-phenyl-1,2,5,6-tetrahydro-pyridine (MPTP).

The reduced capacity to synthesize neurotransmitters that occurs in the aged organism may also potentiate the effect of toxic substances. Carbon disulfide is an organic solvent with a variety of industrial applications that produces neurobehavioural dysfunctions (Wood, 1981). The mechanism of CS2 toxicity seems to be inhibition of dopamine-beta-hydroxylase, the enzyme that converts dopamine to norepinephrine (McKenna & DiStefano, 1977). Since norepinephrine metabolism declines with age, the spectrum of physiological effects regulated by this catecholamine would be expected to suffer greater disturbance, following exposure to CS2 or to pesticides that reduce brain catecholamines such as methyl bromide (Honma et al., 1987), in old rats compared to young ones.

Toxic compounds that serve as neurotransmitter receptor blockers could have their effects on behaviour accentuated, making neuroendocrine control of homeostasis more difficult in the elderly. For example, the formamidine pesticides, amitraz and chlordimeform, have been shown to block alpha-noradrenergic receptors (Costa & Murphy, 1987; Costa et al., 1988) and induce a variety of behavioural disorders (Boyce & Dyer, 1984; Hsu & Kakuk, 1984; Landauer et al., 1984) in rats. Since there is an age-related decline in noradrenergic receptors in several species (Rogers &
Bloom, 1985), exposure to these compounds may have a more pronounced effect on the older organism.

Certain movement disorders associated with senescence may be related to age-related impairments in the brain dopaminergic systems (Marshall & Berrios, 1979). Waddington et al. (1985) observed a decrease in the density of brain dopamine receptors in old rats, with no changes in affinity. In contrast to young animals, the receptors of old animals were not able to respond effectively to long-term treatment. The decrease in dopamine receptor density was selective for the D-2 receptor subtype, although the coupling between D-1 receptors and adenylate cyclase appears to be affected by aging. A similar loss of D-2 receptors in the elderly has also been measured using positron tomography in the living human brain (Wong et al., 1984). An age-related decrease in binding to the serotonin receptor may relate to cerebral dysfunction in the elderly (Shih & Young, 1978).

Studies with rats have indicated that the increased sensitivity of older rats to diazepam is due to pharmacodynamic differences (Guthrie et al., 1987). Pedigo et al. (1981) suggested this could relate to changes in the benzodiazepine/GABA/chloride ionophore complex. Human studies have also indicated that the site of increased sensitivity to the benzodiazepines lies distal to the receptor (Swift, 1985), possibly involving changes in the chloride ionophores.

Alterations in endogenous opioid systems may play a role in some of the behavioural changes observed in the elderly. Binding of dihydromorphine to the opiate receptor decreases in specific areas of the brain in aged rats as compared to young ones (Messing et al., 1980). This reduction is due to a decrease in receptor number, with no change in affinity. Despite a relatively large body of evidence that some receptors and neurotransmitters are at least qualitatively altered with aging, it is still not clear whether and how these changes are causally related to increased sensitivity of the CNS to certain drugs with aging, as described below.

The brain appears to exhibit increased sensitivity to phenobarbital (Kitani et al., 1985b; Van Bezooijen et al., 1989) in both mice and rats. Such enhanced sensitivity with age to an anticonvulsant supports earlier studies with phenytoin (Kitani et al., 1984). It is possible that the aging brain of both experimental animals and humans has increased sensitivity to all CNS depressants. Enhanced brain sensitivity has also been reported for hexobarbital in rats (Van Bezooijen et al., 1989) and oxazepam in mice (Kitani et al., 1986). The aging human brain appears to be more sensitive to nitrazepam (Castleden et al., 1977a) and other benzodiazepines (Reidenberg, 1980). Recently, zonisamide, a new drug whose anticonvulsant properties are distinct from the other drugs, was demonstrated to have an increased anticonvulsant effect in aging mice (Kitani et al., 1987). Taken together, these results suggest that these pharmacodynamic changes with age may reflect a decreased response capability for seizures in the elderly, rather than a specific age effect on all the distinct receptors (Kitani et al., 1986). In fact, the lethal threshold for pentylenetetrazole in mice has been shown to decrease with age (Nokubo & Kitani, 1988), coupled to an increase in the threshold for maximal seizure.

The action of other environmental compounds on CNS function may be more diffuse. Recent studies have demonstrated enhanced sensitivity of old mice to cyanide intoxication (McMahon & Birnbaum, 1990b). Exposure to various metals has been shown to alter a variety
of CNS functions. There has been a particular interest in a potential link between Alzheimer's disease and aluminium toxicity. It was initially reported that the autopsied brains of Alzheimer's patients showed elevated aluminium levels (e.g., Perl & Brody, 1980). However, others have found no such correlation. Marksberry et al. (1981) compared the brains of Alzheimer's patients with older adult controls and found no correlation between the density of aluminium content and the density of neurofibrillary tangles and neuritic plaques. However, a more recent study showed that the brain aluminum levels were significantly increased as a function of age in both control and Alzheimer patient populations (Bjorksten et al., 1989).

Manganese is a metal that causes age-type neuropathy. However, unlike aluminium, it is associated with extrapyramidal disorders, characterized by intention tremor (Donaldson, 1987). In monkeys, the neurological symptoms of choreo-athetoid movement, rigidity and tremor occurred after 18 months of manganese exposure. These clinical signs, in association with severe lesions of the globus pallidus and subthalamic nucleus, resembled Parkinson's disease and suggested a possible link between environmental exposure and occurrence of the disease in aging individuals.

The neurotoxic effects of other metals have been widely studied, particularly those of lead, which has been implicated in reduced intellectual abilities in children (Needleman et al., 1979), slowed reaction time (Hunter et al., 1985), and impairment of other cognitive abilities (Winneke et al., 1982; Hansen et al., 1985). While psychophysiological parameters appear to be relatively insensitive to low levels of lead exposure, several studies have demonstrated both cognitive and emotional effects due to long-term exposure to lead (Hogstedt et al., 1983; Mantere et al., 1984). Long-term exposure to mercury vapour has been reported to interfere with verbal intelligence and memory performance (Piikivi et al., 1984). Hanninen (1982) suggested that abnormalities due to mercury exposure affect the motor system and result in intellectual impairment, a gradual and progressive deterioration of memory function, and emotional disability. Other metals including copper, iron and manganese have also been reported to cause CNS dysfunction (Grandjean, 1983). However, there are no available data on the role of exposure to these metals in dysfunction of the CNS in the aged.

Examples of naturally occurring neurotoxic agents that stimulate nervous system senescence have been reported in certain island populations. For example, the Chamorro peoples of the Marianna Island, specifically Guam and Rota, exhibited a high incidence of amyotrophic lateral sclerosis, Parkinsonism and Alzheimer's-like dementia that was recently linked to their diet. The Chamorro's diet consists in part of a flour made from the seeds of Cycas circinalis. When one of the components of these seeds, beta- N-methylamino-l-alanine (BMAA), a compound similar in structure to excitotoxic amino acids, was fed to macaques, they exhibited signs of motor neuron, extrapyramidal and behavioural dysfunction (Spencer et al., 1987).

It seems that several motor neuron disorders are associated with the appearance of endogenous excitotoxic glutamate agonist-type molecules. One such molecule is 2,3-pyridine dicarboxylic acid (quinolonic acid), which has been isolated from the brains of humans, rabbits and laboratory rodents (Moroni et al., 1984) and has been shown to excite CNS neurons when applied iontophoretically (Perkins & Stone, 1983). An interesting correlate is the fact that...
brain concentrations of quinolinic acid increase with age (Moroni et al., 1984), suggesting that its presence may result in the spontaneous onset of neurodegenerative conditions that are mimicked by environmental neurotoxicants such as BMAA and beta-\(N\)-oxalylamino-\(L\)-alanine (BOAA).

### 3.2.2 Endocrine system

There are several different ways in which the endocrine system and the hormonal signalling operations involved may undergo alterations with age and toxicant exposure. These can be categorized as changes in: (a) the availability of hormones for binding to the target tissues; (b) the reception of the pertinent transmitter or hormonal signal by the target cells; and (c) the nature of the hormonal message.

#### 3.2.2.1 Changes in hormonal availability with age

Age-related or toxicant-induced shifts in synthesis, rate of clearance and rate of secretion will all function to alter hormonal concentrations. Such changes in the size of the available signal pool may have corresponding effects on the magnitude of the response by the target tissue. These changes may reflect a decline with age in the homeostatic controls, which rely heavily on endocrine feedback relationships.

Several toxicants have also been observed to cause changes in circulating hormonal levels (Cooper et al., 1986). Significant reductions in serum testosterone, for example, have been seen following short-term exposure of rats to the plasticizer dinitrobenzene (Rehnberg et al., 1988b) and the pesticide chlordimeform, the latter also causing marked reductions in serum LH, thyroid-stimulating hormone (TSH), \(T_4\) and \(T_3\) levels. The effects, moreover, may be remarkably specific. Following three days of exposure in male rats, the pesticide linuron, for instance, was reported to decrease the serum \(T_4\) level in a dose-related manner, while leaving \(T_3\) and the pituitary and gonadal hormones unaffected (Rehnberg et al., 1988a).

There is also a growing body of evidence for a hormonal influence on toxicant metabolism. A sizeable number of xenobiotics, including both drugs and environmental toxicants, are metabolized by the hepatic cytochrome P450 monooxygenase system (Nebert & Gonzalez, 1987). Components of this system have been found to be influenced by glucocorticoids (Schuetz et al., 1984; Simmons et al., 1987) and markedly affected by sex steroids (Kamataki et al., 1985b) and growth hormone (Yamazoe et al., 1987; Zaphiropouos et al., 1989). Consequently, persistent shifts in the circulating levels of such hormones, as have been reported for the aging animal, could affect the manner in which xenobiotics are metabolized following exposure.

Reported attenuations with age in the rhythms of human and rat serum testosterone (Bremner et al., 1983; Steiner et al., 1984; Tenover et al., 1988), LH (Vermeulen et al., 1989) and GH (Sonntag et al., 1980), among other hormones, can present differences in young-versus-old comparisons, depending on when such sampling is performed. Comparable effects on hormonal rhythms have been reported to occur in response to toxicant exposure. For example, single injections of 2,3,7,8-tetrachlorinated dibenzo-p-dioxin (TCDD) resulted in some evidence of alterations in prolactin and corticosterone rhythms in rats (Jones et al., 1987). It may be that an aging system, while still exhibiting rhythmic hormonal changes, may be increasingly sensitive to their disruption by low toxicant levels.
3.2.2.2 Changes with age in the reception of the signal by the target cells

A general decline in the transmitter regulation of hormonal function may also place an aging animal at increased risk for toxicant exposure (Govoni et al., 1988), given that various environmental toxicants (including, for example, the solvents vinyltoluene, ethylbenzene and styrene, the halogenated hydrocarbon TCDD, and certain heavy metal cations) have been reported to interact with catecholaminergic systems (Govoni et al., 1979; Lucchi et al., 1981; Arfini et al., 1987; Mutti et al., 1988; Russell et al., 1988).

These changes have been observed for both neurotransmitter and hormone receptors. There is some evidence of a decreased responsiveness of target tissues to steroid hormones during senescence. For example, age-related declines in the concentration of the estrogen receptor may reflect a decline in the circulating estrogen levels (Thakur, 1988). Impaired responsiveness could also be due to reduced receptor translocation (Belisle & Lehoux, 1983) or other steps in steroid action which occur after hormone binding. In fact, age-related alterations in glucocorticoid responsiveness are due to both receptor and post-receptor events (Kalimi, 1982). Testicular luteinizing hormone receptor concentration and total content also decrease with age (Amador et al., 1985). In general, receptors for steroids, insulin, glucagon, catecholamines and prolactin appear to decrease in concentration with increasing age in rodents, dogs and humans (Roth, 1979b).

An additional consideration of alterations with age or toxicant exposure in the reception of a hormonal (or transmitter) signal by target cells concerns not only effects on the receptor itself, but changes in the cell's membranes. In the aging rat brain, there is evidence for a progressive decrease in membrane fluidity (Hershkowitz, 1983; Nagy et al., 1983). This is at least partially attributable to alterations in the lipid composition. For example, it has been reported that aging is associated with elevations in cholesterol, sphingolipids and saturated fatty acid chains, all leading to increases in rigidification (Rouser et al., 1972). Since protein activity in the membrane is influenced by the fluidity of the lipid micro-environment, any alterations with age in membrane viscosity may affect not only receptor functions but also enzymatic activity.

3.2.2.3 Changes in the nature of the hormonal message with age

The antigenic site(s) on a hormone recognized by antibodies can be quite distinct from those regions that bind to the receptors and trigger a physiological response in the target tissue. This distinction may have an added importance for studies in aging, since alterations with age in peptide hormone structure have been reported (Conn et al., 1980) that reflect changes in post-translational processes. These effects, moreover, may be influenced by shifts in the steroid hormonal milieu in the older animal (Ulloa-Aguirre et al., 1988). A number of hormones are glycosylated to varying degrees and such differences in their carbohydrate residues may alter biological activity (Ulloa-Aguirre & Chappel, 1982; Warner et al., 1985) and/or plasma half-life (Morell et al., 1971). Consequently, hormonal measures based solely on immunoreactivity per se potentially offer a somewhat inaccurate picture of endocrine alterations with age.
3.2.3 Kidney

The aging kidney appears to be more susceptible than the young one to drug-induced nephrotoxicity as well as to renal ischaemia. In fact, cortical tubules of senescent rats seem more sensitive to oxygen deprivation than do those of young rats (Miura et al., 1987). Thus, increased susceptibility of the aging kidney can be independent of pharmacokinetic effects.

Age-related susceptibility to nephrotoxicity has been demonstrated for numerous drugs including salicylate, acetaminophen, cephaloridine and doxorubicin. Kyle & Kocsis (1985) showed that kidneys from older rats develop nephrotoxicity to a greater extent than do those of young rats following an equal dose of salicylate on a body weight basis. However, the dose to the kidney could be greater in the older rats due to a decrease in the volume of distribution. In addition, McMahon et al. (1990a) has recently demonstrated that at toxic doses old rats produce more reactive metabolites of salicylate than do young ones. The age-related enhancement in cephaloridine nephrotoxicity may also be due to pharmacokinetic changes (Goldstein et al., 1986), although renal cortical slices from old rats demonstrated alterations in active uptake of organic anions and cations following exposure to the antibiotic, which were not observed in young rats. Doxorubicin treatment resulted in greater toxicity in old rats (Colombo et al., 1989). The onset of the delayed nephrotoxicity was noticeably faster in the old rats; this may have been related to higher drug retention in the kidneys of old rats.

Age-related increased susceptibility to acetaminophen nephrotoxicity has been investigated more than that of any other chemical in rats. Tarloff et al. (1989b) stressed that while enhanced sensitivity to acetaminophen nephrotoxicity clearly occurs with advancing age, great care must be taken in choosing the ages for comparison. Beierschmitt et al. (1986a) demonstrated by both functional and histological criteria that susceptibility to acetaminophen-induced acute tubular nephrotoxicity increases with age in rats. This may reflect altered susceptibility rather than an alteration in pharmacokinetic parameters, since the generation of reactive metabolites from acetaminophen decreases with age (Beierschmitt & Welner, 1986). Increasing delivery of acetaminophen to functioning nephrons, whose numbers are decreased in the aged kidney, does not appear to be responsible for the age-related increase in susceptibility (Beierschmitt et al., 1986b).

Alterations with advancing age do not have to result in increasing sensitivity. Studies by Murty et al. (1988b) indicated a decrease in hydrocarbon-induced hyaline droplet nephropathy in male rats during senescence. This male-specific nephropathy is associated with the presence of alpha2u-globulin, whose synthesis is strongly age dependent (Roy et al., 1983). Although gasoline causes extensive nephrotoxicity in young rats, old rats are resistant. In fact, gasoline failed to alter hyaline droplet numbers in aged male rats, which also demonstrated an altered lysosomal response as compared to young rats. This lack of response of aged rats to hydrocarbon-induced hyaline droplet nephropathy suggests that these rats would also be resistant to hydrocarbon-induced renal neoplasia.

3.2.4 Immune system

With advancing age a progressive decline in the concentration of glucocorticoid receptors occurs in the spleen (Roth, 1979a). This alteration may depend either on an age-related modification of the ratio among various subsets of lymphoid cells carrying different
receptor densities or on an intrinsic failure of aged cells to maintain an adequate turnover of receptor molecules. With advancing age, membrane receptors for hormones of low relative molecular mass on lymphoid cells also show alterations, although these are more related to impaired coupling to signal transduction systems (Feldman et al., 1984; Roth, 1988). It has been shown that the number of receptor molecules decreases with advancing age whereas the affinity does not seem to change. In the aged population with altered function of the immune system, it is possible that immunomodulatory agents present in the food potentiate immune dysfunction, making the elderly more vulnerable to age-related diseases.

The effects of immunosuppressive compounds can be identified from toxicological experiments with young adult rodents. These chemicals include organotin compounds, some pesticides, halogenated aromatic hydrocarbons (e.g., hexachlorobenzene and dioxins) and cyclosporin (Poland & Knutson, 1982; Vos & Penninks, 1987; Vos & Luster, 1989; Schuurman et al., 1990; Vos et al., 1990), and they can also potentiate autoimmunity, which is found more frequently in the elderly.

3.2.5 Other tissues and systems

There are few data on pharmacodynamic changes in other tissues and systems during aging. An increase in the sensitivity of the aging human myocardium to digoxin (Chavaz et al., 1974), as well as to intravenous anaesthetics (Dundee, 1979), has been suggested. The stomach, kidney and bone marrow are more susceptible to the adverse effects of non-steroidal anti-inflammatory agents in the elderly than in the young, and cerebral side effects are more common (Huskisson, 1983). The anticoagulation properties of warfarin are also changed in the elderly, with the aged demonstrating increased sensitivity (Shepherd et al., 1979).

Although in most toxicological studies the cardiovascular system is not well examined, there are several chemicals known which can cause cardiovascular toxic or atherosclerotic effects. Such compounds in food include erucic acid in combination with omega-3-linolenic acid, cetolenic acid, brominated vegetable oils, cobalt salts in combination with ethanol, lead, nitrates and high amounts of caffeine (Speijers, 1983). On the other hand, compounds such as saturated fatty acids, odd-numbered cyclopropenoid fatty acids (sterculia acid), ergot alkaloids, halogenated aromatic hydrocarbons and cadmium induce toxic effects on the vascular system and possible potentiating atherosclerotic effect (Speijers, 1989a,b). Conrad & Bressler (1982) investigated the cardiovascular effects of caffeine in elderly men. Caffeine, in doses equal to those contained in 2 to 3 cups of coffee, produces an increase in blood pressure, but has no positive inotropic effect in healthy elderly men.

Kim & Kaminsky (1988) suggested that age plays an important role in modifying susceptibility to the toxic metabolite of fluroxene, 2,2,2-trifluoroethanol. They noted greater effects on the stomach, liver, testicles, brain and kidneys of aged rats as compared to younger animals. Enhanced sensitivity to ethanol intoxication has also been noted in old rats (Guthrie et al., 1987), suggesting altered tissue sensitivity. Acute ethanol hepatotoxicity may be due to enhanced liver susceptibility to the toxin (Rikans & Snowden, 1989).

3.3 Modifying factors

3.3.1 Nutrition
Nutrition has been shown to influence the aging processes in rodents and the occurrence and progression of age-associated diseases in rodents and humans. Moreover, the age-associated changes in physiological processes affect the nutrition of the mammalian organism. However, there is little information on how aging influences the nutritional requirements of humans, a subject that urgently requires scientific study.

Nitrates in foods can be reduced to nitrites in the oral cavity, GI tract and urinary bladder (Tannenbaum et al., 1978). Nitrites react with amines in the stomach forming N-nitroso compounds, many of which have been shown to be carcinogenic in animal studies, and it is difficult to deny their hazard to man (Searle, 1976; Bartsch, 1991).

During commercial processing and domestic preparation, foods may become contaminated with toxic chemicals. For example, smoked and grilled foods contain small amounts of polycyclic aromatic hydrocarbons and a wide variety of phenols and other organic compounds derived from smoke (IARC, 1990). Canned foods can become contaminated with tin or lead.

In a study by Spagnoli et al. (1991), both 4-month-old and 4-year-old New Zealand white rabbits were fed an atherogenic diet. Increased incidence and degree of atherosclerotic lesions were seen in the 4-year-old rabbits. These results showed an increased susceptibility of the older arterial wall to hypercholesterolaemia. Although 4-year-old rabbits are still relatively young, considering the life span of this species (10-12 years), this study suggests that aging arteries might be vulnerable to atherogenic compounds.

Malnutrition associated with inadequate intake or uptake of nutrients in the elderly is caused by several factors. These include socio-economic conditions (Munro, 1984) such as a) ignorance of the need for a balanced diet, b) poverty, c) social isolation, d) physical dependence (disability) and physical inactivity, e) mental disorders, and f) changes in habits, e.g., retirement (Munro, 1984; American Dietetic Association, 1984; Ferro-Luzzi et al., 1988). Other conditions resulting in malnutrition include malabsorption due to a variety of intestinal conditions, alcoholism, and the use of therapeutic drugs that interfere with nutrient utilization and therefore with the toxicity of chemicals (Krupka & Vener, 1979; Kohrs, 1981; Munro, 1984; Chen et al., 1985; Robertson et al., 1988). Malnutrition in much of the aged population can enhance the vulnerability of the elderly to the effects of the toxic chemicals in food. It has also been reported that malnutrition alters pharmacokinetics in different ways depending on the drugs examined (Roe, 1983; Cusack & Denham, 1984). This can be another factor in the altered sensitivity of the malnourished elderly to toxicants. Major deficits or marginal nutrient status occur for proteins, calcium, vitamins A, C and B (thiamin, riboflavin, niacin, B6 and B12) and zinc (American Dietetic Association, 1984; Ferro-Luzzi et al., 1988).

In aged women, a deficiency in calcium is especially associated with osteoporosis (American Dietetic Association, 1984; Munro, 1984; Caraceni et al., 1988; Cauley et al., 1988). The deficiency in iron and zinc might play a role in haematological status and immune function, while a higher intake of aluminium might have neurological sequelae (American Dietetic Association (ADA), 1984). An iron deficiency might make individuals more vulnerable to compounds toxic for the haematopoietic system, e.g., hexachlorobenzene and lead.
Data showing that older individuals have a reduced need for energy, due to slower metabolism and decreased activity, has an important impact on the intake of both macro and micronutrients, because the composition of the diet is based on the average caloric or energy intake (American Dietetic Association (ADA), 1984; Ferro-Luzzi et al., 1988). Considering this reduced need for energy, the elderly often reduce their intake of nutrients.

Dietary manipulation can alter life span in laboratory animals (Barrows & Kokkonen, 1978; Young 1979). Restricting food intake in laboratory rats produces a significant extension of life span (McCay et al., 1935; Ross, 1961). Similar effects have been observed in mice (Weindruch & Walford, 1988; Kubos et al., 1984), as well as in more primitive organisms including fruit flies (Harman, 1981), and nematodes and Neurospora (Harman, 1982). Other studies have indicated that the reduction in caloric intake that accompanies protein restriction, and not the protein restriction per se, is responsible for the increased longevity (Leto et al., 1976; Davis et al., 1983; Schneider & Reed, 1985). Dietary restriction has been shown to be effective when started as late as middle age (Cheney et al., 1983; Kubos et al., 1984; Masoro, 1988). Food restriction appears to act either by influencing primary aging processes or by a general protective mechanism, rather than directly modulating multiple specific pathogenic processes underlying specific diseases (Masoro et al., 1991).

Chronic nephropathy in rats has been reported to be retarded by food restriction (Saxton & Kimball, 1941). Rats fed ad libitum a semisynthetic diet of 21% casein as the protein source exhibited a marked age-associated progression of chronic nephropathy, whereas rats fed 60% of the ad libitum intake developed almost no age-related progression of this disease process (Maeda et al., 1985). Caloric restriction appears to be more important than protein restriction in retarding nephropathy (Maeda et al., 1985). Fat or mineral restriction was found to have no influence on longevity (Iwasaki et al., 1988).

Dietary restriction delays the age-dependent loss of adipocyte responsiveness to hormones, prevents the decline in serum free fatty acid levels, delays the increase in serum cholesterol, and reduces the increasing triglyceride level observed in aging rats (Cooper et al., 1977; Liepa et al., 1980; Masoro et al., 1980; Yu et al., 1980).

Dietary restriction from weaning has been shown to delay the onset and reduced the severity of chronic nephrosis, periarteritis, myocardial degeneration and muscular dystrophy in very old animals (Berg, 1976). Chronic restriction has also been shown to inhibit certain types of tumours, decrease the incidence of neoplasms, and increase tumour latency (Ross, 1976; Weindruch et al., 1982; Weindruch & Walford 1988). Early work using a chemically induced mammary carcinoma model suggested that the primary effect of caloric restriction was on tumour promotion (Weindruch & Walford, 1988). More recent data (Fishbein, 1991) have demonstrated that the initiation of chemical carcinogenesis can also be decreased by caloric restriction. For example, exposure to aflatoxin B1 resulted in less DNA-adduct formation in liver from young calorically restricted male rats than from controls fed ad libitum. Such changes are most probably due to changes in hepatic cytochrome P-450 expression (Fishbein, 1991).

There is ample evidence of better maintenance of T-cell-dependent immunological responses in aging mice chronically
restricted from weaning (Weindruch & Walford, 1988). However, it has been reported that restriction initiated at an adult age can also delay the age-specific decrease in immune function (Fernandes et al., 1977; Friend et al., 1978; Weindruch & Walford, 1988).

Although caloric restriction extends life span in invertebrate and lower vertebrate species as well as rodents (Weindruch & Walford, 1988), as yet there is no definitive evidence whether or not caloric restriction will increase longevity, or decrease neoplastic and degenerative diseases, in higher mammals or in man. There is some evidence that reduced caloric intake in man reduces urinary output of thymidine glycol and 8-hydroxy-guanosine, which implies reduced free-radical-mediated DNA damage (Fishbein, 1991). However, until the mechanisms by which caloric restriction evokes its effect are fully understood, the only way that it can be conclusively proved whether or not caloric restriction does prolong life in higher mammals, is to perform longevity studies in these species. Such experiments, using non-human primates, are underway in the USA (Fishbein, 1991), but it will be some time before definitive data are available.

Chronic disease in the elderly is accompanied by caloric deficit, which in turn causes breakdown of body proteins and negative nitrogen balance as well as the utilization of fat stored in adipose tissues. Dietary protein appears to promote age-associated renal disease in both humans and rats (Brenner et al., 1982).

When illness occurs, nutritional deficiencies frequently become clinically manifest (Rudman, 1987). For example, trauma or a fall causing fractures leads to immobilization resulting in rapid loss of body stores of nitrogen and calcium. This slows down mending of the fracture. Similarly, surgical procedures in the elderly often result in delayed recovery and risks far exceeding those of younger persons. Heart failure and malignancies can lead to cachexia with loss of weight, muscle mass and nutrient reserves. Infection may produce similar changes and intervene to become the terminal event.

3.3.2 Alcohol intake

Ethanol is one of the chemicals most commonly ingested by humans. Its effects on the body are numerous and varied. Loneliness and isolation would seem to foster consumption of alcohol beverages among older persons. The decrease in lean body mass and body water that occurs with aging is responsible for higher blood levels of alcohol in elderly people than in younger adults consuming the same quantities (Vestal et al., 1977).

Elderly individuals have a decreased tolerance for alcohol, due to an increased sensitivity of the CNS to the depressant effect of ethanol. The metabolic effects of ethanol are the result of either the increase in the NADH/NAD⁺ ratio occurring due to ethanol metabolism or to direct toxic effects of ethanol or its metabolite, acetaldehyde. An increase in serum uric acid level (hyperuricaemia) is common during heavy alcohol ingestion, thus inducing acute gouty arthritis in patients with known gout. Hypoglycaemia and hyperlipidaemia occur in patients who are not eating adequately or who are ingesting a high-fat diet with ethanol, respectively. Thrombocytopenia is caused by alcoholism in patients with advanced alcoholic liver disease (IARC, 1988).

The use of drugs increases with aging. Acceleration or inhibition of drug metabolism by ethanol depends on the duration of
ethanol ingestion and the presence or absence of ethanol in the body at the time the drug is ingested (Mezey, 1981). The presence of alcohol in the body causes a decrease in the metabolism of certain drugs, such as antipyrine, meprobamate, pentobarbital and benzodiazepines, resulting in increased bioavailability of the drugs to the CNS and thereby contributing to unwanted side-effects. In contrast, ethanol can increase the metabolism and metabolic activation of certain xenobiotics, such as the known human leukaemogen benzene, thus potentially leading to enhanced toxicity (IARC, 1988).

Chronic ethanol ingestion increases tolerance to CNS depressants in young individuals, but its effect in the elderly is unknown. The concomitant administration of ethanol and barbiturates results in an enhanced depressant effect of these drugs on the CNS and can result in coma or even death. All other sedative-hypnotic drugs tested have either synergistic or additive effects with ethanol. Intellectual deterioration and dementia are common complications of chronic alcoholism. Alcoholic patients show more signs of mental aging at every chronological age (Gaitz & Baer, 1971).

Chronic excessive alcohol ingestion is associated with increased mortality from cancer, cirrhosis, non-malignant respiratory diseases such as emphysema, and accidents (Klatsky et al., 1981; IARC, 1988). However, a decrease in coronary artery disease and mortality is associated with moderate alcohol ingestion. This may be due to increases in plasma HDL-cholesterol and decreases in LDL-cholesterol that occur during ingestion of alcohol (Mezey, 1981). Ethanol consumption is also associated with hypertension and an increased mortality from cerebrovascular accidents (Kozararevic et al., 1980; Blackwelder et al., 1980).

3.3.3 Smoking

Smoking clearly plays an etiological role and produces an acceleration of a wide spectrum of age-associated disease. Smoking contributes to an increased mortality rate (Gupta et al., 1980). It also provides an excellent example of problems faced in the consideration of the environmental impact on aging.

Scientists recognize that smoking presents a complex toxic insult through inhalation. Increased pathology in aged mice has been reported after exposure to cigarette smoke (Matulionis, 1984). The immune response in aged mice exposed to cigarette smoke has been shown to be decreased at some ages but not at others (Keast & Ayre, 1981). The role of smoking in coronary heart disease has been reviewed by Kannel (1981), who observed that the relative effect of cigarette smoking decreases in old age and proposed that this could be due to the selection of a more resistant population. Estrogen-related diseases have also been associated with smoking (Baron, 1984). Reif (1981) has examined susceptibility to lung cancer and concluded that the shape of the susceptibility distribution is determined by the effects of all environmental carcinogens (both known and unknown) to which the population has been exposed, as well as by differences in genetic susceptibility among members of the population. Smokers and non-smokers both get lung cancer, but at different age ranges. Some of these studies suggest that aged individuals are more susceptible to the effects of smoking, whereas others suggest that duration of smoking appears to be the critical factor (IARC, 1990). The smoker is exposed to multiple toxic agents simultaneously, the effects of which are more pronounced in aged individuals. Major aspects of metabolism and pharmacokinetics are altered in aged individuals. Therefore, the
effective dose of any chemical reaching the systemic target tissues in aged humans would be dependent on these perturbations.

3.4 Interactions of chemicals and diseases

3.4.1 Cancer

Cancer morbidity is expected to rise with age and with an increasing percentage of elderly people living in industrialized countries (Magnus, 1982). There is no consensus on the causes of the age-related increase in tumour incidence. Various arguments support the concept that an age-related accumulation of total dose of all carcinogens accounts for tumour induction as a function of age in sensitive individuals (Peto et al., 1975, 1985). One viewpoint is that the sensitivity to carcinogens is stable and independent of age, whereas another is that changes in the internal milieu of the organism, such as the metabolic and immunological shifts of natural aging, provide favourable conditions for tumour development with increasing age (Burnet, 1970; Dilman, 1971).

Comparison of human epidemiological data with in vivo and in vitro animal experimental results is difficult but does allow some limited conclusions. It seems that environmental carcinogenic factors as well as endogenous carcinogens are important causes of increased tumour incidence in old people (IARC, 1990). This conclusion is supported by the increasing incidence of occupational cancer with increased exposure time to carcinogenic agents and by the correlation of lung cancer incidence with the number of cigarettes smoked (Doll & Peto, 1981; Peto, 1986). Humans, in general, have an age-related increase in the incidence of epithelial neoplasms (Doll, 1978), but the relationship of age to incidence of other cancers in different organs varies (Doll, 1973; Moolgavkar & Venzon, 1979; Anisimov, 1987; Dix, 1989). Some tumours appear most frequently in childhood, some increase exponentially with age, and others reach a peak at a certain age and then decline.

The data on cancer incidence among the atomic bomb survivors in Hiroshima and Nagasaki, Japan, have been very informative concerning radiation-related solid tumours as well as leukaemia. Age at time of exposure appears to be a strong determinant of leukaemia risk; the greatest absolute risk was experienced by those who were exposed at ages 0-9 or 50 years and over (Beebe, 1979). Most of the excess cancer deaths from solid tumours among the atomic bomb survivors have occurred among those who were over 35 at the time of the blast.

Analysis of data on the positive correlation between the aging rates of different species with their cancer rates and the observation that these two processes, aging and carcinogenesis, may be initiated and promoted by impairments of gene regulation led Cutler & Semsei (1989) to conclude that both cancer and aging may arise from a common set of genetic alterations. The analysis of the interrelationship between aging and carcinogenesis should be based on epidemiologically and experimentally confirmed data. Epidemiological data, analysed in terms of a multi-stage model (Kaldor & Day, 1987), can estimate the importance of age at onset, duration of carcinogen exposure, and latency in a population. On the level of the organism, carcinogenic agents influence not only the cell, causing genomic damage that leads to neoplastic transformation, but also create in the cell a microenvironment that facilitates proliferation and clonal selection (Anisimov, 1987, 1989). Multi-stage carcinogenesis is accompanied by various disturbances in tissue homeostasis and systems of anti-tumour resistance that, in turn, are under the influence of systemic
(nervous, hormonal and metabolic) factors. How long it takes for 
frank neoplasia to develop depends on the state of those systems at 
the moment of exposure to a carcinogen or tumour promoter and the 
dose.

According to the multi-stage model of carcinogenesis, the 
carcinogen whose effect increases in proportion to age at exposure 
affects the partially transformed cell. In this case the tumour 
incidence would increase and latency would decrease, as compared to 
a population exposed to the same effective dose of carcinogen at a 
young age. For example, application of 7,12-
dimethylbenz[a]anthracene in small doses or 
12-0-tetradecanoylphorbol-13-acetate to the skin of mice of 
different ages caused neoplasms more frequently in older animals 
(Stenbäck et al., 1981; Ebbesen, 1985). Exposure of mice and rats of 
various ages to phenobarbital resulted in hepatocarcinogenesis only 
in old animals (Ward, 1983; Ward et al., 1988). The number of events 
necessary for complete malignant transformation in 15-month-old rats 
under the influence of N-nitrosomethylurea is lower than in 
3-month-old rats (Anisimov, 1988). In every tissue, the number of 
events occurring in the stem cell before its complete transformation 
is variable and depends on many factors, in particular the rate of 
aging of the target tissue and of the regulatory system(s) of the 
tissue (Anisimov, 1987, 1989). This model is consistent with the 
analysis of age-related distribution of tumour incidence in 
different sites in humans and experimental animals (Doll, 1978; Dix, 
1989; Anisimov, 1987).

Epidemiological observations have shown that exposure to some 
carcinogenic agents leads to a rise in cancer incidence independent 
of age at the start of exposure (e.g., smoking), while other agents 
induce more tumours when the exposure begins in the elderly (e.g., 
lung cancer following asbestos exposure) (Kaldor & Day, 1987; IARC, 
1990).

3.4.2 Other diseases

Aging affects the functional capacity and structural integrity 
of many organ systems. Environmental chemicals can also affect 
several target organs. The combination of the influence of aging and 
the toxic effects of chemicals in the environment might potentiate 
the risk for elderly persons. An inherent problem common to all 
research in chronic disease is the dissection of the respective 
roles of time per se from those of primary aging. Hazzard (1985) 
stated that an essential feature of human aging is the change in 
physiological competence across the life span, as reflected in 
homeostatic reserve. Homeostatic reserve declines at an accelerating 
rate with age, normally producing death in old age from 
multifunctional etiologies.

The relationship between aging and atherosclerosis is a prime 
example of this conundrum. It seems most likely that the changing 
picture of atherogenesis in western society has led to a large 
number of people who survive into old age with not only a degree of 
clinical atherosclerosis, but also with other chronic progressive 
diseases, such as chronic obstructive pulmonary disease, immobility 
from osteoarthritis and/or osteoporosis, and mental incompetence 
from Alzheimer's disease, multi-infarct dementia or other 
age-related dementing processes.

These diseases could significantly modify the response of the 
organism to various environmental chemicals by decreasing or 
increasing their susceptibility, followed by an acceleration of
these diseases or induction of new ones.

4. APPROACHES TO EXAMINING THE EFFECTS OF CHEMICALS ON THE AGED POPULATION

4.1 Experimental approaches

4.1.1 Principles for testing chemicals in the aged population

There are two principal approaches to the study of age-related changes of any functional, morphological and/or biochemical parameter, i.e. "longitudinal" and "cross-sectional". Longitudinal studies consist of repeated estimations of any parameter in the same animal in different periods of life. Cross-sectional studies involve separate groups of animals of different ages who are examined at a given point in time. Results obtained using the longitudinal approach may significantly differ from the results from experiments carried out using cross-sectional approaches. In studies on the toxicity of chemicals, it is obligatory that identical conditions be provided and maintained for all the animals. Problems related to the choice of animal species, strain, sex, age, life stage and chemical treatment (both route and dose selection) will be considered below.

4.1.2 Animal models

An extensive discussion of the choice and use of animal models in research has been recently published (Rogers et al., 1991).

4.1.2.1 Animal species

The selection of suitable species obviously involves both practical and economic factors. Animals with a short life span are preferred. However, good life-table information is not available for all species. A knowledge of species-related differences in metabolic pathways and inherent sensitivities is also important in choosing animal species for study. The choice of species should depend on the experimental question as well as homology of response. For example, while closely related isoforms of drug-metabolizing enzymes may exist in different species, their tissue distribution and substrate specificity may vary greatly (Nebert et al., 1991). Such differences in isoform expression, together with reported differences in DNA repair efficiency, could be responsible for species-related differences in an organism's susceptibility to chemical carcinogens (Daniel et al., 1983; Mehta et al., 1984; Anisimov, 1987; 1989).

Regardless of such metabolic differences, mice and rats are most often used in aging research because of a short life span, relative ease of maintenance under defined conditions, wide use in biological research, and suitability for a variety of molecular and genetic analyses. There are extensive life-table information for some strains of mice and rats as well as a data base on their spontaneous pathology. If old animals are purchased from a supplier rather than being maintained in an investigator's own laboratory, it is necessary to obtain information on the lifetime environment of the animals, including housing conditions and dietary history. It may be preferable to keep animals from weaning until the desired age for investigation under the same controlled conditions.

Mammalian species other than rodents have only been used sporadically in aging research, owing to their long life span, lack of life-table information, genetic heterogeneity, restricted availability and high cost. However, it is critical that such larger
species be used to answer certain questions. For example, studies are currently underway to determine if dietary restriction can prolong the lifespan of two different monkey species (Ingram et al., 1990).

4.1.2.2 Animal strain

The choice of adequate rodent strain for experiments on aged animals is critical. The male Fischer-344 rats is a popular model in aging studies because of its size and growth characteristics. However, testicular interstitial cell tumours begin to appear at the age of 18 months in rats fed ad libitum, and by the age of 2 years the tumour incidence approaches 100% (Fishbein, 1991). Recently, a highly significant positive trend with time has been observed for the increasing prevalence of leukaemia, anterior pituitary tumours and thyroid c-cell tumours in both sexes, adrenal pheochromocytomas in males, and mammary tumours and endometrial stromal polyps in female F-344 rats (Rao et al., 1990). Some mouse strains suffer from a single major disease process (e.g., tumours of the mammary gland or liver, or chronic nephropathy), and the presence of this disease in most animals could modify the response to chemical exposure and complicate the interpretation of the results.

In the USA and Japan, Fischer-344 and Sprague-Dawley rats and B6C3F1, Swiss, and CD-1 mice are most frequently used, whereas in European countries Wistar-derived and Brown Norway rats and NMRI and Swiss mice are the most popular strains. Inbred animals have the advantages of greater stability and predictability of response. However, heterogeneity of outbred animals more closely resembles the heterogeneity of human populations. The choice of a certain animal strain also depends on previous experience with these animals, the final choice of an appropriate species and/or strain being dependant on the scientific hypothesis under investigation. Each strain has its own pattern of background pathology, which can be greatly influenced by animal husbandry.

4.1.2.3 Animal sex

Sex-differences in response to chemicals are well known. Thus, the use of both sexes is often necessary in toxicity testing. However, the large gender differences in chemical toxicity and pharmacokinetics that occur in rats become less apparent in old age due to the decreased expression of sex-specific isoforms of the hepatic drug-metabolizing enzymes (Kitani, 1991).

Ovarian status may significantly influence the sensitivity to some chemical agents, modifying the biological response, as been demonstrated for chemical carcinogens (Anisimov, 1971, 1987). It is noteworthy that, when using females of the post-reproductive period, the investigator must be aware that a) the ovaries in females of some species (i.e. rats and mice) are not atrophied and may continue to secrete steroid hormones, and b) some animals may be in persistent estrus, while others may be in anestrus or pseudopregnancy status (Aschheim, 1976). Furthermore, an animal's reproductive history could influence its response to a chemical in later life.

4.1.2.4 Selection of age groups for comparison

The problem of appropriately characterizing the animal's age within the context of its life span is of particular importance in experimental gerontology. Since the aging process causes significant changes in various systems of the organism, there is a need for the definition of some reference points for adequate comparison of the
results of different experiments. The importance of these points is particularly significant in cross-sectional studies. One of the confounding factors in such comparisons between experiments is the need for frequent comparisons of results from different animal species with different life spans (long-lived and short-lived species and/or strains). Many authors have used two kinds of age groups: "mature" or "adult" animals; and "old" animals. However, the true age of "mature" rats in the reports from different investigators fluctuates from 2 to 14 months and the age of "old" animals from 12 to 37 months. The life cycle of experimental animals can be divided into four periods: a) prior to weaning (developmental); b) sexual maturation (maturational); c) reproductive; d) pronounced age-related changes (senescent period) (Zapadnyuk, 1971). All studies should compare animals from multiple age periods.

4.1.2.5 Underlying pathology of animals of different ages

As mentioned previously, the background pattern of pathology must be taken into account in the selection of animal species and strains. A species-, strain- and sex-specific incidence of pathology, whether neoplastic or not, is observed to increase rapidly in the second half of the life span, even for those animals maintained under specific pathogen-free conditions (Burek, 1978; Anismanov, 1987; Frith & Ward, 1988; Fishbein, 1991). Among non-neoplastic diseases in rats, the most frequent are chronic glomerulonephropathy, cardiomyopathy, amyloidosis, and peripheral nerve degeneration. For example, the incidence of spontaneous leukaemia in F-344 rats frequently used in long-term studies may exceed 30%, and the frequency of interstitial cell tumours of the testis may reach 100% in old males. This pathology may significantly modulate the host response to xenobiotics.

4.1.2.6 Transgenic animals

During the last decade it has become possible to add new genetic information to the germ line of experimental animals (Jaenisch, 1988). More recently successful attempts have even been made to specifically alter gene sequences in the mouse germ line, thereby abating or correcting specific gene functions (Capecchi, 1989). In the study of the effects of chemicals on the aged population, transgenic animal models have at least two contributions to make. Firstly, the influence of specific genes on the age-related susceptibility to environmental chemicals can be assessed in the in vivo situation (Vijg & Papaconstantinou, 1990). Secondly, by using transgenic animals harbouring a shuttle vector with one or more mutational target genes, mutagenic effects can be studied in different organs and tissues of animals as a function of age (Gossen et al., 1989).

4.1.2.7 Animal husbandry

In experimental research on the sensitivity of aged animals and the aging process, the husbandry aspects should be defined. The quantitative and qualitative results might depend greatly on the dietary conditions. The dietary composition should meet the minimal requirements for nutrients, minerals, vitamins and (raw) fibre for adult animals according to established and published standards for laboratory animal diets. Another important dietary factor is the quantity to which the test animals have access. They may either receive a restricted diet containing adequate level of nutrients or be fed ad libitum. The choice of diet depends on the questions to be asked.
In addition to the dietary status of the animals, their microbiological status should be defined. In some cases interaction of the chemical with the gut microflora might modify the outcome of the study. Specific-pathogen-free (SPF) animals are preferred. The direct environment of the test animal, including relative humidity, temperature, light and dark cycles, seasonal influence and stress can all influence the final outcome of a study and therefore should be defined carefully (Masoro, 1991).

Differences in animal husbandry may cause large variations in biochemical and pathological effects. This is clearly illustrated by alterations in the background data among different laboratories.

4.1.3 Chemical exposure

4.1.3.1 Dose level

The selection of dose is a difficult issue. The usual requirement is to have a minimum number of test groups (3) plus one control (vehicle) group. This permits the development of a "dose-response" curve and allows for appropriate statistical evaluation of the results. The highest dosage should induce minimal signs of toxicity, bearing in mind that the maximum tolerated dose for young animals could in some cases be toxic for old ones, and that high doses may alter the toxicokinetics of the chemical.

Considering that the body weight of young and old animals could be significantly different, a correct dosage calculation of test substances in comparison groups is an important problem, i.e. whether it is better to normalize per unit of body weight or body surface, or to some other parameter such as lean body mass (Travis et al., 1990). The available data indicate similarity of the results when calculation of dose is performed per unit of body weight or body surface. It should be taken into consideration that the growth and development of various organs may have different rates, and that relative organ weight (and consequently, the effective target dose of the substance) may not be the same in animals of different ages. When the substance is administered in food or water, the consumed quantities should be taken into account, because they may differ in animals of various ages. When the oral or dermal route of administration is used, age-related changes in the extent and rate of absorption may be important. Age-related changes in lung ventilation capacity may also alter the internal dose of a chemical when the inhalation route of administration is used. When looking for portal-of-entry effects, a constant concentration of the agent may be used in all age groups.

4.1.3.2 Route of administration

There is general agreement that the test substance should be administered by the route that corresponds most closely to human exposure. For humans, the main exposure routes are oral, dermal and inhalation, while in animal experiments the oral route is most frequently used. However, when pharmacokinetic studies show that other routes of administration result in equivalent target tissue levels, such alternative routes can be used. The use of injections (subcutaneous, intraperitoneal, intramuscular or intravenous) may be expedient under certain circumstances.

4.1.3.3 Duration of exposure

The question being addressed should guide the design of the study. If the potential risk involves acute exposure of the elderly,
then an acute experimental scenario is required. If human exposure is ongoing, long-term studies may be necessary. Exposure to the test substance in chronic studies should start shortly after weaning and continue for the major portion of the animal's life (at least through the mean life span). IARC (1986) recommended 24 months of exposure for rats and mice, and 18 to 20 months for hamsters when studying the carcinogenic potential of chemicals. Choice of exposure duration based on life-table characteristics would be optimal. However, these guidelines do not apply to experiments when animals of different ages are used at the start of the study. In this case the dose and duration of exposure could differ in groups of young and old animals. However, it is preferable to use identical exposure durations whenever possible.

4.1.4 Non-mammalian models

Many species can be used as models in the study of age-related sensitivity to environmental toxicants. These include fungi (Neurospora crassa and Podospora anserina), protozoa (Paramecium tetraurelia and Tetrahymena pyriformis), rotifers, nematodes (Caenorhabditis elegans, C. briggsae and Turbatrix aceti), and insects (Drosophila melanogaster, Musca domestica and Tribolium confusum) (Committee on Chemical Toxicity and Aging, 1987). Non-mammalian species such as fish and salamanders have been used in aging research (Weindruch & Walford, 1988) and are potentially available for toxicology studies. Several of these models have already been used to examine the effects of chemicals on aging. Because of their short life span, ease of use and relatively low cost, non-mammalian organisms could be important in the initial phases of a test system to identify environmental chemicals that might affect aging.

4.1.5 In vitro studies

For the purposes of screening xenobiotics, the use of in vitro models can result in substantial economy and efficiency. Stock cells and, in some cases, tissue explants can be cryopreserved in large amounts. This permits repeated assays with comparable materials and the sharing of common stocks by numerous laboratories. Moreover, such stocks can be used to investigate cell-to-cell interactions, such as metabolic cooperation and metabolic transformation. Finally, tissue culture approaches can substantially reduce the numbers of animals required for experimentation. Such methods cannot, however, be expected to substitute for the intact animal experiment.

There are three general categories of in vitro methods: organ culture, tissue explants and cell culture. Organ culture involves the short-term maintenance of variable intact segments of tissue, for example, the full thickness of a segment of aorta. In tissue explants, the early migration and proliferation of epithelial and fibroblast cell types can be observed. Cell cultures are of four general types:

(a) primary cell cultures and mass cultures of cells taken directly from the animal, usually after enzymatic dispersion of biopsied tissue;

(b) established, serially passaged cultures with relatively reproducible cycles of growth in early phases, but with limited replicative life span, and with a genetic make-up reflecting that of the donor age;

(c) "transformed" cell cultures with indefinite replicative potential and generally with altered genetic makeup;
established or transformed cell lines into which specific DNA sequences ("transgenes") have been transfected.

Each of these types of models could prove useful for studies of the effects of environmental agents on the elderly. One general approach would be to explore the toxic effect of an agent as a function of donor age, so as to detect unusual susceptibilities of the cells and tissues of aged subjects. Another general approach would be to culture the cells in vitro after in vivo treatments. If a set of behaviours or phenotypes was observed with tissue from young, treated subjects that proved to be comparable to that observed with tissue from old untreated animals, an effect of the in vivo treatments on the aging process could be inferred.

An entirely different experimental paradigm could be based on the hypothesis that established cultures with finite replicative life spans recapitulate the natural history of comparable cell types in vivo - the well-known "in vitro model of cellular aging" first developed by Hayflick & Moorhead (1961). The appropriateness of such models for the study of aging is controversial, since it has been proposed that the attenuation of growth observed in vitro corresponds to terminal differentiation in vivo (Norwood & Smith, 1985; Bayreuther et al., 1988).

Of special interest would be the evaluation of agents that exhibit unusual toxicity to putative stem cells. An excessive depletion of stem cells could seriously compromise the regenerative potential of tissues in aging subjects. In such studies, it would be important to investigate a variety of cell types. Most research to date has concentrated on in vitro aging of cultures of fibroblastoid cells established from the fetal lung or from the dermis of individual subjects of various ages. The precise origin of such cells is not clear. Thus, with such cells it would be difficult to compare age-related changes in vivo with those observed in vitro.

A final experimental paradigm would be to use postreplicative terminally differentiated cells in culture in order to investigate agents for their potential to accelerate age-related alterations observed in vivo. Such cell types might be derived through in vitro terminal differentiation, or from normal or transformed embryonic neuroblasts or myoblasts. A major concern, however, would be the extent to which the experimental milieu reflected in vivo conditions.

4.1.6 Statistical considerations

For statistical treatment of the results of short-term testing, the statistical methods that are generally available may be used. However, for statistical analysis of the results of long-term testing, in particular carcinogenicity testing, the comparison of results from treatment groups with different survival rates is a major issue. In these cases the recommendation of IARC (Peto et al., 1980; Gart et al., 1986) could be applied. All the tumours found at necropsy must be evaluated as "fatal" or "incidental." This approach permits one to conduct a comparison between young and old animals despite the very different patterns of survival from those expected. A crude analysis, ignoring the fact that young animals survive longer than old ones, will overestimate the ratio of tumour incidence in young and old animals. Conversely, a "death-rate" analysis, treating all tumours as if they were fatal, will over-correct for the effects of differences in survival on the
incidence of tumours discovered at the autopsy of animals that died of unrelated causes. Bias can be eliminated only if tumours that were discovered in an incidental context are analysed by the prevalence method, while tumours discovered in a fatal context are analysed by the death-rate method (Peto et al., 1980; Gart et al., 1986).

4.1.7 Extrapolation of animal data to humans

Extrapolation of animal data to humans may be either qualitative or quantitative. On the basis of experimental results, the weight-of-evidence approach can help predict whether a given substance may be considered dangerous for humans. Such information includes data on the pharmacokinetics of a chemical, its cytotoxicity and other toxic properties, as well as the data obtained in in vitro and short-term tests. Of primary importance for quantitative extrapolation, a dose-response relationship must be detected in experimental animals. An important stage of quantitative evaluation is extrapolation of the data obtained from exposure to rather high doses to lower doses to which humans may be exposed in the environment. It is necessary to take into account biological differences, both in pharmacokinetics and pharmacodynamics, between the test species and humans. For example, a dose taken per unit of the body surface area, or its concentration in the daily ration, includes a correction factor for species sensitivity and allows extrapolation of experimental doses to man (Mantel & Schneiderman, 1975; Turusov & Parfenov, 1986; Travis et al., 1990). Of course, species sensitivity to the action of some chemicals may also vary significantly.

Various physiological and mathematical models have been proposed for extrapolation of toxicological effects. Most of these models refer to carcinogenesis (Turusov & Parfenov, 1986; Swenberg et al., 1987; Clayson, 1988). The predictive nature of these models for non-carcinogenic end-points remains to be determined.

4.2 Epidemiological and clinical approaches

4.2.1 Disease pattern of aged population

In order to study the pattern of disease occurring in the elderly, the first question is "What illnesses are most common and important among the elderly?" The answer can be most easily provided in terms of diseases that cause death, hospitalization or visits to a doctor's surgery.

The assessment of health status among the elderly on an international basis is essentially limited to the use of mortality data, since these are the only comprehensive data available. In the developed countries, roughly 50% of all deaths occurring between the ages of 65 and 74 years are attributable to cardiovascular diseases. Among males in this age group, ischaemic heart disease accounts for 25% of deaths, while 11% are due to cerebrovascular diseases. For women, 20% of deaths are attributed to ischaemic heart disease and about 15% to stroke. Cancer accounts for another 25% of deaths among men and women aged 65-74, lung cancer being the cause of 10% of all deaths among elderly males. Roughly 7% of deaths are due to respiratory diseases and 3% to external causes (WHO, 1989).

The trends in the four leading causes of death, i.e. malignant neoplasms, heart diseases, cerebrovascular diseases and respiratory diseases, within the age range 65-74 showed that, in selected developed countries between 1950-1954 and 1980-1984, death rates
from all malignant neoplasms rose slightly for men but remained essentially unchanged for women except in France. A more marked decline in mortality is apparent for heart disease in the USA and Australia where death rates have fallen by 25-30% since the late 1960s. Male mortality has fallen in France and Japan, but has risen in Hungary. A similar pattern of change is also apparent for females. Mortality from stroke has also declined in most countries, although the timing and extent of the duration has varied from country to country. For example, the decline in Japan occurred ten years earlier than that in Australia. In the United Kingdom, France and USA, rates have been falling since the 1950s. The trend in mortality from respiratory diseases is less clear, there being little evidence of sustained and comprehensive decline. However, it is apparent that there has been some progress in reducing mortality from these diseases in Australia and the United Kingdom over the last decade (WHO, 1989).

It must be recognized from the outset that mortality data do not always accurately reflect the underlying morbidity and are particularly inappropriate in the case of many conditions for which the fatality rate is low, yet which are important causes of morbidity among the elderly. Data on causes of death are also less reliable for the elderly than for other age groups owing to the multiple pathological conditions often present at the time of death. Nevertheless, with few exceptions, comprehensive morbidity data are not available for the elderly.

Data from the USA shows that cardiovascular diseases cause the largest proportion of hospitalization in the elderly. As a group, diseases of the digestive system account for the second most common diagnostic category while neoplastic diseases (90% malignant) are the third most common cause of hospitalization. The remaining causes are diseases of the respiratory system, injury or poisoning, and diseases of the genitourinary or musculoskeletal systems (White, 1989). In a study from Shanghai, China, the common diseases of hospitalized patients over 65 years of age in the 1950s, when arranged in order of frequency, were hypertension, coronary artery disease, chronic bronchitis, prostate hypertrophy, femur fracture, pulmonary tuberculosis, diabetes mellitus, cholelithiasis and tumours of various organs. Coronary artery disease, pneumonia, hypertension and chronic bronchitis became the leading causes of hospitalization in the 1970s (Zhu et al., 1982). In the 1980s, data from other parts of China (Jiangxi, Liaoning, Xinjiang) showed that chronic bronchitis or pneumonia was the most frequent cause of hospitalization for elderly patients, followed by hypertension and coronary artery disease (Xu et al., 1986; Shen et al., 1987; Xiong, 1989).

Data from the USA shows that diagnoses arising from visits to the doctors surgery by people aged 75 or older, when arranged in order of frequency, are hypertension (17.6%), chronic ischaemic heart disease (9.5%), diabetes mellitus (6.7%), osteoarthritis (6%), cataracts (5.1%), heart failure (4.4%), cardiac arrhythmia (3.6%), arthropathies (3.6%), glaucoma (2.8%), hypertensive heart disease (2.6%), angina pectoris (2.3%), chronic airway obstruction (2%) and neoplasms (1.4%) (White, 1989).

4.2.2 Assessment of effects of environmental chemicals in the elderly population

There are more limitations in clinical studies than in animal toxicology studies. Firstly, the conditions in the study are not easily controlled. Secondly, presumably harmful effects to the
subjects under study need to be avoided in designing the protocol. The following approach is suggested.

Comprehensive, multidimensional functional assessment of the elderly should be carried out. This involves analysis of the physical, psychological, social, environmental and other aspects of functioning (Zarit et al., 1985; Kane, 1987). Measurements of the ability to perform the activities of daily life should be obtained. Physical functioning can be measured by a combination of diagnosis, symptom description, health reporting, days in bed or hospital during a specific period, and reported pain or discomfort. The subject's orientation with respect to time, place and person, as well as short-term and long-term memory, should be assessed. Psychological measures are used to evaluate depression, anxiety, loneliness and sense of mental well-being, and a psychiatric history should be recorded.

Clinical assessment involves a complete physical examination and selective laboratory or instrumental examinations. As most environmental chemicals affect certain parts or organ systems of the body, laboratory or instrumental examination of these particular parts or organ systems should be performed. The purpose of clinical assessment is to find any structural or functional abnormalities that may occur during the course of exposure to the environmental chemicals.

Measures of environmental chemicals in the human body, such as the determination of their concentration or that of their metabolites in blood, body fluids or tissues (including nails, hairs, etc.), faeces, urine and expired air, should be conducted. Biomarkers of exposure, such as haemoglobin adducts, may be used when available.

The results of the above-mentioned assessments may be correlated and analysed to arrive at a conclusion as to whether the environmental chemicals have harmful effects on the subjects studied.

4.2.3 Acute episodes

There are few epidemiological reports on the effects of exposure to environmental toxicants on the aged population. Several acute air pollution incidents resulted in marked increases in illness and death, mostly among the elderly. One such incident occurred in the Meuse River Valley in Belgium in 1930 when accumulating air contaminants trapped by an inversion caused the death of 63 people and illness in 6000 residents. An incident in 1948 in Donora, Pennsylvania, USA, resulted from a similar inversion that covered a wide area. Of the population of 14,000, 20 died (compared with the expected two deaths for the same period) and 43% fell ill. Again, the elderly were the most seriously affected. In London, 4000 excess deaths were attributed to smoke and sulfur dioxide in the fog of 1952, and an incident in 1962 caused 400 deaths above the normal value for the period. Similar episodes of fog-induced mortality in various places have been described (Amdur, 1986). In all these episodes, the most vulnerable people were the elderly, and the victims were usually suffering from acute bronchitis and pneumonia, resulting in acute respiratory failure. However, detailed and systematic study of the effects of environmental chemicals on the elderly is still lacking (Amdur, 1986).

4.2.4 Concerns for the aged population
The health conditions of the elderly are quite different from those in the early or middle age of human life. Many chronic diseases that begin at an early age extend into advanced age. Some pathological conditions occurring in middle age may become symptomatic in the elderly. Certain medical problems are clearly more prominent among the elderly, e.g., cancer of the prostate, temporal arteritis and osteoarthritis. Illness in older people often involves multiple organ systems that are interrelated by symptoms, physical findings, functional capacity and treatment. The emotional and social consequences of the physical conditions are also related. Furthermore, the manifestations of aging processes that usually comprise degeneration, both structural and functional, may sometimes be difficult to differentiate from those of diseases. Aging processes and existing diseases, as well as social and environmental factors, act together to make the assessment and care of the elderly complex and difficult.

As far as the effects of chemicals upon human health are concerned, the elderly are subjected to long-term exposure to environmental chemicals. Some exposures occur daily. The source may be polluted air, water, food or products made of inorganic or organic chemicals with which people frequently come into contact. Some exposures occur during occupational activities in which people are engaged for many years. The cumulative effect of continuous or repeated exposure to chemicals may result in pathological changes and clinical manifestations found in later stages of life. In addition, the structural and functional changes of aging, usually degenerative in nature, make the elderly more vulnerable to adverse effects of environmental chemicals.

As mentioned above, the multiple disease status in the elderly is usually accompanied by polypharmacy. In developed countries, the consumption of drugs by people over 65 years of age accounts for 25-50% of total drug consumption (Vestal, 1978). The most commonly used are neuropsychiatric and cardiovascular drugs, anti-inflammatory analgesics and diuretics. Since these drugs are used for the relief of symptoms rather than the cure of diseases, repeated or sustained prescribing is the rule. Under such conditions, interactions between environmental chemicals and drugs should be carefully considered.

4.3 Biomarkers of aging

The term "biomarker of aging" arose in conferences sponsored by the Fund for Integrative Biomedical Research (Regelson, 1983) and the National Institute on Aging (Reff & Schneider, 1982). The Committee on Chemical Toxicity and Aging (1987) defined a biomarker of aging as "a biological event or measurement of a biological sample that is considered to be an estimate or prediction of one or more of the aging processes". Baker & Sprott (1988) offered the following specific features for a biomarker of aging: (a) nonlethal; (b) highly reproducible within and across species; (c) reflects physiological age or some basic biological process of aging or metabolism; (d) displays significant alterations during relatively short time periods; (e) is crucial to the effective maintenance of health and prevention of disease; and (f) reflects a measurable parameter that may be predicted at a later age. Development of a panel of biomarkers would assist in assessing the effects of environmental chemicals that modulate aging processes in laboratory animals and man.

There is currently a significant research effort, especially with rodent species, to establish batteries of biological markers of
aging (Allaben et al., 1990). But how can one differentiate alterations that are simple functions of chronological time from those that are the results of intrinsic aging or of intrinsic aging coupled with the effects of chronological time? One approach has been that of comparative gerontology. Starting with a group of taxonomically related species that vary substantially in their maximum life-span potentials, one simply asks if the rate of change of the putative biomarker reflects the maximum life-span potential of the species.

From a practical point of view, various biomarkers may be used for the measurement of aging. For different levels of integration of an organism, different parameters may be used. At the subcellular and cellular levels, measurements of macromolecular lesions, the degree of collagen cross-linking, the level of lipofuscin accumulation, specific enzyme activities, the number of particular hormone receptors, numbers of cell divisions, etc. may be investigated as biomarkers. Tissue and organ weights, cellularity, growth or some functional activity (muscle strength, visual acuity, secretion rate) may be considered. The functional activity of motor, cardiovascular, nervous, endocrine, immune, haematopoietic and other systems and subsystems may be considered at the systemic level as potential biomarkers of aging. In addition, at the level of the whole organism, the functions of the main integrative systems should be addressed.

Test batteries that attempt to measure functional age (Webster & Logie, 1976), physiological age (Hollingsworth et al., 1965) and biological age (Furukawa et al., 1975; Nakamura et al., 1982; Voitenko & Tokar, 1983; Reis & Foethig, 1984; Dubina et al., 1984; Hochschild, 1989) have similar conceptual underpinnings in that they utilize the great variability in performance that emerges among adults of the same chronological age in standardized, age-sensitive tests. Studies using this approach have been conducted on human populations, and similar measures have been employed in a few rodent studies (Hofecker et al., 1980; Dubina et al., 1983). This approach can be viewed as the performance variability model of biological age. It has considerable intuitive appeal and, in many cases, statistical elegance. An individual may be judged biologically younger if he is performing better than expected for his chronological age. However, in their extensive and critical reviews, Costa & McCrae (1980, 1985) revealed many conceptual and methodological flaws in this approach.

Biomarkers of aging, which are markers of susceptibility, may have their greatest utility in the study of the effects of environmental chemicals on the processes of aging. The effects of environmental chemicals on the elderly would be assessed most efficiently by using biomarkers of effect (NAS, 1989).

5. CONCLUSIONS

a) With increasing age, humans become more vulnerable to environmental challenges due to the deterioration of physiological and psychological processes. Therefore, it is likely that the elderly will be more susceptible to the harmful effects of environmental chemicals.

b) The elderly are heterogeneous with respect to the extent of deterioration of physiological and psychological processes.

c) Few, if any, of the hundreds of thousands of environmental chemicals have been tested for increased toxicity in the Aged Population, principles for evaluating the effects of chemicals (EHC 144, 1992)
elderly.

d) Some of the many age-associated diseases may cause an increased susceptibility to the harmful action of specific environmental chemicals.

e) The use of animal models for aging research requires special considerations, such as housing conditions, diets, monitoring for infectious agents, and specifically defined pathologies. It is also necessary to assess several age ranges based on life-table information in order to distinguish the senescent from the mature and developing animal.

f) The selection of the animal model should be based on the likelihood of providing information of relevance to specific problems in humans.

g) The characteristics of the elderly result from intrinsic aging processes, environmental factors, and other life-span events. Therefore, the study of the elderly may not provide information on basic aging processes.

h) Both the size of the aged population and the number of chemicals in the environment will undoubtedly increase over the next few decades. It is therefore expected that the adverse effects of chemical exposure on the elderly will increase in importance as a health care issue.

6. FURTHER RESEARCH

a) Experimental, epidemiological and clinical data should be obtained on:

* the toxicity, mutagenicity and carcinogenicity of environmental chemicals in old individuals as compared to young adults;

* the effect of age on the pharmacokinetics and pharmacodynamics of environmental chemicals;

* the long-term effect of environmental chemicals on molecular, cellular and physiological parameters as potential biomarkers in the elderly.

b) New models should be developed for assessing the susceptibility of the elderly to environmental chemicals as compared to young adults. Such models should be suitable for assessment of particular consequences and relevant to humans, and could include:

* non-mammalian and mammalian models (e.g., Drosophila, fish, rabbits, mini-pigs);

* transgenic animal models;

* cell lines with specific genetic characteristics.

c) For each study the appropriate applicability and use of experimental models and techniques must be validated:

* animal models should be well-defined in terms of survival, pathology and husbandry;

* human subjects should be examined carefully for subtle
disease status, medical history, life-style and socio-economic status.

* standard procedures for measuring molecular, cellular and physiological parameters should be defined (and developed if necessary) in order to prevent misinterpretation.

d) The effects of environmental chemicals on the processes of aging remain to be evaluated. A special scientific workshop should be devoted to this topic.

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APPENDIX 1. Background Papers

Anisimov, V.N., Approaches to examine the effects of chemicals on the aged population: experimental approaches.

Birnbaum, L.S., Basis of altered sensitivity of the elderly to chemicals - pharmacokinetics and pharmacodynamics.

Cooper, R.L., & Goldman, J.M., Alterations in susceptibility to toxic compounds in the aged central nervous system and endocrine system.

Dilman, V.M., Theories and mechanics of aging.

Fabris, N., Systemic biology of aging.

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Li, S., Aged population: demographic, life expectancy, and lifestyle.

Likhachev, A.J., Age related peculiarities of repair of DNA damage with carcinogens.

Martin, G.M., Definitions on aging.

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Speijers, G.J.A., Groups at risk and their chemical exposure as well as nutrition as a source of chemicals and as a confounding factor.

Zhu, J.R., Approaches to examine the effects of chemicals on the aged population: epidemiological and clinical approaches.

See Also:

Toxicological Abbreviations