

HYPOTHALAMUS PITUITARY AND AGING

Edited by

ARTHUR V. EVERITT, Ph.D.

JOHN A. BURGESS, M.B., B.S., F.R.A.C.P.

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Edited by

ARTHUR V. EVERITT, Ph.D.

*University of Sydney
Sydney, Australia*

and

JOHN A. BURGESS, M.B., B.S., F.R.A.C.P.

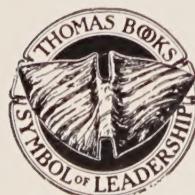
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To Joyce and Margaret
and
to the elderly of all nations

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FOREWORD

THE ANCIENT PROBLEM: what is the primary cause of aging and whether its velocity can be influenced, is unsolved. It is—however—the material of experimental research.

The enormous evolution of our knowledge on the many different influences of endocrine organs on physiological and pathological processes, stimulates study of the relationships between these two functional territories. The central nervous regulation via hypothalamic centers and then via pituitary hormones, leads certainly to a search for more intimate connections.

However, the difficulties in understanding such relationships must be enormous, since we do not understand the metabolic, enzymatic or functional changes of aging—let alone those which are caused by endocrine organs. The way to understanding is still the collection of experimentally proven facts.

It is a brave decision of the editors to stimulate discussion. No doubt they do a service in helping to understand not only single facts, but also the connections between them. Are the general changes caused by aging influenced by hormonal stimuli, are they basically related or are they accidentally interfering with each other?

Stimulation of further research will be one of the consequences of this material.

F. VERZÁR

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A. V. EVERITT

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HYPOTHALAMUS
PITUITARY
AND AGING

CHAPTER I

INTRODUCTION

ARTHUR V. EVERITT and JOHN A. BURGESS

THE SITE OF THE PHYSIOLOGICAL control of aging has been sought in almost every organ of the body. When the functions of the endocrine glands became known late in the 19th century, the early endocrinologists believed that general aging of the body could be secondary to aging of the endocrine system. One of the first workers with this view was Brown-Séquard, who in 1889 injected himself with a suspension of dog testis. He reported an astonishing degree of rejuvenation, but subsequent tests performed on both human and animal subjects failed to confirm his observations.

The pituitary gland is an obvious choice as a regulator of aging, because of its major role in the control of most other endocrine glands as well as regulating the processes of growth, reproduction and metabolism. Simmonds (1914) was probably the first to associate aging with a loss of anterior pituitary function. Similar and related observations were made by other workers (Aschheim and Zondek, 1927; Pribram 1927; Lucien, 1929; Aschoff, 1937). There have developed two schools of thought, one that aging is accelerated by pituitary deficiency as above, and the second that aging is hastened by pituitary hyperfunction. Most of the recent data support the latter view. This does not mean that physiological aging is due to pituitary disease, but rather its rate of development may be regulated by a normally functioning pituitary (Everitt, 1966).

Since the pituitary gland is controlled by centers in the brain, especially the hypothalamus, the central control of aging may reside in the hypothalamus as suggested by Groen (1959), Dilman (1971) and Frolkis, *et al.* (1972). The strong association between brain weight and longevity in animals (Sacher, 1965) may lend support to this hypothesis.

The chapters which follow describe the aging process in general, as well as aging in the hypothalamic-hypophyseal-peripheral endocrine system, and review the effects of hypothalamic, pituitary and other endocrine factors in aging. In particular, fresh thoughts on hypothalamic-hypophyseal relationships in aging have been contributed by several international authors.

The editors have consciously encouraged a diversity of approach and of subject matter within the framework of this aspect of aging research in

the hope that this may not only add to the presently limited background information on this subject, but that also the different styles of approach may stimulate increasing search and research into the multi-faceted and intriguing development of aging in human and animal alike.

The breadth of approach and the enthusiasm of the contributors in examining the subject of aging in man and animal augurs well for the rapid development of understanding in this previously neglected field. It is the hope of the editors that bringing together such a breadth of contributions from the most clinical to the molecular level may spark some of the necessary work needed in understanding the problem of aging.

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CHAPTER 2

THE NATURE AND MEASUREMENT OF AGING

ARTHUR V. EVERITT

SUMMARY

AGING MAY BE DEFINED as a progressive deterioration of the organism after maturation, which increases the probability of death. Aging processes are universal, intrinsic, progressive and deleterious changes with time. With increasing age there is an anatomical involution, a physiological decline, a rising incidence of pathological lesions and a decreasing resistance to death.

The cause of aging is unknown. It is believed that there are multiple causes. It is likely that the primary control of aging is built into the DNA of genes, whose expression is programmed as a function of time. The rate of aging and the maximal duration of life appear to be determined by the interaction between the genetic program in the cell and environmental factors, such as radiation, temperature, nutrition, stress, etc. The interaction between the genetic program and the environment may be mediated through the hypothalamic-pituitary-peripheral endocrine system.

The measurement of biological age is difficult. There are two ways of measuring the aging process, one is to study mortality in a population and the second is to measure the impairment of particular physiological functions in the individual with advancing age. A number of investigators have produced batteries of physiological parameters to measure aging of the whole body.

INTRODUCTION

There are many different ways of looking at the aging process. The view depends on the method of observation. For example, in the aging human kidney, the anatomist usually sees a decline in weight (Roessle and Roulet, 1932), the physiologist records a fall in glomerular filtration rate (Davies and Shock, 1950), the histologist notices a decrease in the number of glomeruli (Moore, 1931), the biochemist observes an increased activity of peptidase (Abderhalden, 1942-1944), and the pathologist may report a rise in the incidence of nephrosclerosis (Oliver, 1952). Each observer is looking at only one facet of the aging process. It is essential to see the whole picture, or else false conclusions may be drawn. It is also necessary to con-

sider the influence on the kidney of aging processes in other organs, such as the cardiovascular system.

In most organs, but not all, the most striking age changes are physiological involution and pathological development.

DEFINITION OF AGING

One of the greatest barriers to progress in gerontology is the lack of agreement on how to define aging. Marott Sinex (1966) says "definitions have often buried within them a prejudgment of the fundamental nature of the aging process."

Grmek (1958) refers to two breadths of meaning:

1. A *broad meaning*, in which aging is regarded as a progressive, irreversible change of the organism, from conception until death. This includes both the developmental and involutorial phases of the life cycle.
2. A *narrow meaning*, in which aging is described as the involution of the organism as a function of time.

Most gerontologists favor the narrow view of aging as an involutorial process. Nevertheless, there is little doubt that the involutorial phase may be influenced by the rate of development as suggested by the experiments of McCay and co-workers (1935). There are number of processes, such as basal metabolic rate and growth rate, which decline progressively throughout the life cycle. Maynard Smith (1966) pointed out that a developmental process leads to an adult adapted for survival, whereas an aging process leads to loss of adaptation and to death. The term "senescence" refers only to the involutorial phase.

Aging is often regarded as the *progressive deterioration of the organism after maturation*. This deterioration involves a decline in vigor (Comfort, 1959), a loss of abilities (Simms, 1950), a decrease in adaptation (Strehler, 1959), an involution (Warthin, 1929), mental, physical and functional deterioration (Bennett, 1968), a decline in the production of free energy (Calloway, 1964), a loss of information (Comfort, 1969a), an increased susceptibility to disease (Curtis, 1963) and many other deleterious changes.

The *increase in mortality rate* is accepted as an essential feature of aging by most authors (Simms, 1950; Medawar, 1952; Strehler, 1962; Curtis, 1963; Comfort, 1964; Maynard Smith, 1966; Shock, 1968). Death is the end result of aging. However, death is invariably from disease and not old age (Simms, 1946; Cameron, 1955). Aging probably renders the tissues more susceptible to the diseases which terminate life (Curtis, 1963).

It is generally assumed that a long life is associated with a slow rate of aging. This has been demonstrated only in the case of food-restricted rats,

whose life duration is increased (McCay, *et al.*, 1935) and whose collagen fibers in tail tendon age at a slower rate (Chvapil and Hruza, 1959; Giles and Everitt, 1967). However, there have been a number of reports of a dissociation between the life duration and the rate of collagen aging. For example, life duration is shortened but collagen aging is not accelerated in the X-irradiated rat and mouse (Verzár, 1959; Darden and Upton, 1964) the hypophysectomized rat (Everitt and Cavanagh, 1965; Verzár and Spichtin, 1966) and the rat with chronic lung disease (Everitt, 1969).

There is no doubt that the frequency of pathological lesions increases with age, but the relationship between pathology and aging remains unsettled. Terminal pathology is usually associated with old age, but may develop independently of the aging process.

DEFINITION OF AGING. Aging or senescence is defined as a progressive deterioration of the organism after maturation, which increases the probability of death.

CRITERIA OF AGING

Strehler (1959, 1962) defined aging as a process which is universal, intrinsic, progressive and deleterious.

1. *Universality.* The change occurs in all older members of the species. This eliminates most diseases of old age, such as arteriosclerosis and cancer, which are not present in all individuals.
2. *Intrinsicality.* Aging is a built-in process, which takes place, even when all environmental influences are eliminated.
3. *Progressiveness.* The onset of the process is gradual and the change is accumulative.
4. *Deleteriousness.* The change must shorten life.

SURVEY OF AGE CHANGES

The aging process may be studied by either the cross sectional or the longitudinal method. In the cross-sectional method a parameter, such as basal metabolic rate, is measured at the one time in groups of subjects of different ages. For example, 30 and 75 year olds may be compared. In the longitudinal method, serial measurements are made in the same individual over the whole or portion of the life span. The cross-sectional method is criticized (Shock, 1967) because the age differences observed may be due to other variables, such as life experience, work history, disease incidence or selection for longevity, rather than age.

Age changes are most apparent in those organs or tissues that do not renew their cells (Verzár, 1964), as for example in the brain, where the neurone count falls throughout life (Brody, 1955). However for most organs there is a growth phase which is succeeded by the decline phase referred

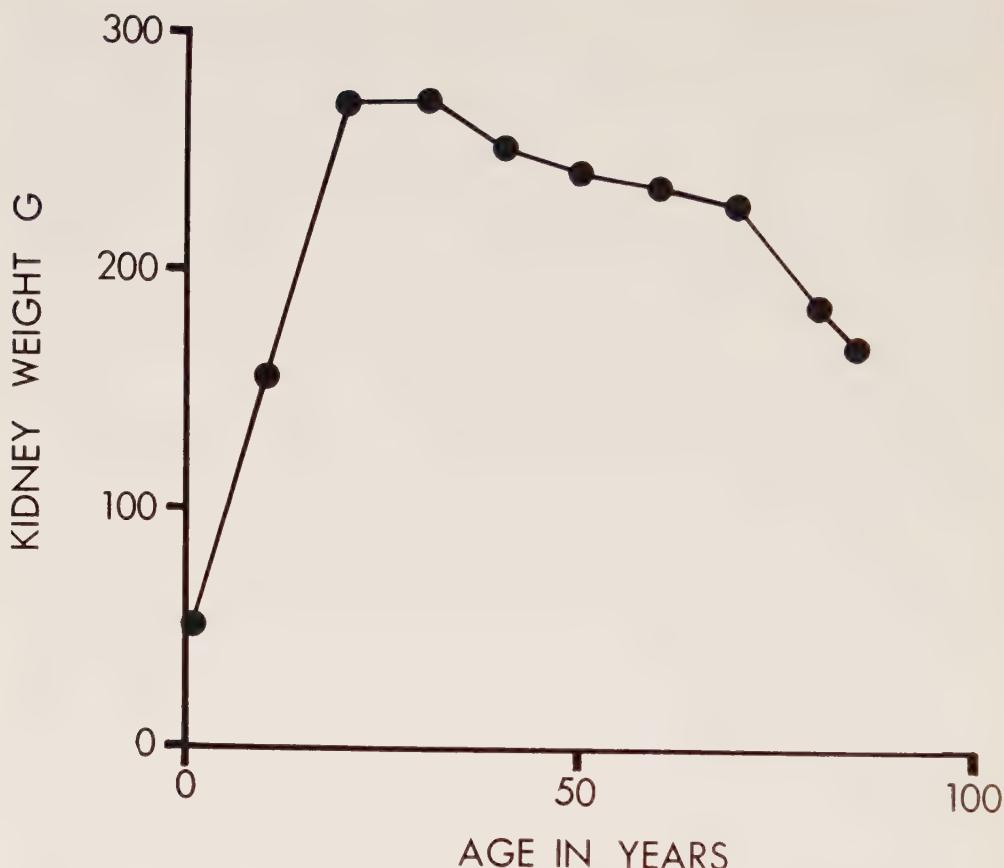


Figure 2-1. The growth and decline of kidney weight with age in man (from Roessle and Roulet, 1932).

to as aging. This is seen typically in the growth and decline of the kidney (Fig. 2-1).

Anatomical Involution

Examination of human autopsy material reveals a decrease in the weight of most organs (thyroid, adrenal, testes, ovary, uterus, liver, kidney, pancreas, brain, skeletal muscle) during the second half of the life cycle (Roessle and Roulet, 1932; Oliver, 1949; Korenchevsky, 1961). The variation with age in the weight of the kidney is shown in Figure 2-1. The decrease in organ weight in old age is apparently due to the loss of cells and functional units like the nephrons in the kidneys. A decrease in the mitotic rate of reverting post-mitotic cells with age (Korenchevsky, *et al.*, 1953) contributes to the decreased cell counts in many aged tissues.

Physiological Decline

Accompanying the loss of cells and functional units in organs, is a decline in the level of organ function. The studies of Shock and his colleagues (Shock, 1962 and 1968) demonstrated a progressive fall in a large number of physiological parameters between the ages of 30 and 70 years in man (Fig. 2-2).

The percentage decline in resting cardiac output was 30 percent, renal plasma flow 50 percent, glomerular filtration rate 31 percent, vital capacity 44 percent, maximum rate of muscular work 30 percent, and basal metabolic rate 16 percent.

There are, however, no decrements with age in those physiological characteristics of blood which determine the environment in which body cells exist (Shock, 1960). When measured under resting conditions, there is no appreciable age change in blood volume, pH, sugar level, osmotic pressure or electrolyte content.

It is clear that aging does not affect all physiological systems to the same extent.

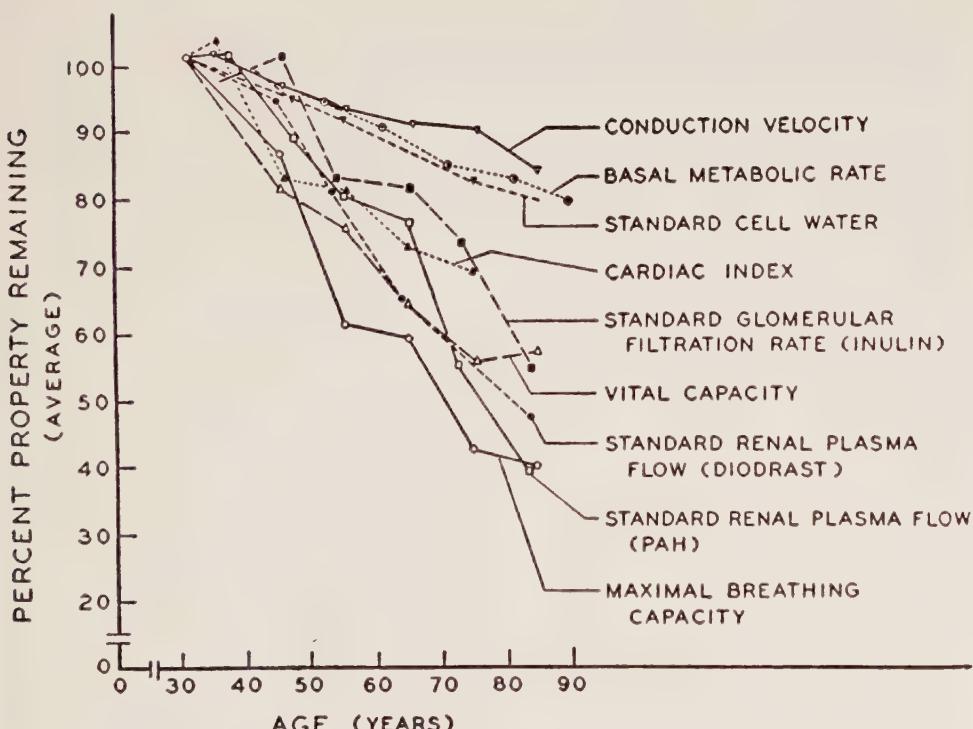


Figure 2-2. The decline in human functional capacities with increasing age (from Strehler, 1959).

Biochemical Changes

The anatomical and physiological decline in old age can be related to the loss of cells from organs. It is likely that senescent changes in individual cells may also contribute to the functional decline. However it has been very difficult to demonstrate biochemical age changes within the cell.

Analyses of the gross chemical composition of human tissues show decreases with age in total body water, lean body mass, total cell solids and total exchangeable potassium which is the principal cation of cells (Bakerman, 1969). However such gross studies are unable to show whether the chemical changes are due to cell loss or to aging of the cell.

The basal metabolic rate (as measured by oxygen consumption or heat production per square meter of body surface per hour) of the whole body diminishes gradually throughout life in man (Shock, 1955; Korenchevsky, 1961). However, when oxygen consumption is reported in terms of intracellular fluid volume, there is no decrement with age (Shock, *et al.*, 1963). As intracellular water is a measure of the protoplasm in cells, the decline in metabolic rate is taken to indicate a loss of protoplasm with increasing age. Once again this age change is apparently associated with cell loss, although there could be loss of protoplasm before the cell dies.

The oxygen uptake of most mammalian tissue slices (based on wet weight, dry weight or nitrogen content) decreases with age, according to most investigators (Barrows, 1956; Korenchevsky, 1961). However, most of the decrement occurs during the growth period. In adult rats between the ages of 12 to 14 months and 24 to 27 months a decrease was observed in the endogenous oxygen consumption of kidney but not liver (Barrows, *et al.*, 1958). The age decrement in kidney metabolism appeared to be the consequence of a reduced number of cells or quantity of protoplasm per unit wet weight (Barrows, 1960).

Studies of enzyme activity per cell in rat tissues have generally not succeeded in demonstrating age differences. Barrows and Reeder (1961) failed to show any impairment in the synthesis of intracellular enzymes in old rats. Adelman (1971) however maintains that the induction of intracellular enzymes is age-dependent.

Age differences in the synthesis of RNA and protein in a number of rat tissues have been studied by Kanungo, *et al.* (1970) and indicate that the proportion of effective mRNA decreases in old age.

Although biochemists have not clearly demonstrated impairment of cellular function in old age (Kohn, 1971), there is no doubt that cellular changes can be seen with the light and the electron microscope. For example, lipofuscin pigments accumulate in cells as they age. Some of these changes are physiological and others pathological.

Increase in Pathological Lesions

The frequency of pathological lesions in various tissues rises progressively with age in man (Howell and Piggott, 1951; Cameron, 1955; Kohn, 1963; Howell, 1968) in the rat (Simms and Berg, 1957; Berg, 1967), in the mouse (Tucker and Baker, 1967) and in other vertebrates. The rise with age in the frequency of lesions in the rat may be seen in Figure 2-3.

In old age there are usually a number of morbid processes present in the body capable of terminating life (Howell and Pigget, 1953; Zeman, 1962; Kohn, 1963).

Rise in Mortality Rate

As the body ages, the probability of dying increases. The force of mortality rises with age and so the expectation of life falls. The mortality curve for a human population is seen in Figure 2-4. There are comparatively few deaths during the first half of life, but from middle age onwards there is a steady rise in mortality. This subject has been treated in detail by Comfort (1964).

THEORIES OF AGING

There are many different theories of aging. This is caused largely by the difficulty of testing these theories, very few of which can be evaluated scientifically. The laboratory testing of a theory of aging may take months or years, according to the rate of aging in the experimental animal being used. In man such a test could take a century to perform.

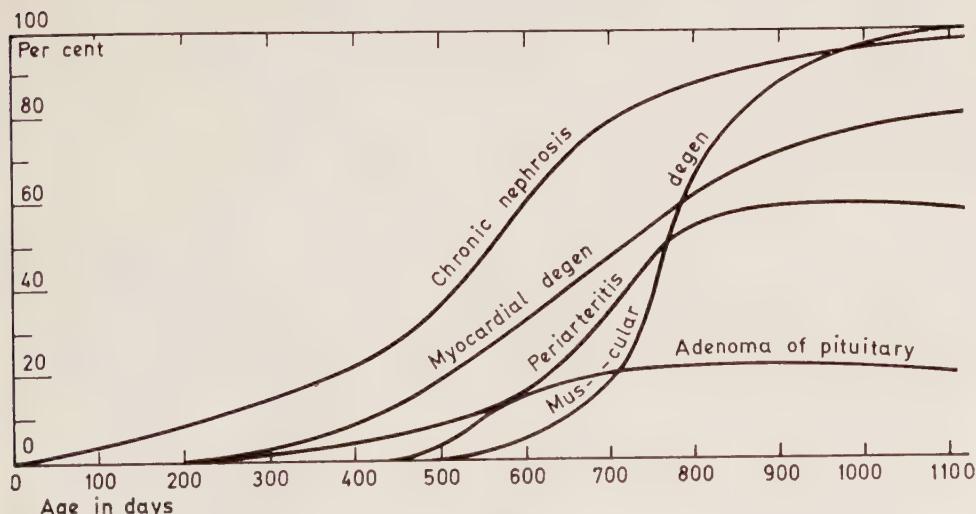


Figure 2-3. The percentage of rats in each age group having lesions of 5 major diseases (from Simms and Berg, 1957, reproduced by permission of *Journal of Gerontology*).

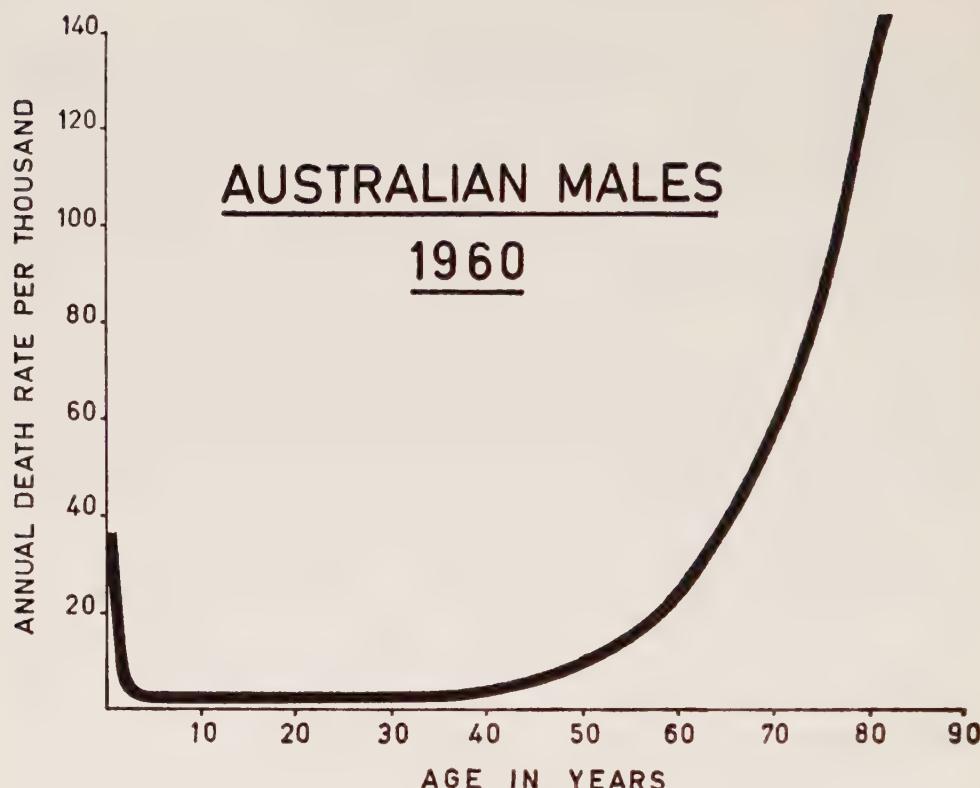


Figure 2-4. Age specific death rates of Australian males in 1960 (from Roche, 1964).

Some of the more important theories of aging have been discussed by Carpenter and Loyn (1968), Bjorksten (1969), Bender, *et al.* (1970), Comfort (1970), Kanungo (1970), Goldstein (1971), von Hahn (1973), Burnet (1974), Hayflick (1974) and Hershey (1974) in his monograph *Lifespan and Factors Affecting It*, Springfield, Thomas.

Genetic Control

It is generally agreed that aging and longevity are genetically determined. Each animal species has a characteristic life span (Comfort, 1964). In man there is a positive correlation between the life duration of parents and their offspring (Pearl and Pearl, 1934). Monozygotic twins usually have almost identical life durations, whilst dizygotic twins exhibit a greater difference in longevity.

Evolution of Senescence

Weismann (1891) regarded aging or senescence as a product of evolution. He believed that protozoa are immortal and that senescence and death first appeared in the metazoa. However, Jennings (1945) showed that nat-

ural death does occur in protozoa. The evolutionary theories of aging have been discussed by Edney and Gill (1968) and Guthrie (1969).

Other workers maintain that senescence is subject only to indirect natural selection. Senescence is thought to be bound to characters such as body size (Bidder, 1932) or brain size (Sacher, 1959), which are subject to natural selection. Williams (1957) contended that senescence evolved because of selection acting on youthful vigor, which is negatively correlated with senescence.

A third group of authors consider that senescence is not a direct product of natural selection. In their view evolution selects features only during the reproductive phase of life (Medawar, 1952) and the adaptation so achieved is of value only in infancy and early adult life (Strehler, 1960). Natural selection is probably not able to remove harmful late acting genes from the population (Haldane, 1941; Medawar, 1952). Senescence may thus be due to the accumulation of harmful mutations.

Defects in Genetic Design

Strehler (1960) has described four deficiencies in the design characteristics of the genetic constitution, which could result in senescence. These are:

1. *Inadequacy of design.* The genes do not provide sufficient defense against the forces which cause senescence.
2. *Errors of design.* Senescence-producing genes have appeared through mutation and have not been eliminated.
3. *Omissions in design.* Selection pressure may have been too weak to evolve genes for defense against deterioration in old age.
4. *Contradictions in design.* A gene may have a beneficial effect early in life, but be harmful late in life.

Classification of Theories

Theories of the basic mechanisms of aging are of two types: "control" and "random" theories (Lints, 1971). In the "control" theories aging is under the control of both the genome and the environment. Examples of "control" theories are the genetic-program theory and the rate-of-living theory. The "random" theories hold that random cell damage is responsible for the events characteristic of aging and death. The somatic mutation theory is an example of a random-error theory.

Program Theory

Aging is regarded as a genetically programmed phenomenon just like development. Comfort (1964) said "the organism must pass through a fixed sequence of operations, metabolic or developmental, the rate of its passage

determining the life span." There is postulated a timing mechanism which normally switches genes on or off at fixed times, thus, throughout life there is a continuous flow of information from the DNA in genes through messenger RNA to the site of protein synthesis (Barrows, 1966). As a result of playing out the genetic program, senile changes appear and lead to death. The observation that fibroblasts senesce and die after a limited number of divisions in cell culture (Hayflick, 1966) has been used as evidence for the program theory. Some workers are now of the opinion that senescence is not the result of programmed death, but the lack of a genetic program to maintain the life of the organism (Comfort, 1970).

Somatic Mutation Theory

Several authors suggested independently that aging may result from random mutations in the DNA of somatic cells (Danielli, 1956; Curtis and Gebhard, 1958; Szilard, 1959; Faila, 1960). The mutations are cumulative and introduce errors into the coded information needed for protein synthesis.

Evidence supporting this hypothesis is of several types. Curtis, *et al.* (1966) have shown that the incidence of atypical chromosomes increases with age in rat liver. Species with high mutation rates have short life spans (Curtis, 1966). Exposure to ionizing radiation increases the number of somatic mutations in rats and mice, accelerates the onset of the diseases of old age and shortens their life (Curtis, 1966).

Burnet (1974) believes that somatic mutation is an intrinsic process in which errors are produced by the enzymatic machinery responsible for DNA replication and repair.

Error Theories

Molecular errors can occur at any stage in the transmission of information from DNA to the protein being synthesized. According to Medvedev (1967) aging is due to the accumulation of these errors. Orgel (1963) pointed out that an error in the amino acid synthetases could lead to a cytoplasmic "error catastrophe" in protein synthesis. Support for Orgel's hypothesis comes from an experiment of Holliday (1969) on the fungus *Podospora*. He showed that the incorporation of certain amino acid analogues shortened the lifespan of *Podospora*. However, it is now accepted that the cell can normally repair errors.

Immunological Theory

Walford (1962) suggested that mutated cells stimulate immunological reactions, that would eventually destroy the individual's own tissues. Both the incidence of autoimmune disease and the titer of autoantibodies in-

crease progressively with age up to 80 years in man (Jacobs, *et al.*, 1969; Whittingham, 1970). Walford (1966) tested his theory by giving mice Imuran, an immunosuppressive agent. The treated mice lived only 10 percent longer. This suggests that if the immunological theory is valid it is concerned only with part of the aging problem. There is now evidence that immunosuppression is not desirable because it increases the incidence of tumors (Comfort, 1970; Burnet, 1973).

Burnet (1970) emphasized the importance of the immunological surveillance of tissues as a means of eliminating defective cells. As the immune system is depressed with age, faulty cells accumulate thereby increasing the numbers of tumors and nonfunctional cells. Aging may be related to weakness of the thymus-dependent immune system. Full development of the thymus-immune system depends on pituitary and thyroid hormones (Fabris, *et al.*, 1972).

Cross-Linkage Theory

Mutations and other errors in cells may be due to cross-linkage between macromolecules. Bjorksten (1941, 1968) maintains that aging is caused by cross-linkage of proteins and DNA. Bjorksten (1969) has listed a number of cross-linking agents found in the human organism. Some examples are acetaldehyde, glyceraldehyde, succinic acid, orthoquinones (from adrenaline), copper, calcium and free radicals. Milch (1965) showed that many of the aldehydes formed during intermediary metabolism are able to increase the cross-linkage of collagen. DNA becomes covalently bound to histones in old age (von Hahn and Verzár, 1963).

There is considerable evidence to support the cross-linkage theory. The cross-linkage of collagen molecules satisfactorily explains the increased structural stability of collagen fibers with advancing age (Verzár, 1957). Increasing cross-linkage of the connective tissue network between a capillary and a tissue cell would impede the transport of nutrients and waste products (Lansing, 1952; Sobel and Marmorston, 1958). This could explain the decline with age in the efficiency of most physiological processes (Kohn, 1972). Probably the best evidence for the cross-linkage theory is that food restriction reduces the cross-linkage of collagen in rat tail tendon (Chvapil and Hruza, 1959; Giles and Everitt, 1967) and at the same time increases life duration (McCay, *et al.*, 1935). However inhibition of collagen cross-linkage with the lathyrogen β -amino propionitrile has not consistently increased the life duration of rats (Kohn and Leash, 1967; La Bella, 1968). Also increasing the cross-linkage of collagen with gold salts failed to increase the mortality of rats (Deyl, 1968). Thus the evidence supporting the cross-linkage theory is strong but not conclusive.

Free Radical Theory

Harman (1956) proposed that aging may be due in part to free radical reactions. When Harman (1968) fed butylated hydroxytoluene to mice to remove free radicals the animals lived significantly longer. The fact that radiation which shortens life also causes the formation of free radicals, lends support to this theory. Free radicals may act by damaging mitochondria, the site of biological oxidation (Harman, 1972).

Control Theories

Lints (1971) drew attention to the control theories of aging. These are theories which are concerned with the control of error accumulation, mutation, cross-linkage and other basic cellular age changes. Some of these theories explain how genetic expression may be controlled by environmental factors, both internal and external.

Genetic Theory

According to this theory there are specific genes which control the aging program. The factors which control the expression of the aging genes have yet to be identified (Strehler, 1972). These may be hormones (Adelman, *et al.*, 1972), metabolites or some other factors.

Rate of Living Theory

Pearl (1928) concluded from his studies on *Drosophila* that life duration is inversely proportional to the rate of energy expenditure or the metabolic rate. Strong support for this theory comes from experiments in which the energy intake in food is decreased (by food restriction) or increased (by exposure of mammals to low temperature). McCay, *et al.* (1935) and other workers (see Berg in Chap. 3) have shown that calorically restricted rats live longer than fully fed rats. When the energy intake of rats is raised by lifelong exposure to low temperature the life span is significantly shortened (Johnson, *et al.*, 1963). It follows from this theory that the more work the body does, as measured by energy turnover, the faster it wears out. Senescence and cell damage are thus seen as by-products of metabolism.

Hypothalamic-Pituitary Hormone Theory

Everitt (1973) suggests that environmental factors interact with aging genes through the mediation of hormones. The neuro-endocrine system (hypothalamus-pituitary-peripheral endocrines) is of prime importance in controlling the influence of the environment on body function. Consider the example of rats living in a cold environment. In these animals the low

temperature stimulates the hypothalamic-pituitary-thyroid axis leading to an increased secretion of thyroxine, the major metabolic stimulant. The resultant increase in energy turnover accelerates aging and shortens the life of the rat (Johnson, *et al.*, 1963). Whether thyroxine acts directly on the aging genes or indirectly through some metabolite cannot be determined at this stage. In a similar manner overstimulation of the hypothalamic-pituitary-adrenocortical axis and/or hypothalamic-pituitary-ovarian axis by exposure to stress (Selye and Tuchweber, Chap. 29; Christian, Chap. 16) or by repeated breeding (Wexler, Chap. 17; Árvay, Chap. 18), likewise accelerate the aging process.

Hypothalamic Elevation Theory

According to Dilman (1971) intrinsic aging of the hypothalamus is the cause of aging in other organs and systems of the body. It has been shown by Dilman (Chap. 32) for human subjects that with increasing age the hypothalamus becomes less sensitive to feedback suppression by glucose, estrogens and adrenocortical steroids. Dilman maintains that the elevation of the hypothalamic threshold and the resultant imbalance of the internal environment leads to homeostatic failure and the age-related pathology characteristic of old age.

Hypothalamic Disregulation Theory

According to Frolkis (Chap. 31) disproportionate age changes in the hypothalamus result in hypothalamic disregulation of the organism, thereby causing the development of age-related pathology.

Conclusions

It is too early to draw firm conclusions on the mechanism of aging. Nevertheless it is believed that there are multiple causes of aging. Tissue aging is seen to originate in random cell damage due to crosslinkage, somatic mutation or other errors (Fig. 2-5). The aging process appears to be programmed and to be primarily under genetic control. Unprogrammed aging is mainly due to environmental factors, which act at least in part by mediation of the hypothalamus and the pituitary hormones. The aging process is probably also controlled by an immunological surveillance mechanism which removes defective cells. The genetic and the immunological control may be influenced by hormones.

THE MEASUREMENT OF AGING

There are three ways of measuring the aging process. The first is to study the ages at death in a population of individuals, the second is to

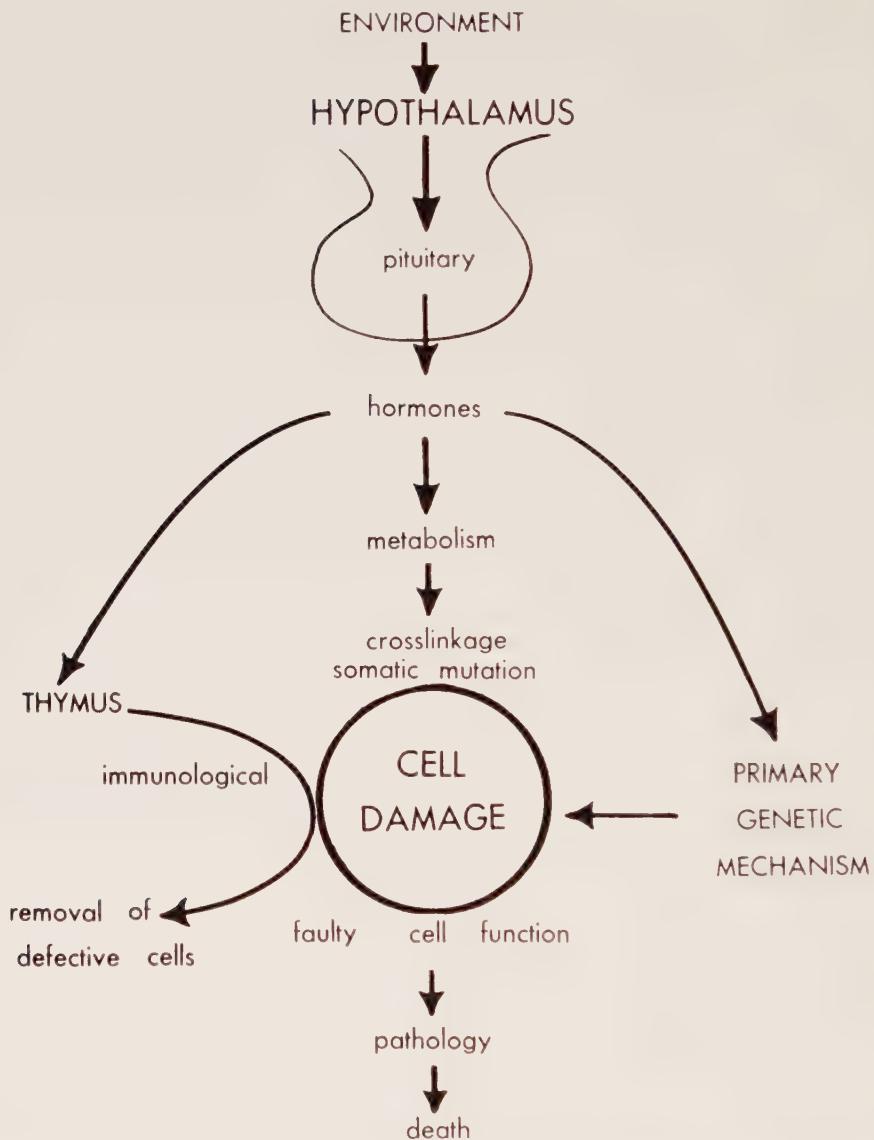


Figure 2-5. Tissue aging is seen to originate in random cell damage due to crosslinkage, somatic mutation or other errors. This process appears to be primarily under genetic control. Environmental factors affect aging probably via the hypothalamus, the pituitary and metabolism. There may be an immunological surveillance mechanism to remove defective cells (from Everitt, 1974).

measure the impairment of particular physiological functions with increasing age (Maynard Smith, 1966) and the third way is to measure the development of pathological lesions (see Berg, Chap. 3).

Mortality

The primary measure of aging adopted by most workers is the mortality of the population, as judged from the survival curve. Life tables are available for man, rat, mouse, thoroughbred horses, sheep, certain fish and many invertebrates including the fruit fly (*Drosophila*), the housefly, the rotifer and nematode worms.

It is usual in longevity studies to report survival quantitatively as the mean life duration \pm the standard error of the mean. The maximum life duration should also be given (Hollander, 1972). For example during the last century the successful control of many infectious diseases has greatly increased the mean life duration in many human populations. However there has been no change in the maximum life duration, because these measures have not affected the aging process.

A study of the shape of the survival curve may give information about the type of aging present in the population (Comfort, 1959; Casarett, 1964).

In a wild population of small birds or mammals the mortality rate is so high that few animals ever show signs of senescence. Such a wild population has an almost constant mortality rate and hence the survival curve shows a logarithmic decline (curve W in Fig. 2-6). A similar survival curve may be seen in mammals kept under poor environmental conditions, or subject to a high incidence of age-independent causes of death, such as an intercurrent infectious diseases or genetic diseases occurring early in life (Casarett, 1964).

In a domestic population (man, laboratory rat or mouse) living under good conditions, the mortality rate is not constant, but increases with age as a logarithmic function (Gompertz, 1825). In such a population the individuals age, that is they have a high resistance to dying. There are few deaths until middle age and hence the survival curve approaches a rectangular form (curve D in Fig. 2-6). This curve indicates a low incidence of age-independent causes of death and a high incidence of age-dependent causes (Casarett, 1964).

Where the aging process is retarded (curve R in Fig. 2-6) as in the food restriction experiments of McCay, *et al.* (1935) the survival curve is merely the same shape, but the time scale is expanded and so the decline appears at a later age. This could also be called delayed aging.

In precocious aging (curve P in Fig. 2-6) there is a simple displacement to the left without change in shape or slope (Casarett, 1964). The decline

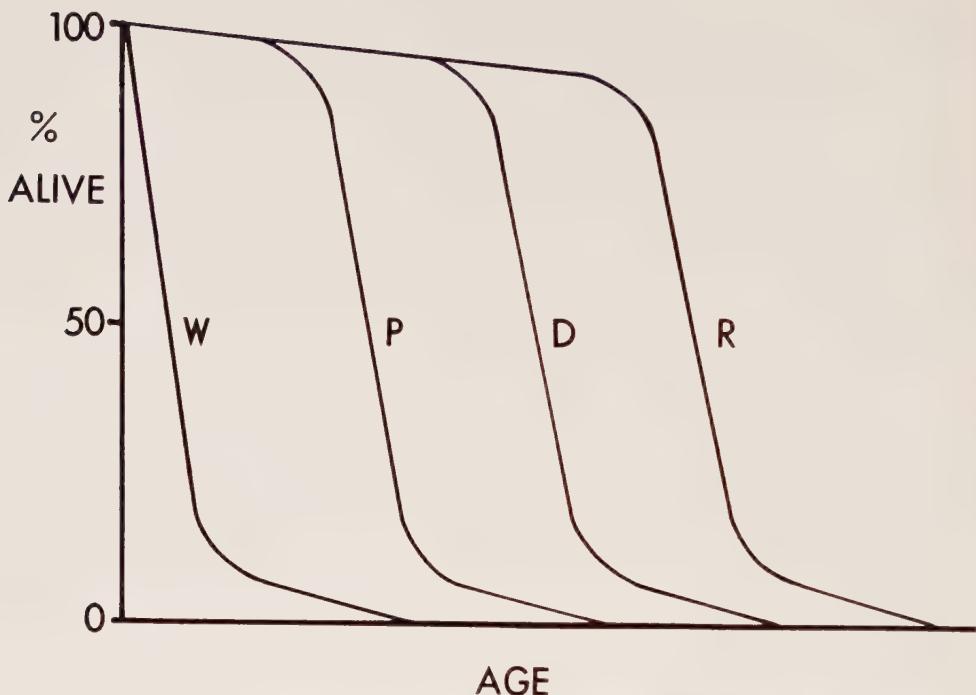


Figure 2-6. Survival curves in a wild population (W), in precocious aging (P), in a domestic population (D) and in retarded aging (R).

appears at an early age as in the case of total-body irradiation (see Berg, Chap. 3).

When lifespan is used as a measure of aging, it is essential to know the principal causes of death. For example, life shortening in mice could be due to the development of a specific disease rather than accelerated or precocious aging. However, the experimental prolongation of life beyond the currently recognized maximum for the species is most probably due to retarded aging (Kohn, 1971).

Physiological Indices

Many age changes have been suggested as parameters of aging. The selection of parameters depends on the criteria used. Probably the best criteria of aging are those proposed by Strehler (1962), namely *universality*, *progressiveness*, *intrinsicality* and *deleteriousness*. These previously discussed criteria appear to be satisfactory for many age-associated phenomena such as visual acuity, muscular work capacity, serum cholesterol and systolic blood pressure. Deleteriousness is the most difficult criterion to assess (Strehler, *et al.*, 1959).

Other criteria of aging have been listed by Sobel and Marmorston

(1958). Two of these criteria (numbers 2 and 5) are difficult to satisfy and to measure. The criteria are:

1. *The process must change at a measurable rate during aging.* This is an essential requirement.
2. *The progression of this change must advance the aging process itself.* This is difficult to assess, because of the lack of experimental data.
3. *The progression of this change must also occur in a situation which is known to advance aging.* Overeating, which is known to advance aging, should accelerate the progression of the age change.
4. *The process must be irreversible.* This is an essential requirement.
5. *The event must be representative of the whole organism rather than an individual organ.* The only global parameters of aging recognized so far are those which measure the decline in the "active protoplasmic mass" (Bourlière, 1970).

Bourlière (1963 and 1970) specified two further criteria:

1. The parameter should undergo a large change with age.
2. The test should be as simple as possible, whilst remaining reliable.

The present author (Everitt) believes that *the parameter should not be labile*. In other words the parameter should not fluctuate with changes in the environment, nor respond immediately to the treatment under study. For example, heart rate undergoes a moderate decrease with age in the rat (Grad, 1953; Everitt, 1958b). However, heart rate is so labile that a reliable estimate is difficult to make in an excited animal. Further, heart rate falls soon after either hypophysectomy or thyroidectomy. Thus heart rate cannot readily be used as an index of aging in either the hypophysectomized or thyroidectomized rat. Very stable parameters such as brain weight or the physical properties of collagen are more reliable indices of aging, although they are not very functional parameters.

Loss of Body Mass and Protein

Body weight increases during growth and declines in old age. In man the loss of weight and the decrease in height are small (Mueller-Deham and Rabson, 1942; Bourlière, 1963 and 1970; Shock, *et al.*, 1963; Forbes and Reina, 1970) and so are of rather limited use as indices of aging. However, in the rat (Everitt, 1957) and the mouse (Robertson and Ray, 1919; Lindop, 1961) the senescent loss of weight is relatively large and has been proposed as a measure of aging (Fig. 2-7). The senescent decline in body weight is probably caused by terminal disease in the rat (Berg, Simms and Everitt, 1963).

There is a steady loss of protein from the human body throughout

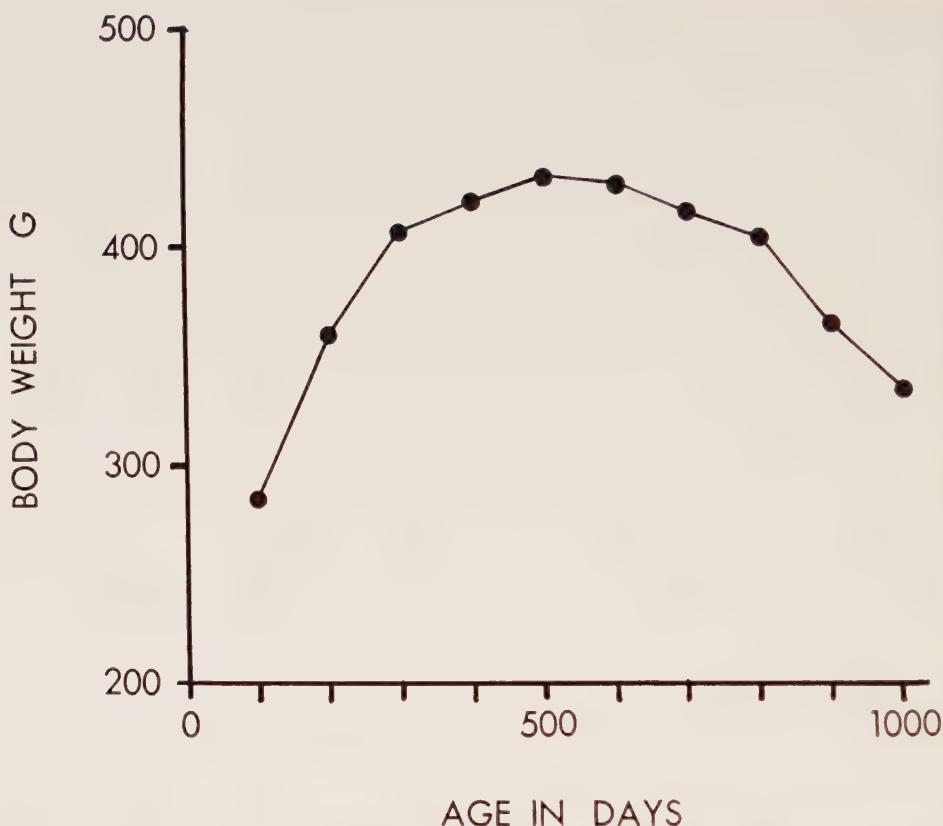


Figure 2-7. The growth and senescent loss of weight in 25 male Wistar rats surviving more than 1000 days.

adult life (Sobel, 1966). This loss is very difficult to measure directly. An estimate of the loss of muscle tissue may be obtained by measuring the urinary excretion of creatinine, a metabolite derived from creatine phosphate in muscle. In man creatinine excretion decreases at the rate of 0.77 percent per year beginning from the early third decade (Norris, *et al.*, 1963; Sobel, 1958). However Parot (1965) questioned its value in healthy old subjects. In the rat creatinine excretion declines from middle age onwards (Everitt, 1958a; Neumaster and Ring, 1964) and is well correlated with the loss of muscle mass and whole-body potassium (Neumaster and Ring, 1964).

Whole-body potassium has been used to measure lean body mass or total cell mass (Allen, *et al.*, 1960; Forbes and Reina, 1970; Novak, 1972) because 90 to 98 percent of the potassium is found in the cells (Sobel, 1966). Naturally occurring K contains 0.012 percent of the radioactive isotope K^{40} , which may be assayed with a whole body counter. Allen, *et al.* (1960)

recorded a progressive decline in body K^{40} between the ages of 20 and 80 years. The measurement of K^{40} is very rapid, taking less than 1 minute per individual, but the apparatus for this test is too expensive for most medical laboratories.

The quantity of intracellular water may also be used as a measure of total cell mass. Intracellular water space is calculated as the difference between total body water (antipyrine space) and extracellular water space (thiocyanate space) and shows a significant decline with age (Shock, *et al.*, 1963).

The work of Shock and his colleagues (Shock, *et al.*, 1963) demonstrated that the basal oxygen consumption of the body decreased with age at the same rate as intracellular water. They therefore concluded that the decline in BMR with age is a measure of the loss of functioning tissue with

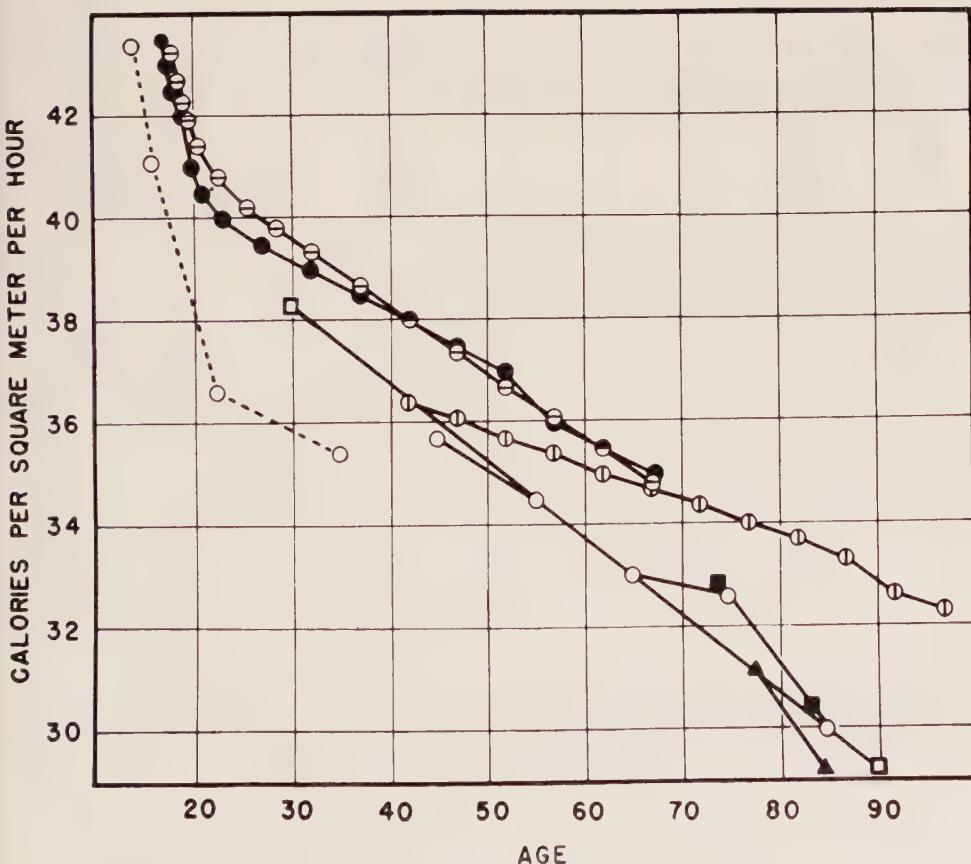


Figure 2-8. The decline in the basal metabolic rate with age in man from N. W. Shock, "Metabolism and Age," *Journal of Chronic Diseases*, 2:687 (1955), Pergamon Press Ltd.).

increasing age. This conclusion is supported by the work of Allen, *et al.* (1960), who showed a high correlation between the K^{40} count and the BMR, and thus the BMR may be a convenient measure of the total mass of active protoplasm. Age differences in BMR are shown in Figure 2-8.

In the rat, protein is being lost in the urine at an increasing rate throughout life (Everitt, 1958a). This increase is large (Fig. 2-9) and is associated with the development of renal disease (Berg, 1965).

Renal Decline

There are large decreases in renal function in man with increasing age (Shock, 1952 and 1968). Both renal plasma flow (diodrast clearance) and glomerular filtration rate (inulin clearance) show large linear decrements between the ages of 30 and 80 years. The age variation in glomerular filtration rate is shown in Figure 2-10. Shock (1968) concludes that the senes-

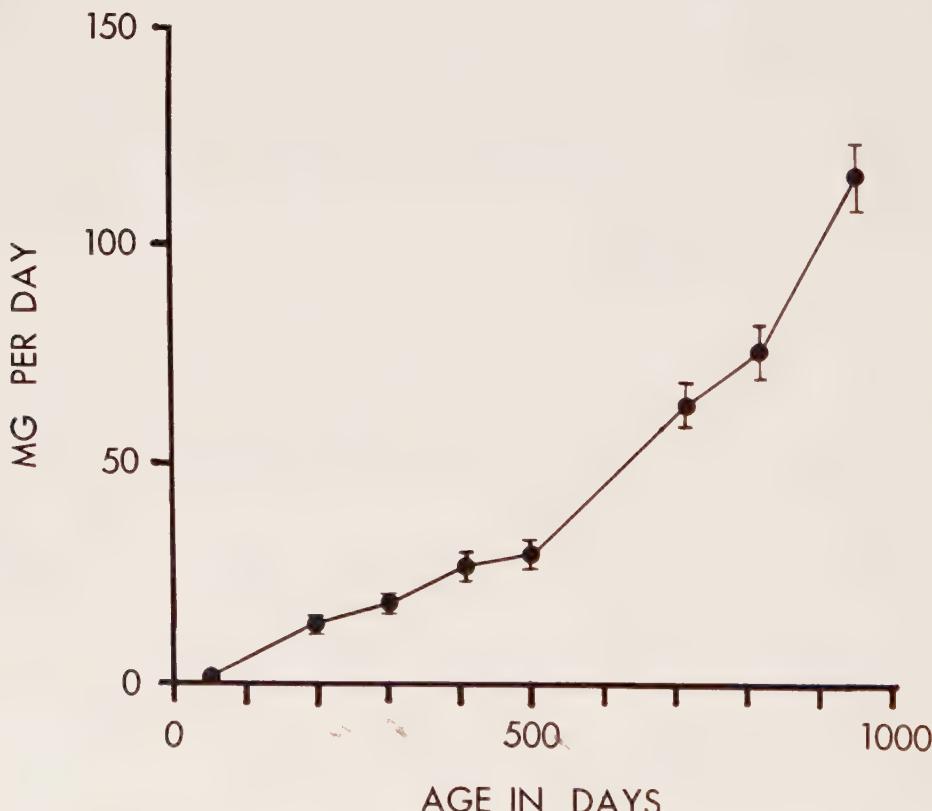


Figure 2-9. The rise with age in the daily excretion of protein in the urine of the male Wistar rat. The mean \pm S.E. are recorded for 18 rats followed throughout life.

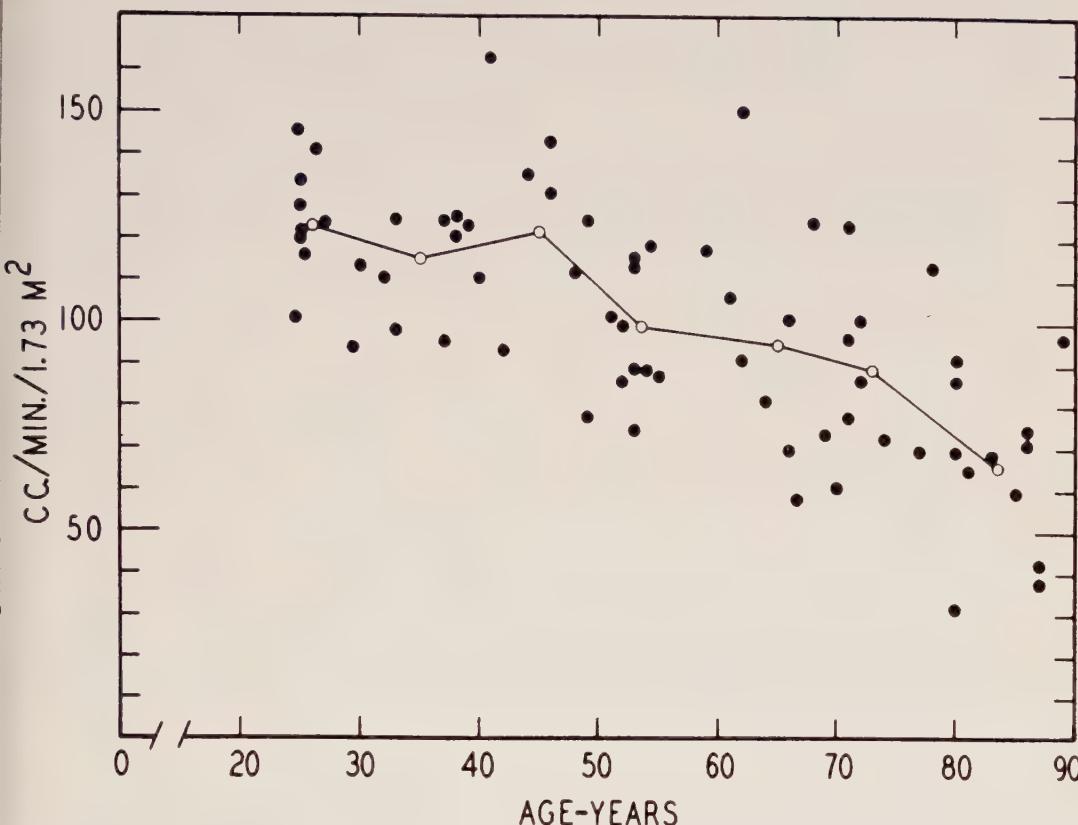


Figure 2-10. The decline in the resting glomerular filtration rate (inulin clearance) with age in man (from Shock, 1952).

cent changes in renal function are due to the gradual loss of irreplaceable nephrons with advancing age.

Similar tests of renal function have been performed in the aging rat (Gregory and Barrows, 1969).

Cardiovascular Decline

The resting cardiac output, as measured by the dye dilution technique, undergoes a large linear decline between the ages of 20 and 80 years (Brandfonbrener, *et al.*, 1955). The cardiac output at 80 years is about half that at 20.

The rise in systolic blood pressure with age (Fig. 2-11) has been widely used as an index of aging (Benjamin, 1947; Murray, 1951; Bourlière, 1963; Hollingsworth, *et al.*, 1965; Conard, *et al.*, 1966). This age change appears

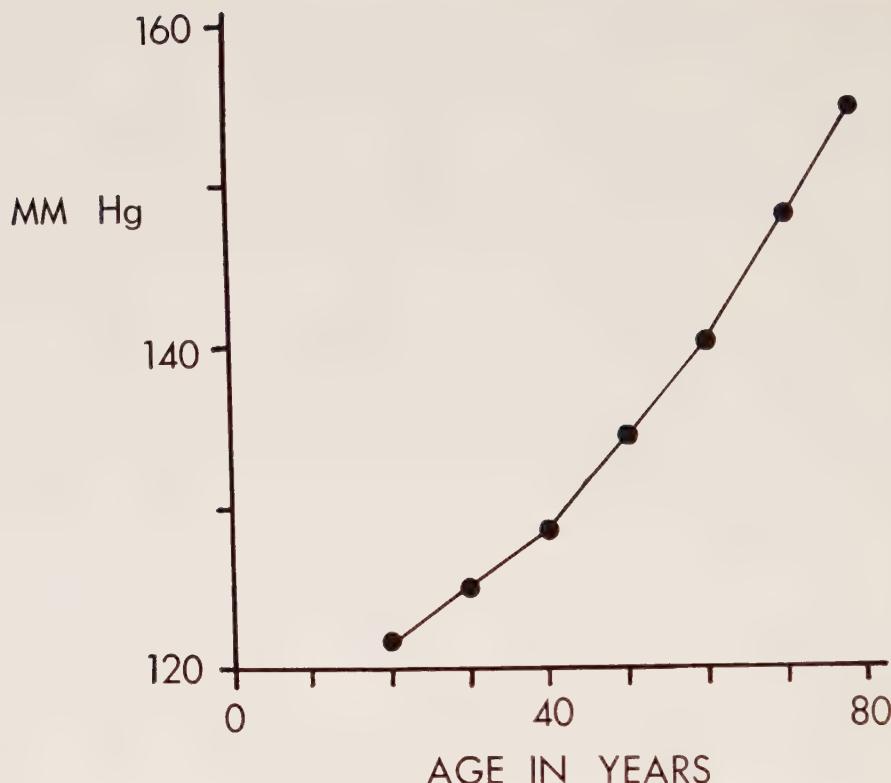


Figure 2-11. The rise in the mean systolic blood pressure with age in man (from the National Center for Health Statistics, Series 11, No. 4, 1-40, 1964, cited by Shock, 1968).

to be an adaptation to the decline in cardiac output and the development of atherosclerosis.

Respiratory Decline

The decrease in vital capacity (the maximum volume of air expired by the lungs) with age has been used as a test of physiological aging (Norris, *et al.*, 1956; Bourlière, 1963; Hollingsworth, *et al.*, 1965). There is a 40 percent reduction between the ages of 30 and 75 years (Fig. 2-12). When a dynamic functional measurement is made, such as the maximal breathing capacity, the age decrement is greater (Norris, *et al.*, 1956).

Muscular Work Decrement

The capacity for performing muscular work undergoes a large decrement between 30 and 80 years (Shock, 1962), as may be seen in Figure 2-

13. Tests of muscular strength or work capacity have been used as a measure of physiological aging by a number of investigators (Murray, 1951; Bourlière, 1963; Hollingsworth, *et al.*, 1965; Conard, *et al.*, 1966).

Neurophysiological Decrements

Visual acuity (Bernstein and Bernstein, 1945; Murray, 1951; Hollingsworth, *et al.*, 1965; Conard, *et al.*, 1966; Lederer, 1969; Fisher, 1973) and auditory acuity (Bunch, 1929; Murray, 1951; Hollingsworth, *et al.*, 1965;

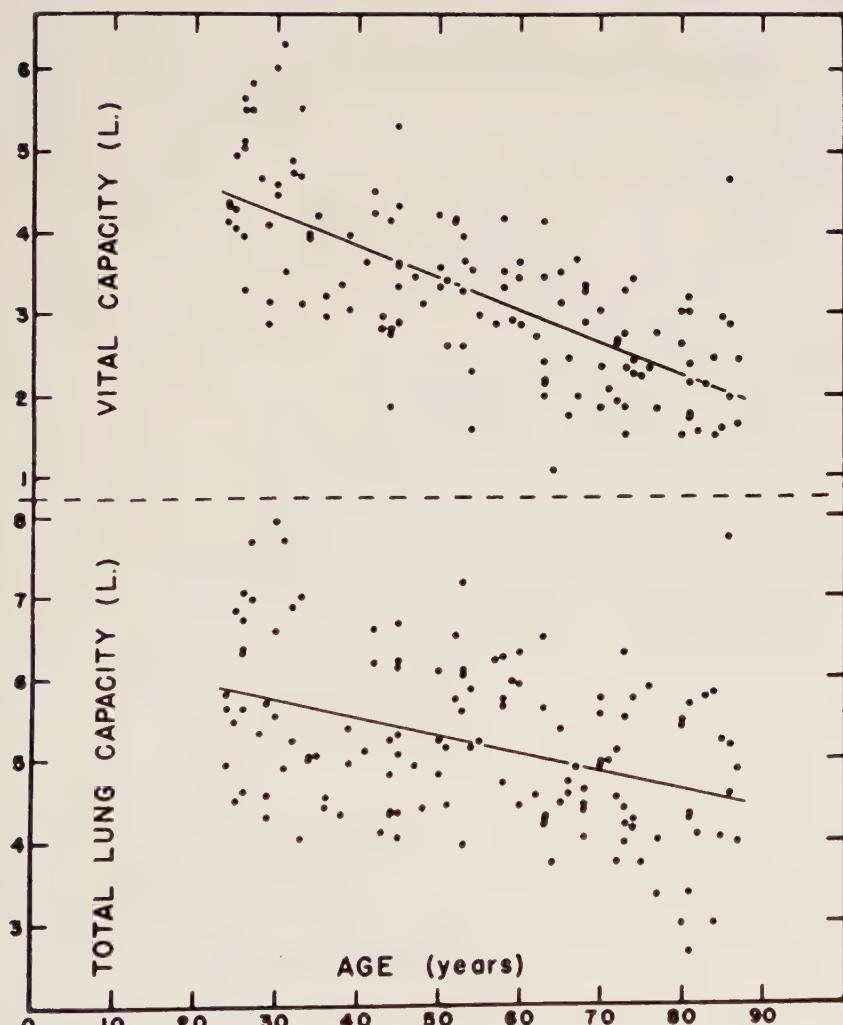


Figure 2-12. The decline in the vital capacity and total lung capacity with age in man (from Norris, *et al.*, 1956, reproduced by permission of *Journal of Gerontology*).

Conard, *et al.*, 1965) have been used as tests of aging. The age decrement in visual acuity is shown in Figure 2-14.

Tests of vibratory sense and reaction time have also been used by Hollingsworth, *et al.* (1965 and 1969) and Conard, *et al.* (1966).

Psychological Decrement

Mental deterioration is a major feature of human aging. Performances on complex mental tests show a decline that increases with the complexity of the task (Shock, 1960). Part of the decrement in scores for intelligence

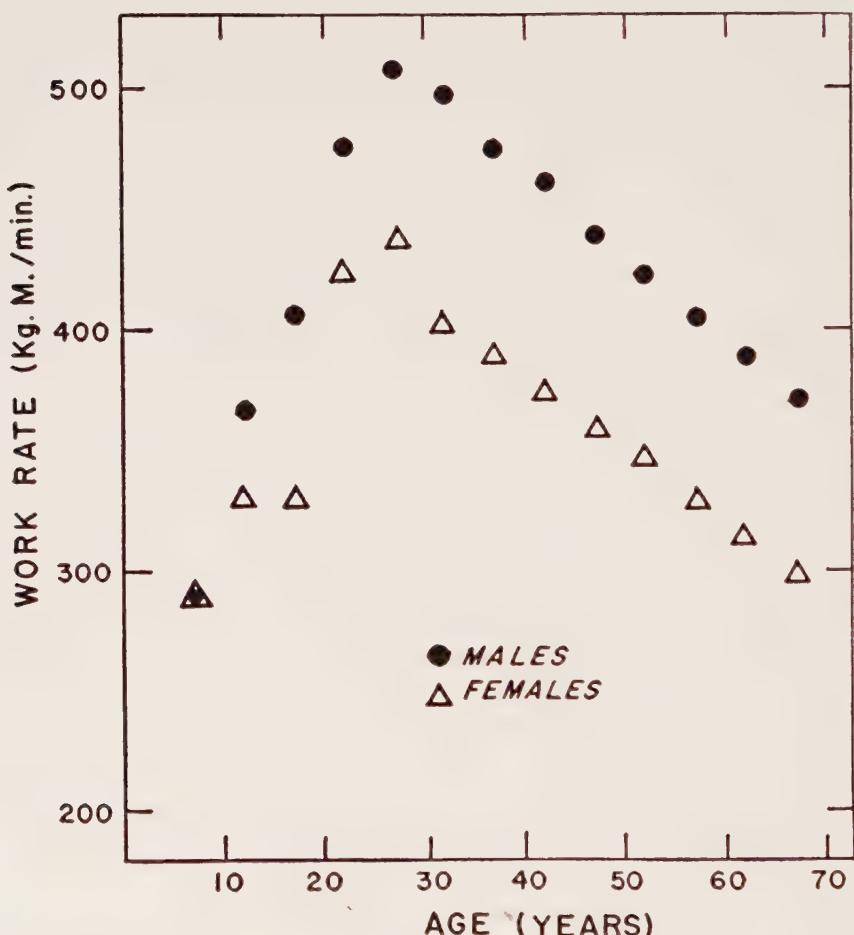


Figure 2-13. The growth and decline in muscular work rate with age in man. (From A. H. Norris, and N. W. Shock: Exercise in the adult years—with special reference to the advanced years. In W. R. Johnson (Ed.): *Science and Medicine of Exercise and Sports*. New York, Harper and Row, 1960.

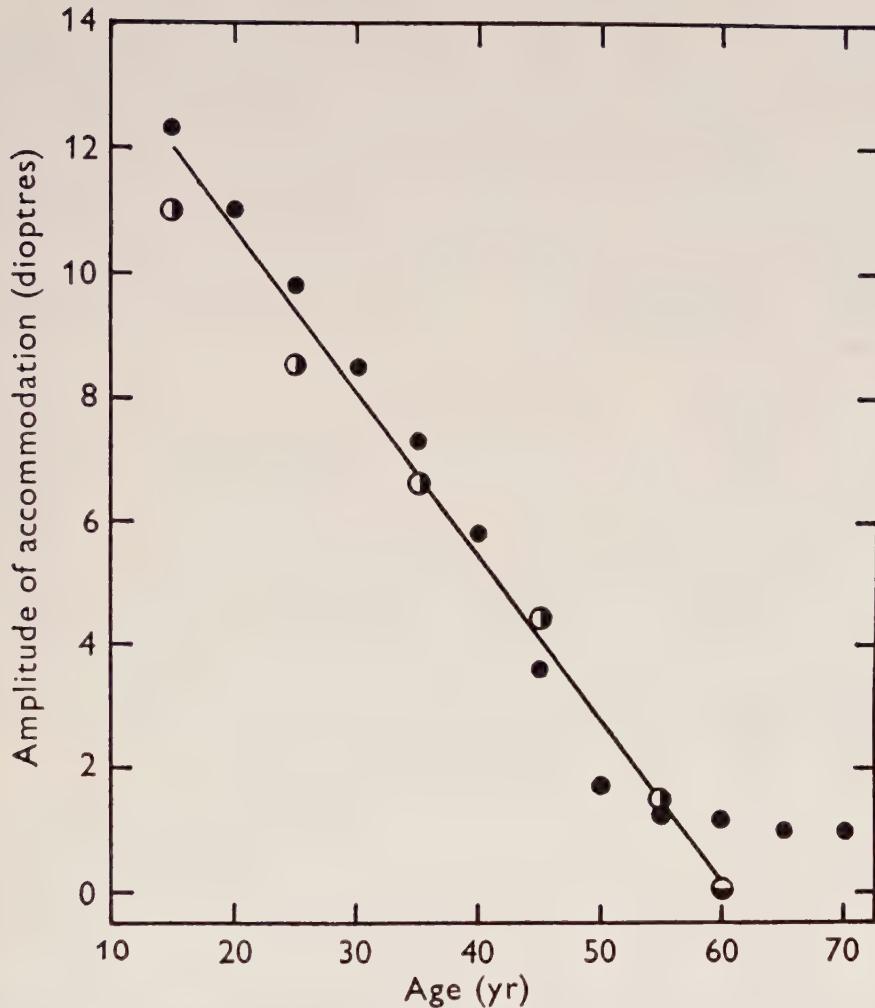


Figure 2-14. The decline in the amplitude of accommodation of the eye lens with age in man (from Fisher, 1973).

tests on elderly people is due to the smaller amount of schooling they have received. Another weakness of most psychological tests is that they have been designed for young adults. Tests of psychological age have been reviewed by Bourlière (1970).

It is possible to test learning and memory in rats with the help of T-mazes (Verzár-McDougall, 1957).

Endocrine Decrements

Tests of thyroid and adrenocortical function have been explored by Shock and his collaborators (Shock, 1968).

Gaffney, *et al.* (1962) showed that the rate of uptake of I^{131} by the thyroid within the first 6 hours after administration was slower in old age. There was no age difference in I^{131} uptake after 24 hours, because of the slower renal excretion of I^{131} in old age. Measurements of thyroxine degradation rate (Gregerman, *et al.*, 1962) reveal a 50 percent decline between the ages of 20 and 80 years. A measured quantity of labelled iodine is administered intravenously and its disappearance from the circulation followed over a 2-week period. Other tests of thyroid function are discussed by Hales, *et al.*, in Chapter 27.

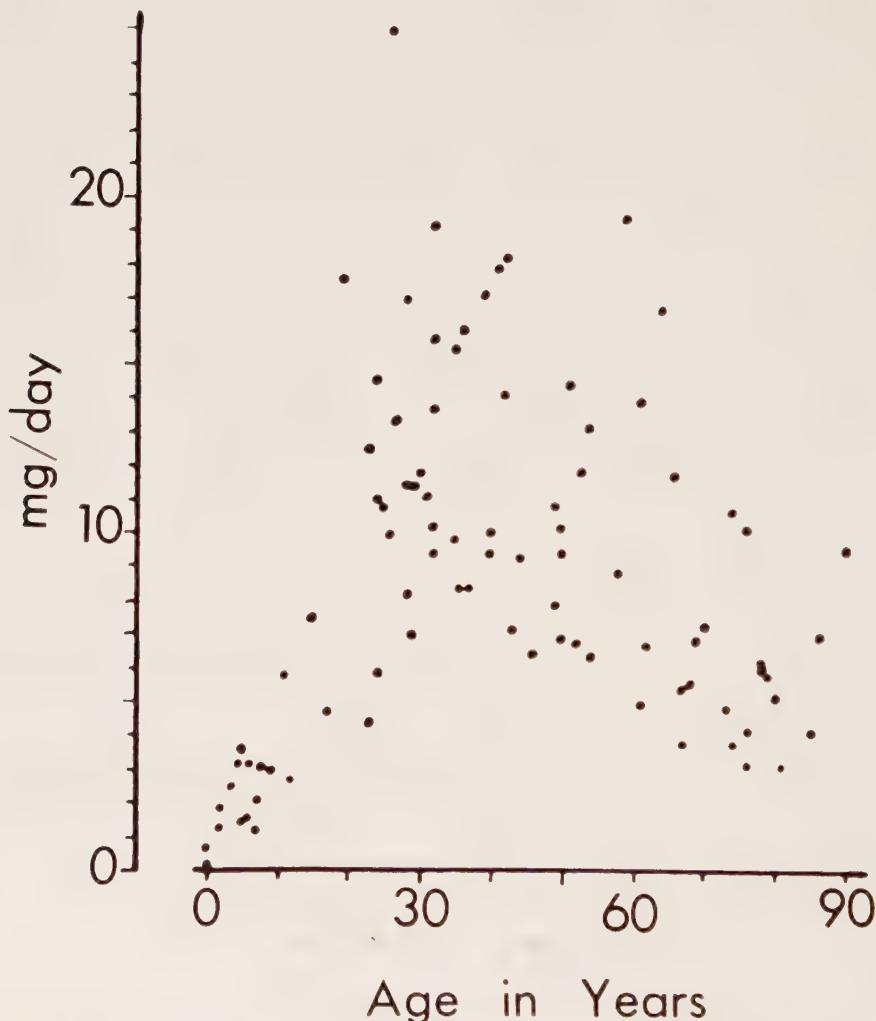


Figure 2-15. The growth and decline with age in the excretion of 17-hydroxy-corticosteroids as shown from measurements on 90 healthy men of different ages (from Borth, *et al.*, 1957).

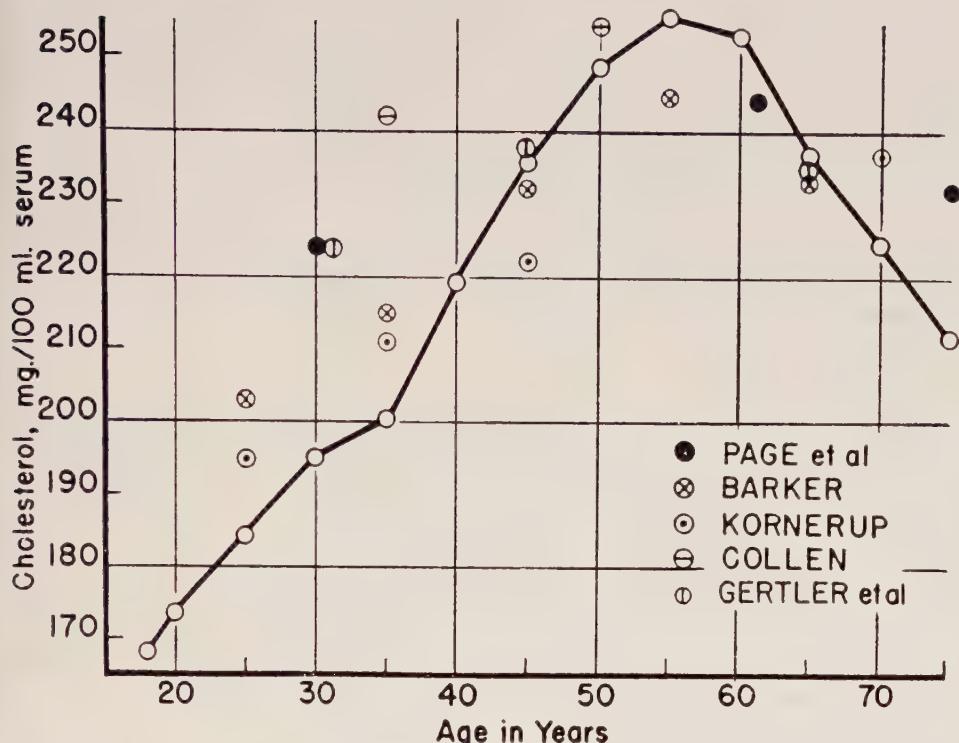


Figure 2-16. The growth and decline in the serum cholesterol level with age in healthy men (from Keys, 1952, reproduced by permission of *Journal of Gerontology*).

The decline in the urinary excretion of 17-ketosteroids is well known (Hamburger, 1948; Kirk, 1949; Kowalewski, 1950). Measurements of the plasma levels of conjugated 17-hydroxycorticoids and 17-ketosteroids demonstrate significant decrements with age (Hochstadt and Reichenbach, 1961). Age differences in the excretion of 17-hydroxycorticoids measured by Borth, *et al.* (1957) are shown in Figure 2-15.

Tests of hypothalamic pituitary function are described by Lazarus and Eastman in Chapter 6. Apart from changes with age in the basal secretion of anterior pituitary hormones, there are also differences in response to stimulation by pharmacological and stressful stimuli.

Aschheim (Chap. 19) describes a biological test of aging of the hypothalamic regulation of the estrous cycle in the rat.

Dilman (1972) measures aging in the hypothalamus by 1) the resistance to inhibition by the negative feedback of glucose, estrogen and cortisol and 2) the loss of normal rhythmic activity like the diurnal variation in the blood cortisol level.

Lipid Metabolism

The serum cholesterol level has been used as a measure of biological aging (Bourlière, 1963; Hollingsworth, *et al.*, 1965; Conard, 1966). The cholesterol level increases with age until late middle age (Fig. 2-16) and then declines in old age (Keys, 1952; Swanson, *et al.*, 1955).

The total fat content of the body increases by 50 percent in both sexes between the ages of 30 and 70 years (Young, *et al.*, 1963; Forbes and Reina, 1970). Skinfold thickness is often used to estimate the thickness of the subcutaneous fat layer (Brozek and Kinney, 1960; Bourlière, 1970).

Collagen Age Tests

Verzár (1955) developed the first biological test of aging using collagen fibers from rat tail tendon. These fibers may be removed from the living animal under anesthesia. As the rat ages its collagen fibers become progressively tougher. When heated in Ringer solution at 65°C, collagen fibers contract. Verzár found that the tension of thermal contraction increased with age (Fig. 2-17), up to 3 years in the rat and 17 years in the cat (Takács and Verzár, 1968). Variations of this test (Boros-Farkas and Everitt, 1967) include measurements of contraction, relaxation or rupture in chemical solutions such as potassium iodide, sodium perchlorate or urea.

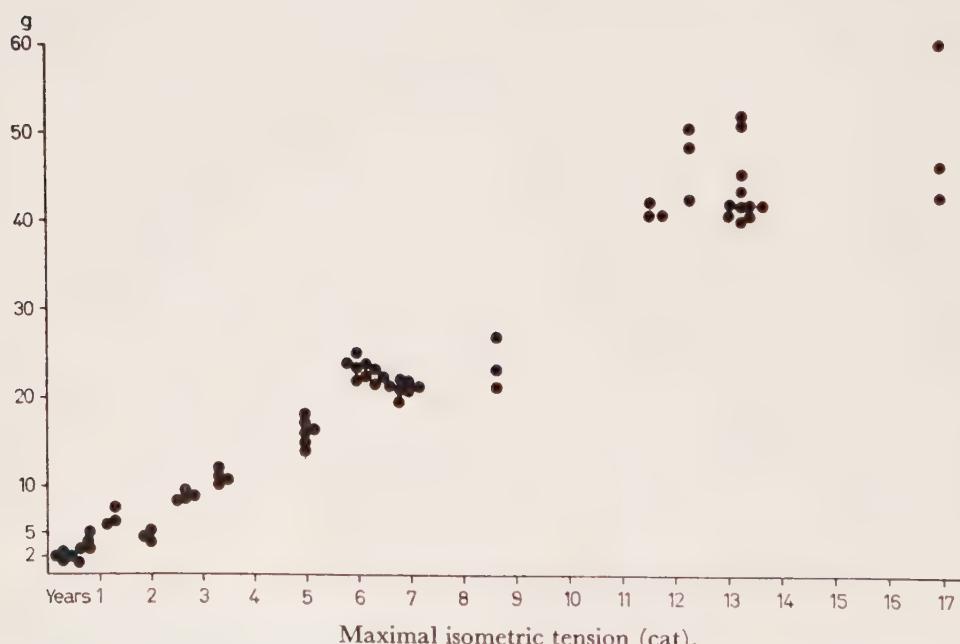


Figure 2-17. The relation of age to isometric thermal contractile tension in cat tail tendon fibers in Ringer solution at 62 to 95°C (from Takács and Verzár, 1968).

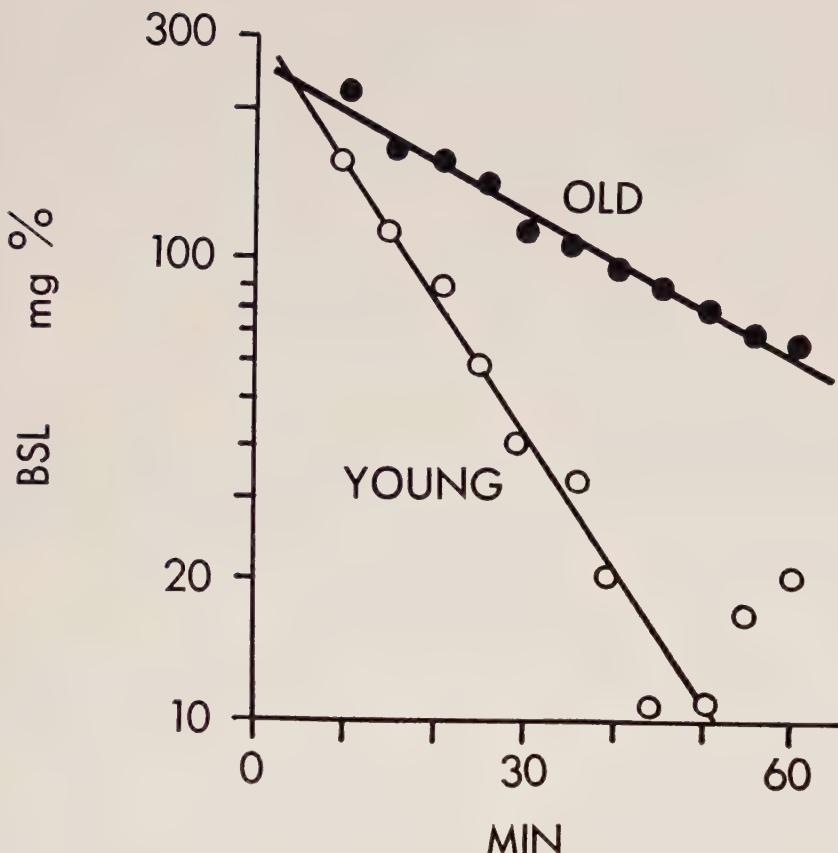


Figure 2-18. The glucose-insulin tolerance test in an old (86 years) and a young (26 years) subject (from Silverstone, *et al.*, 1957).

In man, Sobel and Marmorston (1956 and 1958) proposed that the ratio of hexosamine to collagen in skin could be used as a measure of biochemical age. A specimen of skin is obtained from the gluteal region with a dermal punch. The ratio of hexosamine to collagen declines with age. This test of aging has been used by several other investigators (Clausen, 1962; Anderson, 1965). Kohn (1971) measures the enzyme digestibility of tendon.

Skin elasticity has been measured by the time required for a standard "pinch" of skin to flatten. Hollingsworth, *et al.* (1965) used this test as an index of physiological aging. There is a large rise with age in the flattening time.

Adaptation Tests

The body is able to adapt itself to changes in the environment. However, the capacity for adaptation declines with age, and consequently a

longer time is required to re-establish equilibrium in old age. If measurements are made before adaptation is complete large age differences may be observed. Flückiger and Verzár (1955) found that the drop in body temperature of rats exposed to a low atmospheric pressure (350 mm Hg) for 24 hours was greater in old rats due to their poorer adaptation.

After a standard amount of exercise old men require a longer time for the heart rate to return to normal than do young men (Norris and Shock, 1960). In Figure 2-18 it can be seen that the fall in the blood sugar level following the intravenous injection of 5 units of insulin plus 25 g glucose is much slower in old subjects (Silverstone, *et al.*, 1957).

Aging in the Whole Body

There are two ways of measuring aging in the whole body. The first is to measure the decline in the total "active protoplasmic mass" (Bourlière, 1970) the second is to obtain a composite measure of aging by combining parameters of aging in different organs, that is to use a battery of tests (Comfort, 1969b).

The total protoplasmic mass as discussed earlier, may be measured in man by determining the whole body potassium, intracellular water or more simply the basal metabolic rate (Shock, 1968; Bourlière, 1970). In the rat whole body potassium, urinary creatinine excretion or even body weight may be used.

A battery of tests has been employed by a number of investigators (Murray, 1951; Bourlière, 1963 and 1970; Hollingsworth, *et al.*, 1965; Conard, *et al.*, 1966; Comfort, 1969b; Ries, 1972). Conard and his colleagues employed a battery of 14 tests to study aging in a population of Marshall Islanders who were accidentally exposed to radioactive fall-out in 1954 (Conard, *et al.*, 1966). The tests involved special sense organs (visual acuity, ocular accommodation, arcus senilis and hearing loss), neurological function (vibratory sense, reaction time, and rapidity of movement), integument (skin looseness, skin elasticity, and hair graying), muscular strength, cardiovascular test (systolic blood pressure), serum cholesterol and body potassium. In a similar way Hollingsworth, *et al.* (1965) used a battery of 9 tests to study aging in the Hiroshima survivors. "Physiological age," a composite of parameters, as measured by Hollingsworth and Conard shows little correlation with chronological age until after 40 years. That is, the composite of parameters is strongly correlated with mortality and therefore with pathological development.

It may be possible to distinguish pathological and physiological parameters from the shape of the age curve, since a number of parameters of cardiovascular disease reach a peak in middle age (Webster, 1974). Patho-

logical age can be estimated using a battery of tests which indicate pathological changes such as hypertension, electrocardiographic abnormalities, glucose tolerance test, liver function test, etc. (Ogura, 1967). In the assessment of biological age it is necessary to consider both the incidence of pathology and the physiological age. At a first approximation biological age may be regarded simply as the sum of the pathological and physiological ages (Everitt and Webster, 1974).

CONCLUSIONS

The characteristic features of aging are the progressive involution of bodily functions, accompanied by a diminishing resistance to pathological change which ultimately leads to death.

Human biological age may be evaluated by estimating the total mass of active protoplasm (which declines with age) from the total body potassium⁴⁰ test or more simply by measuring the basal metabolic rate. It is also possible to estimate biological age by using a battery of parameters, each test measuring a different aspect of body function.

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CHAPTER 3

PATHOLOGY AND AGING

BENJAMIN N. BERG

SUMMARY

IN THE PRESENT CHAPTER aging is discussed with relation to the spontaneous diseases that develop in senescent rodents. Although the age changes in tissues leading to disease are obscure, the aging process can be modified by external factors such as calorie restriction or ionizing radiation. Dietary restriction delays the onset of lesions and increases life expectancy in rats whereas irradiation has an opposite effect in mice. This occurs in strains having a wide variety of spontaneous neoplasms as well as non-neoplastic conditions. The different experimental models used for these studies are described. Whether disease is the result or the cause of aging is an unsolved problem, but data obtained under controlled experimental conditions indicate how the aging process may be modified.

INTRODUCTION

Little is known about the age changes in tissues leading to the onset of disease. Without such basic information the study of age-disease patterns is restricted largely to a description of tissue alterations once they have occurred with the passage of time. Although intrinsic changes attributable to an aging process have not been demonstrated, there is convincing experimental evidence that two external forces, namely, dietary restriction and exposure to ionizing radiation can respectively retard or hasten the onset of disease and thus lengthen or shorten the lifespan of rodents. The experimental models used by different investigators to demonstrate these effects will be described in the present chapter.

EFFECTS OF DIETARY RESTRICTION ON DISEASE AND LIFESPAN

Comparative Studies

In 1939 McCay, *et al.* observed that the lifespan of an inbred strain of Osborne-Mendel rats was lengthened by calorie restriction. They used drastic underfeeding which resulted in severe retardation of growth and maturity. The diet was complete except for calories and resulted in a 60 percent reduction in body weight of rats surviving 1,000 days. Moreover their animals had a high incidence of lung and ear infection, a condition that

interferes seriously with the validity of nutrition experiments. In a subsequent paper, McCay, *et al.* (1943) reported a reduction in the incidence of the major diseases of calorie-restricted senescent rats, principally lymphosarcoma of the lung, chromophobe adenoma of the pituitary and chronic nephrosis (Saxton, 1945; Saxton, *et al.*, 1948). Lesion incidence and survival rates were recorded for the combined sexes but not separately for each sex. This could influence the results since the lifespan of female rats is longer than that of males and terminal disease sets in later.

Ross (1961, 1965 and 1969) studied the long-term effects of calorie-restricted purified diets on the longevity of male Sprague-Dawley rats of the Charles River strain, and found that a diet containing 22 percent protein and 54 percent sucrose lengthened the lifespan to a greater extent than other combinations. The diet was considered to be low in protein and carbohydrate though the ratio is generally accepted as optimum for rat growth and reproduction. Calorie intake was reduced by about 60 percent from the amount consumed by the control, a level of restriction resulting in growth retardation comparable with that obtained by McCay.

The principal spontaneous neoplasms included malignant lymphomas, fibrosarcomas, fibromas, and islet-cell tumors of the pancreas. Total tumor incidence was lowered by food restriction, particularly by the 22 percent protein-54 percent sucrose diet (Ross and Bras, 1965). Evidently, underfeeding was most effective with this proportion of protein to carbohydrate. In another series of experiments Ross, *et al.* (1970), confirming an earlier observation by Saxton, *et al.* (1948), reported that the incidence of pituitary adenoma in old rats was reduced by drastic underfeeding and attributed the inhibitory effect to protein deficiency. However, with less severe dietary restriction Berg and Simms (unpublished observation) found no significant change in the number of pituitary neoplasms.

Data on frequency of non-neoplastic conditions were limited to nephrosclerosis (Bras and Ross, 1964). Ad libitum-fed controls received a commercially prepared diet whereas calorie-restricted rats were fed purified diets. The incidence of renal lesions increased exponentially with age and was reduced by food restriction, irrespective of protein-carbohydrate ratios. Histologic changes were first seen at 600 days and consisted of varying degrees of capillary basement membrane thickening followed by obliteration of glomerular tufts.

A high incidence of massive bronchiectatic pneumonia and ulceration of foot pads (due to prolonged confinement in small cages with wire-mesh bottoms) was noted. Complications of such magnitude could have an important influence on the experimental results.

To determine the relation of disease to aging and longevity it is necessary to know the time of onset of lesions, rate of development from the

early to the late stages, and incidence at various ages (Simms and Berg, 1957). Carrel (1931) observed that "a tissue consists of a society of complex organisms which does not respond in an instantaneous manner to the changes of the environment. It may oppose such changes for a long time before adapting itself to the new conditions through slight or profound transformations. To study it at only one instant of the duration is almost meaningless. The temporal extension of a tissue is as important as its spatial existence." Designation of a single major disease as the cause of death without recording other pathological conditions found at autopsy fails to provide the information needed to follow the progress of disease with age. Quantitative data supplying this information were reported by Berg and co-workers (Berg and Simms, 1960 and 1961; Berg, *et al.*, 1962a, b. and 1963) in studies on the time of onset of lesions and longevity, and on the effects of dietary restriction.

Effects of 46 Percent Dietary Restriction on Growth

Sprague-Dawley rats of both sexes derived from a colony free from chronic respiratory disease or *Salmonella* infection were used. Animal quarters were air conditioned and maintained at uniform temperature and humidity, and automatically regulated 12-hour periods of light and darkness were provided. Cages with solid bottoms were used to prevent ulceration of the foot pads. The young were weaned at 28 days of age and were selected so that equal numbers with the same weight were represented in each experimental group. A commercially prepared diet without added vitamin D was supplied at two levels: 1. *ad libitum* (unrestricted) and 2. 54 percent of the *ad libitum* intake (46% restricted) started at weaning. Body weight of the restricted rats was reduced by about 40 percent below the weight of the unrestricted group (Fig. 3-1). Due to better food utilization in males (Morris, *et al.*, 1933; McCay, *et al.*, 1935) the weight curve of the restricted males was nearly identical with that of the unrestricted females, although the food consumption of the males was 20 percent lower (Berg, 1960). Reduction of body weight in both sexes was due largely to the absence of the excess body fat which is stored by *ad libitum*-fed animals. Skeletal size based on tibia length was reduced by 7 percent and body length by 10 to 13 percent as compared with the measurements of the unrestricted groups. Restricted males having the same body weight as unrestricted females had larger skeletal measurements. Although the proximal epiphyseal cartilage persisted in senescent rats, unrestricted or restricted, there was no evidence of osteogenic activity. This is contrary to the widely held belief that rat growth is continuous throughout life. Sexual maturity was attained at about 50 days of age in both restricted and unrestricted females. *Ad libitum*-fed rats, particularly males, became sluggish, obese and

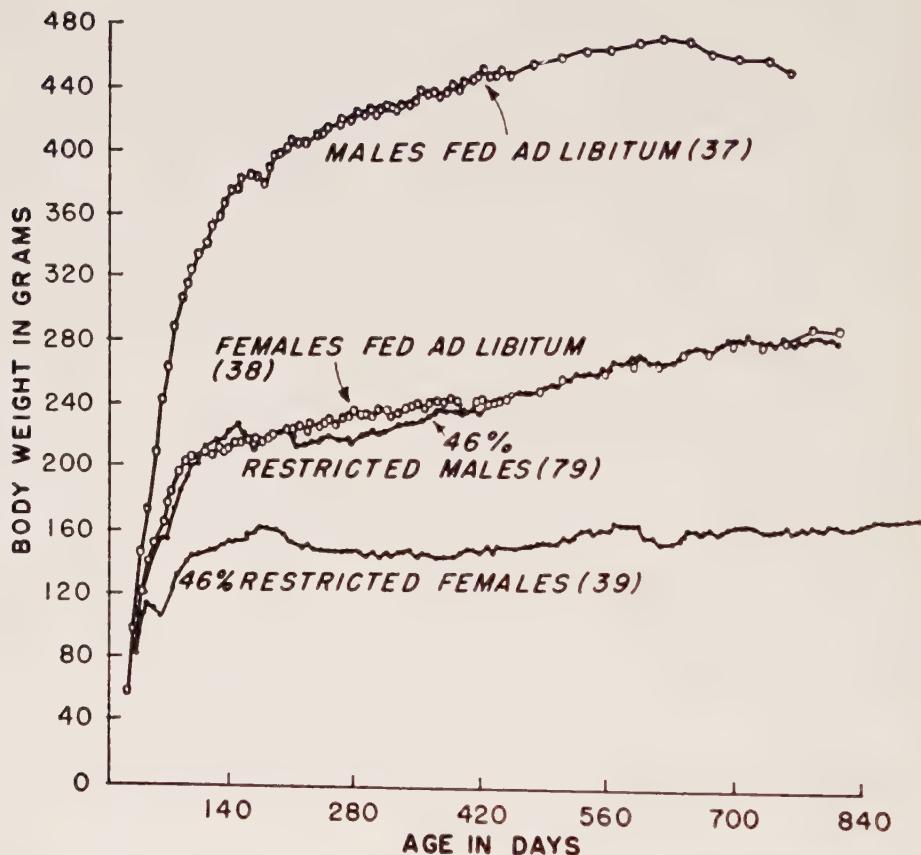


Figure 3-1. Body weight curves of rats fed *ad libitum* and on 46 percent restriction. Note nearly identical curves of restricted males and unrestricted females (From Berg (1960), reproduced by permission of the *Journal of Nutrition*).

untidy with advancing age, whereas restricted animals remained sleek, and active, and resembled young adults. In contrast with McCay's severely retarded rats, 46 percent restriction had little effect on growth or maturity and maintained animals in an excellent state of nutrition. By balancing food intake with energy requirement the overweight of *ad libitum*-fed rats was prevented.

Pathology

Data on microscopically observed lesions were obtained from rats at various ages. Non-neoplastic changes were graded as early, moderate or severe. Since lifespan was extended by dietary restriction, most of the observations on restricted animals were made when they were still in good condition, whereas *ad libitum*-fed rats were moribund or had died at the time of examination. Tissues were fixed in Zenker's fluid and after paraf-

fin imbedding sections were stained with hematoxylin and eosin. Special stains were used for the nervous system and to demonstrate basement membrane changes in the kidney.

The major non-neoplastic diseases were nephrosis, polyarteritis nodosa (periarteritis), myocardial degeneration, skeletal muscle degeneration, and radiculoneuropathy (Berg, 1967). Spontaneous tumors were chiefly of the benign type, the more common being mammary fibroadenoma in females, adenoma of the thyroid, chromophobe adenoma of the pituitary, pheochromocytoma of the adrenal, and islet-cell adenoma of the pancreas. Malignant tumors included sarcoma of the subcutaneous tissue, fibrosarcoma of the liver, carcinoma of the thyroid, and unclassified neoplasms of the central nervous system.

Nephrosis developed earlier than other diseases. Initially, thickening of capillary basement membranes was observed in the glomerular tufts. In addition, adjacent convoluted tubules were often dilated and contained proteinaceous casts. At a more advanced stage, hyalinization and fibrosis of glomeruli, thickening and fraying of Bowman's capsule, and adhesions between the latter and the tufts occurred. There was also marked dilatation of the tubules in the cortex as well as in the medulla. Scattered groups of atrophied and degenerated tubules were also seen in the cortex, and such areas were often infiltrated by lymphocytes. Finally, the parenchyma was replaced by cystically dilated tubules filled with casts, most of the glomeruli had disappeared, and the remaining ones were hyalinized or sclerotic. Interstitial fibrosis was present in varying degrees. Except for medial thickening of an occasional artery, the vessels were unaltered. However, Wilens and Sproul (1938b) found a high incidence of polyarteritis in their rat strain and suggested that the renal lesions could be related to impairment of the circulation. Hydrothorax, ascites, hypertension (Berg and Harmison, 1955), and uremia were late complications of the disease. Lesions similar to those just described were also observed by Saxton and Kimball (1941) and by Snell (1967). These investigators, as well as Wilens and Sproul (1938b) did not report the early glomerular changes seen in our rat strain.

The presence of protein in the urine, generally regarded as normal in the rodent (Bernstein, 1966) appeared to be a manifestation of nephrosis in our rat strain, the degree of proteinuria corresponding roughly with the severity of lesions (Berg, 1965). In early life, the proteins consisted of α_2 globulin and β globulin. Albumin appeared when the concentration reached a level of 20 mg/100 ml and increased as the disease progressed. Ultimately the electrophoretic pattern resembled that of the serum proteins. Accompanying the proteinuria, hyperglobulinemia, reversal of the A/G ratio and hypercholesterolemia developed with advancing age. Perry

(1965) observed proteinuria, hyperglobulinemia and hypoproteinemia in Wistar rats.

Polyarteritis nodosa is characterized by segmental necrosis and inflammation of vessel walls. Lesions were observed in many organs but the pancreatic, mesenteric and spermatic arteries were chiefly affected. The pathological changes corresponded with those described by Wilens and Sproul (1938b) and by Yang (1965). The disease was characterized by remissions and exacerbations as suggested by the presence of lesions of varying severity in a single vessel or in different ones. Microscopically observed changes during the acute stage of the disease consisted of intense leucocytic infiltration of the adventitia and media with degeneration and necrosis of the vessel wall, disruption of the internal elastic lamella, fibrinoid necrosis of the intima and thrombosis. After subsidence of the acute inflammatory reaction, invading fibroblasts converted the vessel into a fibrous nodule, or an aneurysm developed. In rare instances, an aneurysm ruptured and fatal hemorrhage ensued. Intestinal infarction was never observed.

Myocardial changes were localized in the papillary muscles and subjacent wall of the left ventricle, the base, the right anterior papillary muscle at its attachment to the interventricular septum, the apex, and around the branches of the coronary arteries. The latter were normal in our rat strain whereas Wilens and Sproul (1938a) found narrowing of the lumen and thickening of the wall of the coronary vessels in the Mendel-Sherman strain, and Wexler (1964) described a variety of changes in four different strains that had been bred repeatedly. Microscopically, lesions showed atrophy and degeneration of muscle fibers with varying degrees of fibrosis, unaccompanied by an inflammatory reaction.

Skeletal muscle degeneration developed later than the previously described diseases, and involved the gastrocnemius, adductor, and spinal muscles (Berg, 1956; Andrew, *et al.*, 1959; Bourne, 1960). Distribution of microscopically observed lesions was patchy and varying degrees of severity were seen in a single muscle. The early stage of the disease was characterized by loss of cross striations and an increased number of sarcolemma nuclei arranged in parallel rows along the sheaths. More advanced changes included degeneration and fragmentation of muscle fibers, fatty infiltration, aggregates of nuclei resembling giant cells, and mast cell proliferation. In the late stage, the anatomical pattern was completely lost and only empty sarcolemma sheaths and fragments of fibers remained. No inflammatory reaction occurred. The lesions were similar to those observed in vitamin E deficiency (Pappenheimer, 1948), but were neither prevented nor cured by long-term treatment with α tocopherol (Berg, 1959).

A type of radiculoneuropathy characterized by myelin degeneration developed in the cauda equina and other spinal roots of a high percentage

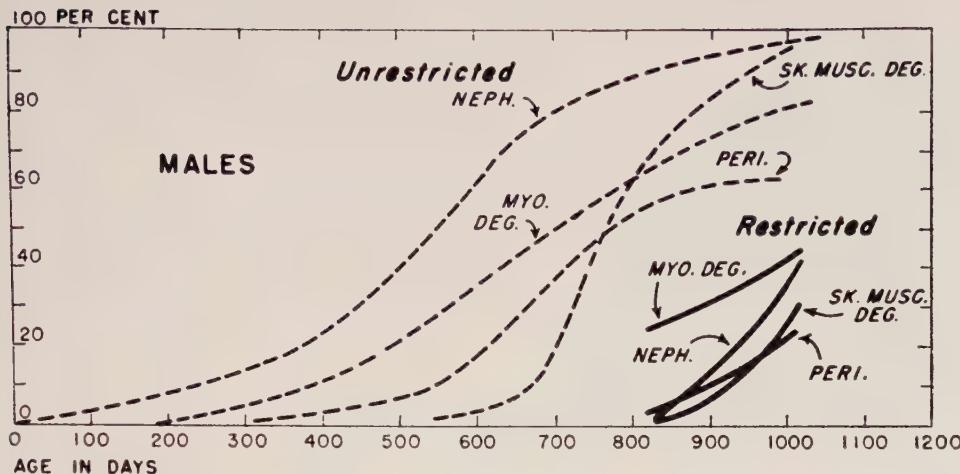


Figure 3-2. Sigmoid-shaped curves representing total incidence of lesions of four major diseases, namely, nephrosis (NEPH.), periarteritis (PERI.), myocardial degeneration (MYO. DEG.), and skeletal muscle degeneration (SK. MUSC. DEG.), in unrestricted and restricted male rats. The delaying effect of dietary restriction on onset of lesions is shown (From Berg and Simms (1965), reproduced by permission of the *Canadian Medical Association Journal*).

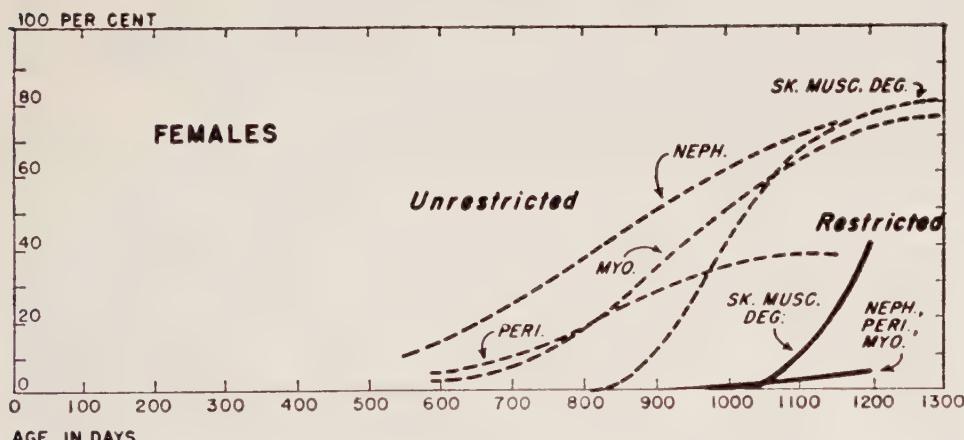


Figure 3-3. Sigmoid-shaped curves representing total incidence of lesions of four major diseases in unrestricted and restricted female rats. The delaying effect of dietary restriction is shown. Onset of lesions is later in female rats than in males, unrestricted or restricted (compare with Fig. 3-2) (From Berg and Simms (1965), reproduced by permission of the *Canadian Medical Association Journal*).

of senescent rats (Berg, *et al.*, 1962a, b). The disease was unrelated to skeletal muscle degeneration. Early lesions showed scattered internodal swelling of myelin sheaths, and later there was segmental myelin degeneration, macrophage formation, and variable sheath cell proliferation. Axones were rarely involved.

Incidence and Time of Onset of Lesions

Analysis of the data on microscopically observed lesions of the four major diseases included 1. incidence and severity at various ages 2. time of onset 3. time interval between early and late changes and 4. the effect of age on the probability of onset of new lesions (Simms and Berg, 1957 and 1962).

The sigmoid-shaped curves in Figures 3-2 and 3-3 show the total incidence of lesions of all degrees of severity for each disease and for the two sexes, at different ages (Berg and Simms, 1965). Radiculoneuropathy was not included because of insufficient data. It is seen that each condition has a characteristic time of onset and rate of development, and appears later in female rats than in males. Representative curves of the relative incidence of early, moderate and severe lesions of myocardial degeneration and skeletal muscle degeneration in male rats are shown in Figures 3-4 and 3-5. By measuring the horizontal distance between the curves, the time required for a lesion to develop can be estimated roughly. From the graphs it is evident that during adulthood the progress of disease as mea-

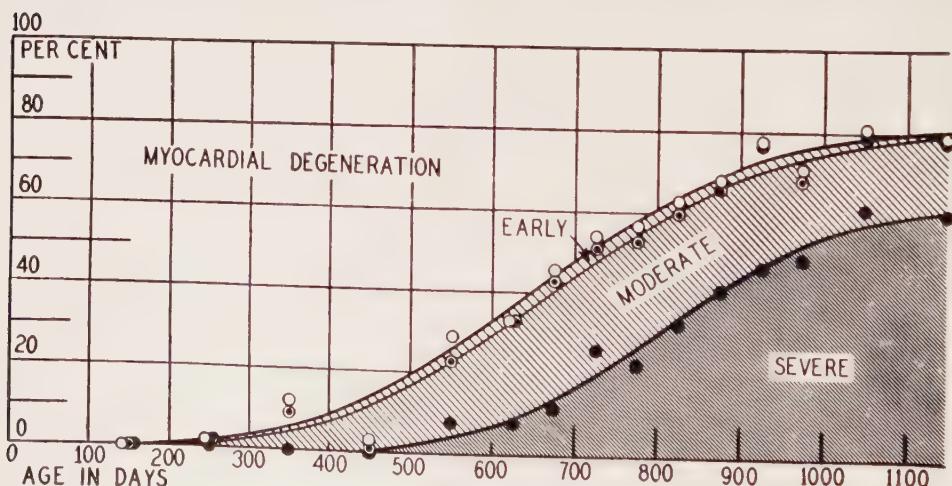


Figure 3-4. Incidence of myocardial degeneration in male rats, expressed as percentage of animals having lesions of different severity at given ages. The top curve represents the total of all degrees of severity (From Simms and Berg (1957), reproduced by permission of the *Journal of Gerontology*).

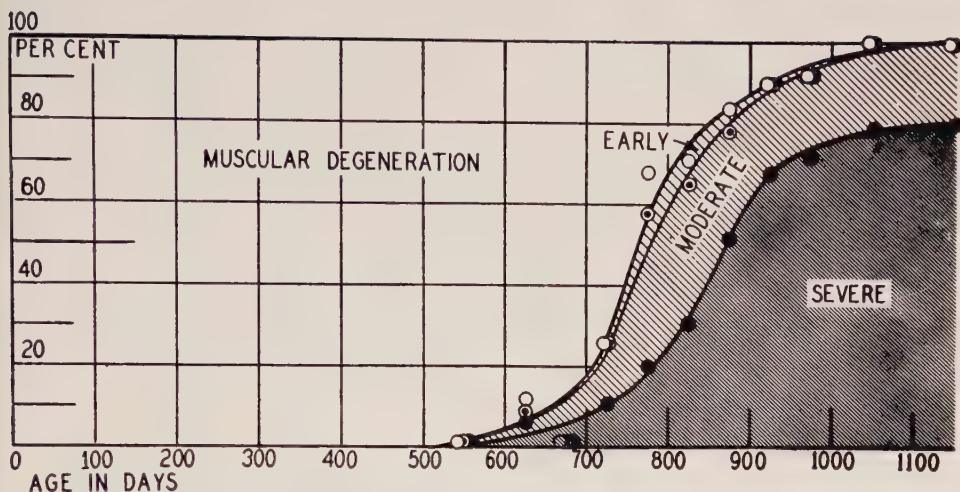


Figure 3-5. Skeletal muscle degeneration in male rats. Note late onset and rapid increase of lesions (compare with Fig. 3-4) (From Simms and Berg (1957), reproduced by permission of the *Journal of Gerontology*).

sured by the time interval from early to late lesions does not change significantly with age. It may be concluded, therefore, that the increase in mortality associated with aging is the result of greater incidence of lesions rather than a change in duration of disease.

Since tumors could not be graded according to time of onset, rate of development or severity, data were limited to total incidence in older rats. At 800 days of age, neoplasms were found in 58 percent of male rats and in 41 percent of females, and at older ages the incidence was 80 to 90 percent in both sexes.

Life Expectancy and Lesion Onset

Age distribution of survival rates (Fig. 3-6 and 3-7) shows that the life-span of male rats is shorter than that of females. The difference in life expectancy of the two sexes is also seen when probability of death is plotted logarithmically against age (Gompertz, 1825). The divergent upper straight line curves for *ad libitum*-fed rats in Figures 3-8 and 3-9 show that the life expectancy of males is 802 days as compared with 930 days for females (Simms and Berg, 1962).

Probability of death and probability of onset of lesions are closely related. This is shown by the solid straight line in Figure 3-10 which is based on data for mortality with age plotted logarithmically against the occurrence of microscopically observed lesions at various ages (Simms and Berg, 1957). It is evident, therefore, that increased mortality and onset of new

lesions are parallel functions of age. It also follows that the forces that hasten or retard the age changes in tissues leading to disease determine the lifespan. This is illustrated by the increased longevity resulting from dietary restriction.

Incidence of lesions of the four major diseases in unrestricted and restricted rats is compared in Figures 3-2 and 3-3. The onset of all lesions was delayed in the restricted groups as evidenced by a shift of the curves to older ages, and survival rates were correspondingly increased (Figs. 3-6 and 3-7). The retardation of lesion onset and the lengthening of lifespan were greater for female rats than for males (Figs. 3-8 and 3-9). By extrapolating the straight line curves of mortality plotted against age to meet each other

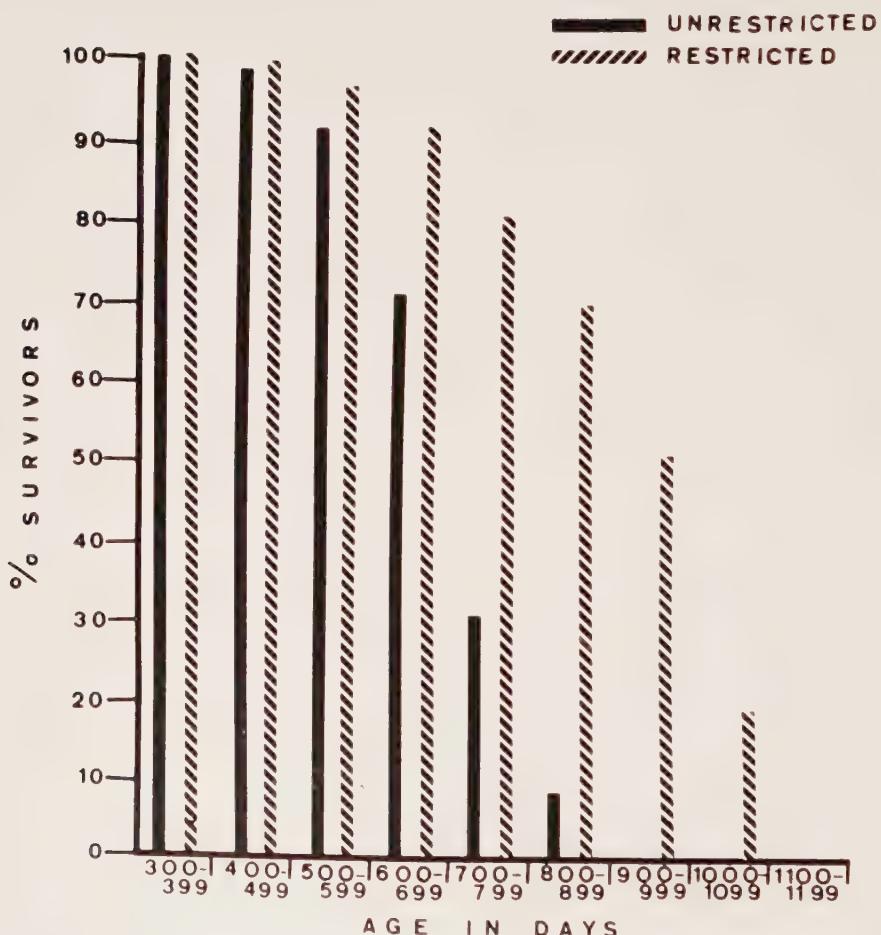


Figure 3-6. Age distribution of survival rates of male rats, unrestricted and restricted.

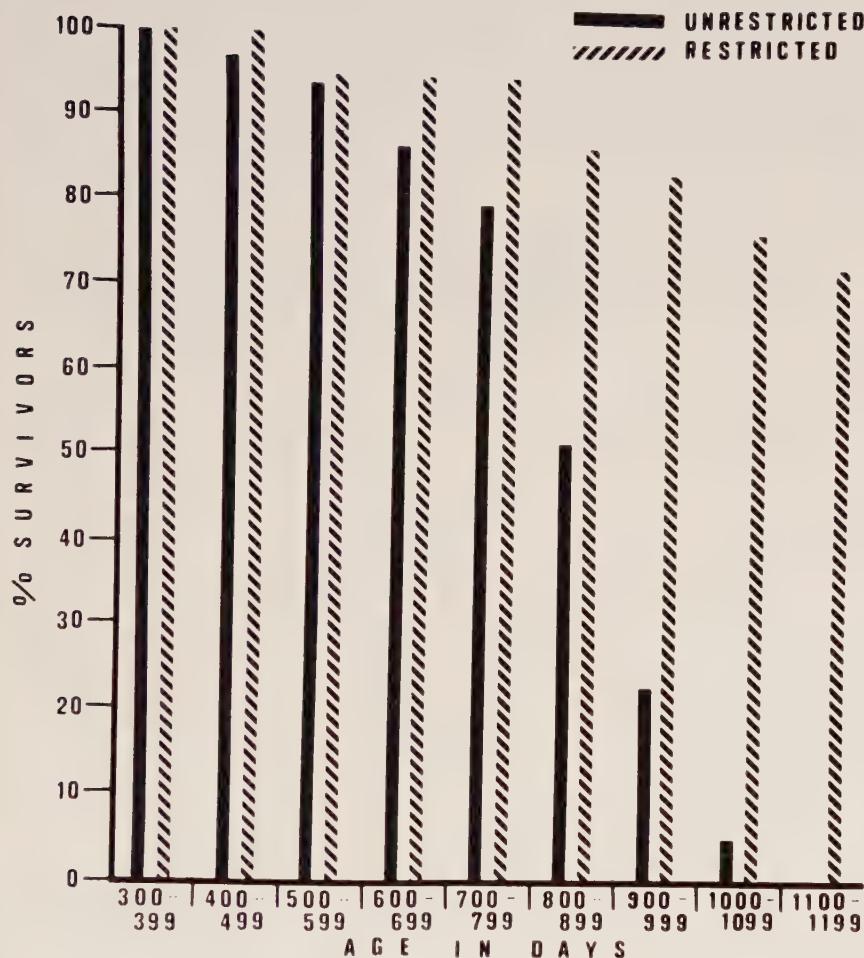


Figure 3-7. Age distribution of survival rates of female rats, unrestricted and restricted.

at age zero (time of birth), and calculating life expectancy from the equation shown in Figure 3-8 it is found that the life expectancy of the restricted males was increased by 25 percent (from 802 to 1,005 days) as compared with 39 percent (from 930 to 1,294 days) for the restricted females. Thus, it appears that age of onset of disease and lifespan are different for the two sexes unrestricted or restricted. However, the factor responsible for the difference could be nutritional rather than hormonal. This suggestion is based on the finding that lesion onset (Figs. 3-2 and 3-3) and survival rates (Figs. 3-6 and 3-7) are substantially the same for both sexes when the body weight of male rats is maintained by dietary restric-

tion at the level of unrestricted females (Fig. 3-1). Due to better food utilization by males, the latter required a lower daily intake than females (10.5 g vs. 13.0 g).

The incidence of spontaneous neoplasms was reduced by food restriction but the effect was only temporary (Berg and Simms, 1961). However, a longer lasting effect was obtained by Saxton (1945, 1948), and Ross and

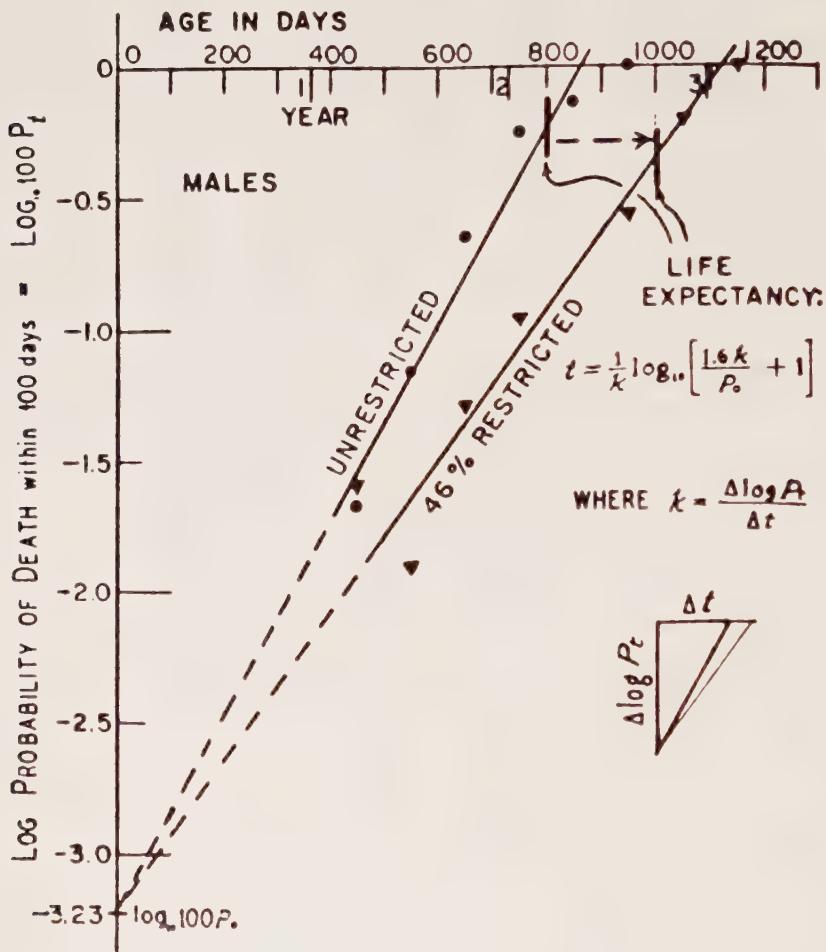


Figure 3-8. Mortality data for male rats, both unrestricted and restricted, plotted as logarithm of mortality rate against age. Each value of 100 P_t (probability of death) is equal to the number of deaths in each 100-day age period, divided by the number of rats alive at the beginning of that period. Slope of lines gives values of k and these values were used in computing the life expectancy of each group of rats (From Simms and Berg (1962), by permission of *Geriatrics*).

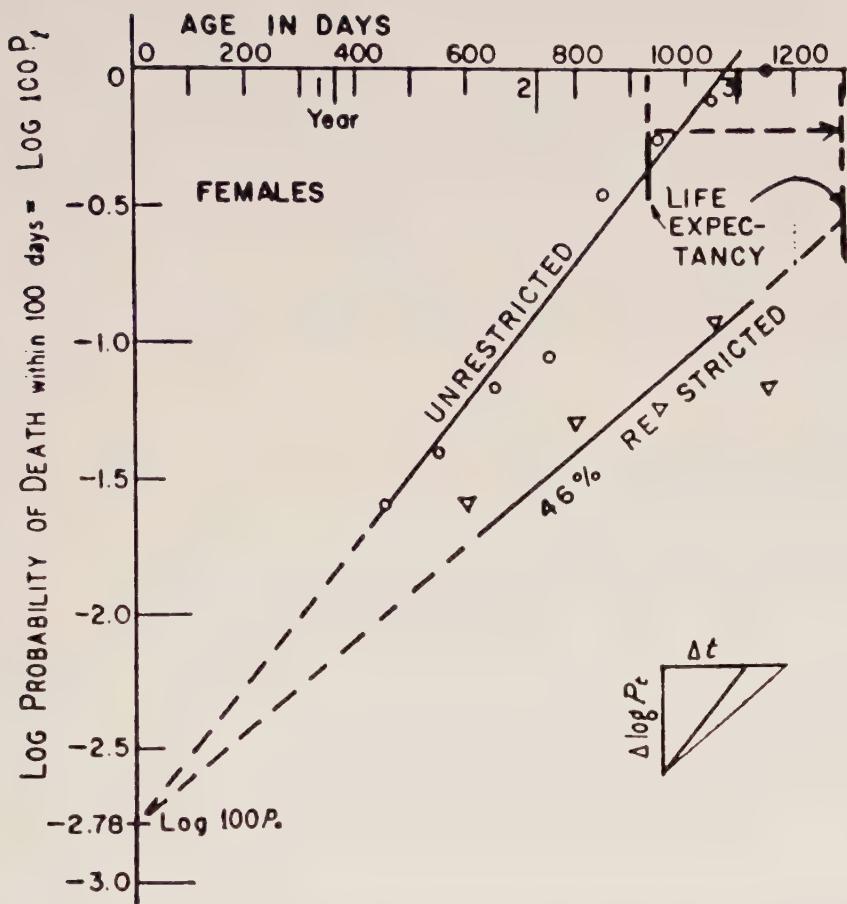


Figure 3-9. Mortality data for unrestricted and restricted female rats (From Simms and Berg (1962), reproduced by permission of *Geriatrics*).

Bras (1965) who used more severely restricted diets. In our series (Berg and Simms, 1961) at 800 days, total tumor incidence decreased from 58 to 26 percent in males, and from 41 to 12 percent in females, while the percentages at older ages returned to higher values. Mammary fibroadenoma was an exception in that the tumor was completely inhibited by dietary restriction in females over 1,100 days old (Fig. 3-11). A similar inhibitory effect on mammary carcinoma in mice was described by Tannenbaum (1940) and Tannenbaum and Silverstone (1953).

From these observations there appears to be a relationship between body growth and tumorigenesis. Dietary restriction leading to retarded skeletal development and delayed sexual maturity has a greater inhibitory influ-

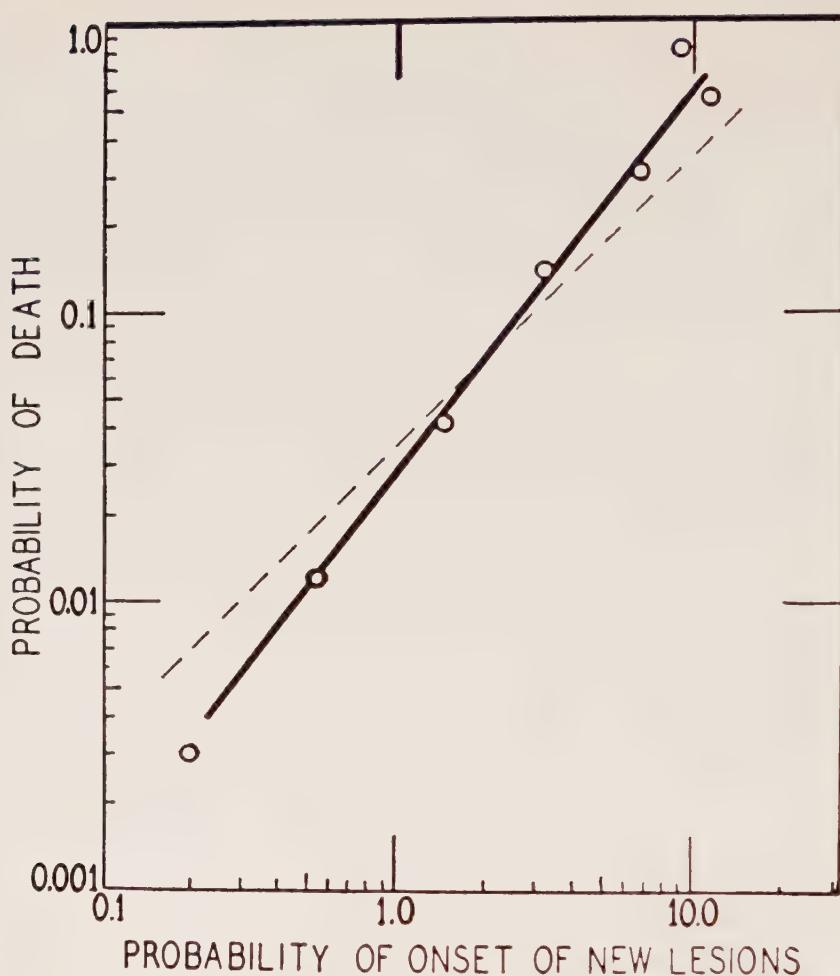


Figure 3-10. Relationship between probabilities of death and of onset of new lesions. Slope of perfect correlation is shown by dotted line (From Simms and Berg (1957), reproduced by permission of the *Journal of Gerontology*).

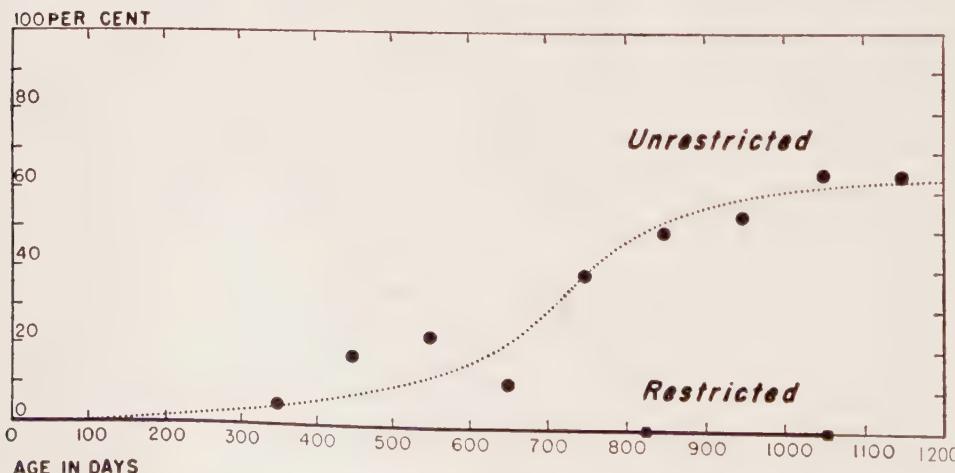


Figure 3-11. Incidence of mammary fibroadenoma in unrestricted and restricted female rats. Note absence of tumors in restricted rats. The smooth dotted curve has a sigmoid shape similar to those of four other major diseases shown in Figures 3-2 and 3-3 (From Berg and Simms (1965), reproduced by permission of the *Canadian Medical Association Journal*).

ence on neoplasia than a level of intake that does not impair growth or attainment of maturity.

Effect of Thyroxine on Nephrosis

In contrast with the delaying effect of dietary restriction on nephrosis, thyroxine hastens the onset of the disease and accompanying proteinuria, hyperglobulinemia and hypercholesterolemia (Berg, 1966). Since metabolic rate is lowered by underfeeding (Best and Taylor, 1961) and increased by thyroxine, it appears that a metabolic factor can influence the progress of the disease. Everitt (1959) observed no significant change in the lifespan of male rats treated with small doses of thyroxine for 200 days.

Conclusions

For each of the major diseases of the rat there is a typical latent period before changes are seen microscopically and each condition has a characteristic sigmoid-shaped curve of lesion incidence with advancing age. The duration of disease measured by the time interval between the early and late stages remains relatively constant throughout adult life. Probability of death parallels probability of onset of lesions. Dietary restriction at a level that has little effect on growth or maturity, but prevents the storage of excess body fat, retards the development of disease and thus extends the lifespan. Evidently, the age changes which govern the susceptibility of tissues to disease can be modified by calorie intake. The aging process also appears to be influenced by a hormonal factor. This is indicated by the later onset of disease in female rats and the longer lifespan as compared with males. However, the possibility that the difference is nutritional in nature rather than sex-dependent cannot be excluded. When the body weight of male animals is kept by dietary restriction at the level of unrestricted females, the age of lesion onset and the life duration are comparable for the two sexes. Because of better food utilization, male rats on a 20 percent lower intake maintain an equal body weight with females.

RADIATION EFFECTS ON DISEASE AND LIFESPAN

Extensive studies by Lorenz, *et al.* (1954), Upton, *et al.* (1960), and Lindop and Rotblat (1961a) demonstrated the life-shortening effect of ionizing radiation in mice. Although the source of radiation, intensity of exposure, and mouse strain used by these investigators differed, the overall results were similar.

Lorenz exposed 50- to 85-day-old LAF₁ mice throughout their life to radium C gamma radiation at rates of 0.11 to 8.8 r per day, with an accumulated dose range of 180 to 2,900 r. The principal spontaneous tumor was

derived from lymphoid tissue (Echenbrenner and Miller, 1954). Incidence of the neoplasm with various intensities of exposure plotted against time in female mice is shown in Figure 3-12. At 4.4 and 8.8 r the curves are shifted to earlier ages while there are no significant differences at lower doses. Substantially the same radiation effects were observed in both sexes.

Frequency of papillonephritis, the principal non-neoplastic disease of LAF₁ mice showed no correlation with dose of radiation and age. The renal lesions were similar to those described by Dunn (1944) in this mouse strain, and consisted of amyloid deposition between the collecting tubules, necrosis at the tip of the papilla, cystic dilatation of glomeruli and tubules, and interstitial fibrosis.

Other common conditions in the LAF₁ strain used by Lorenz included mammary neoplasms, tumors of the lung and chronic dermatitis.

The survival curves for male and female mice combined (Fig. 3-13) show a progressive decrease in survival time with increasing daily dose, with the exception of the animals that received 0.11 r 8-hr day. However, the decreased death rate of the latter group was not statistically significant. There was no predominant cause of death with different doses of gamma radiation.

The weight curves for male mice are given in Figure 3-14. With the ex-

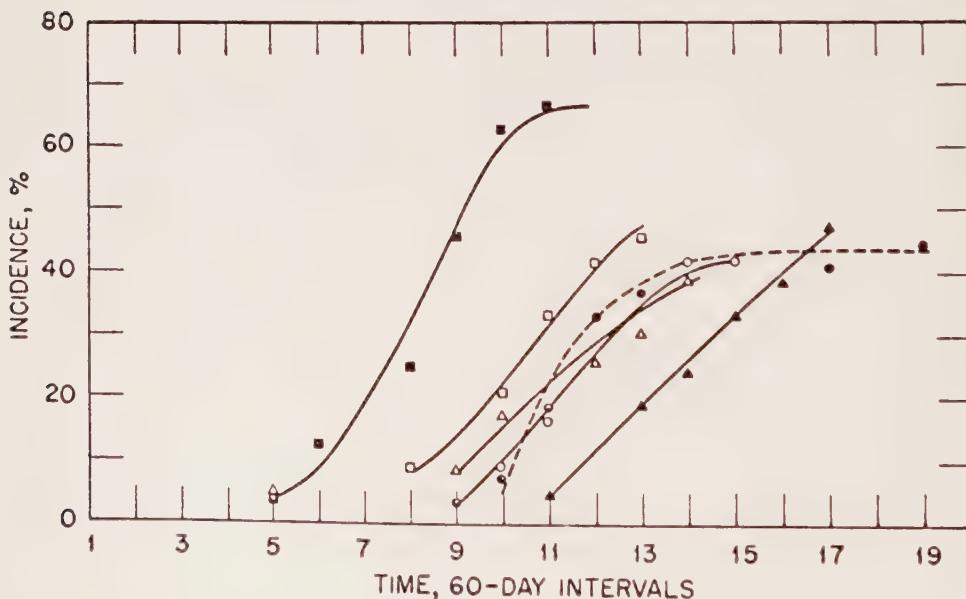


Figure 3-12. Incidence of lymphoid tumors in female LAF₁ mice.

- controls,
 - ▲ 0.11 r/8-hr day,
 - 1.1 r/8-hr day,
 - △ 2.2 r/8-hr day,
 - 4.4 r/8-hr day,
 - 8.8 r/8-hr day.
- (From Lorenz, *et al.* (1954), reproduced by permission of the Atomic Energy Commission).

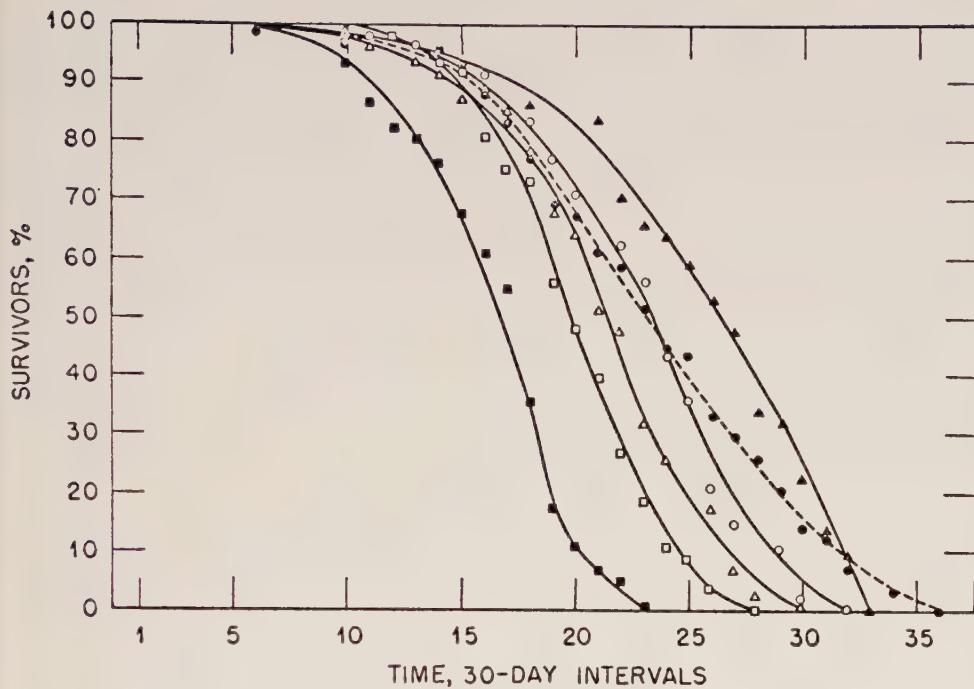


Figure 3-13. Survival rate curves of LAF₁ mice (males and females combined) showing percentage of survivors at 30-day intervals. Symbols denote the same levels of radiation as in Figure 3-12 (From Lorenz, *et al.* (1954), reproduced by permission of the Atomic Energy Commission).

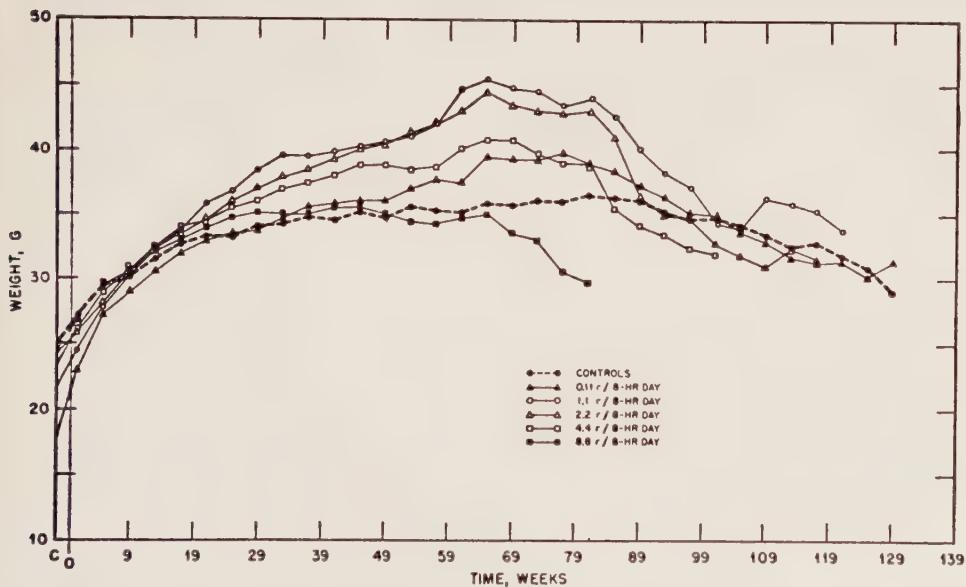


Figure 3-14. Weight curves of male LAF₁ mice exposed to varying doses of gamma radiation from radium C (From Lorenz, *et al.* (1954), reproduced by permission of the Atomic Energy Commission).

ception of the group exposed to 8.8 r, all curves of the other groups are higher than the control. Maximum weight is reached at about 69 weeks. Then a decline sets in and body weights return to the level of the control group. A similar but less pronounced effect was observed in females. Lindop (1965) noted a weight increase in mice after a single whole-body exposure to 50 r of gamma radiation from x-rays. She measured food intake and found no difference in the amounts eaten by irradiated or nonirradiated animals. This investigator also observed skeletal enlargement of her irradiated mice and suggested that pituitary dysfunction could be the cause of increased body size. Both Lorenz and Lindop noted deposition of excess body fat in their irradiated animals. With due reservations regarding the significance of findings in rodents with different metabolic patterns, it is noteworthy that overweight and obesity in rats and mice predispose to premature onset of disease and shortened life expectancy.

Lindop and Rotblat (1961b) studied the long-term effects of a single whole-body exposure of male and female 30-day-old SAS 4 mice to MeV x-rays at 9 dose levels (50 to 780 r). A single cause of death was assigned to each animal. About 40 percent of male deaths were ascribed to leu-

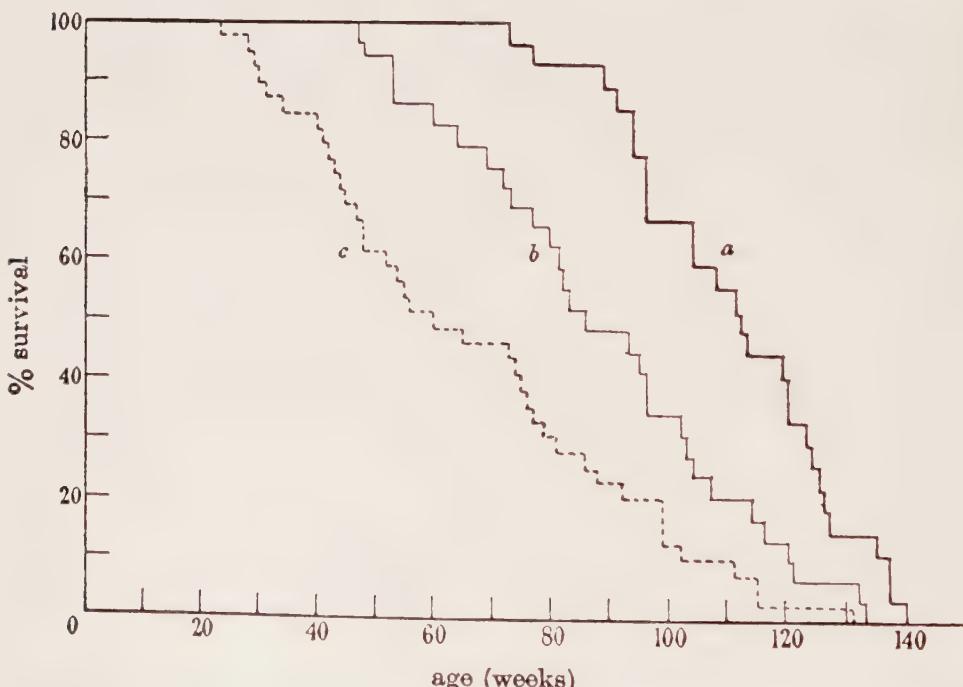


Figure 3-15. Age distribution of deaths from leukemia in male SAS/4 mice: a, control group; b, 198 r group; c, 457 r group (From Lindop and Rotblat (1961), reproduced by permission of the *Proceedings of the Royal Society*).

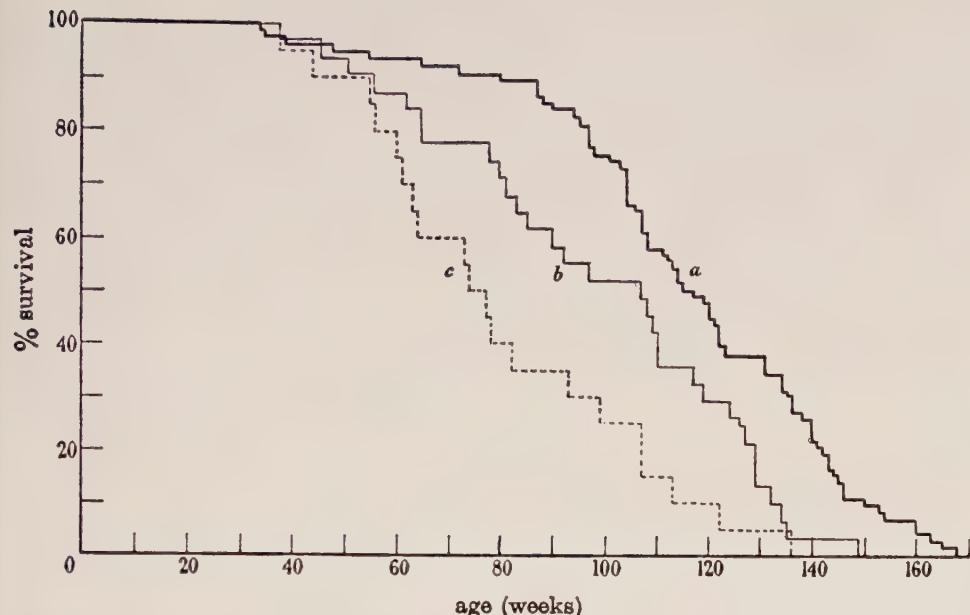


Figure 3-16. Age distribution of deaths from renal disease in male SAS/4 mice; a, control group; b, 198 r group; c, 457 r group (From Lindop and Rotblat (1961), reproduced by permission of the *Proceedings of the Royal Society*).

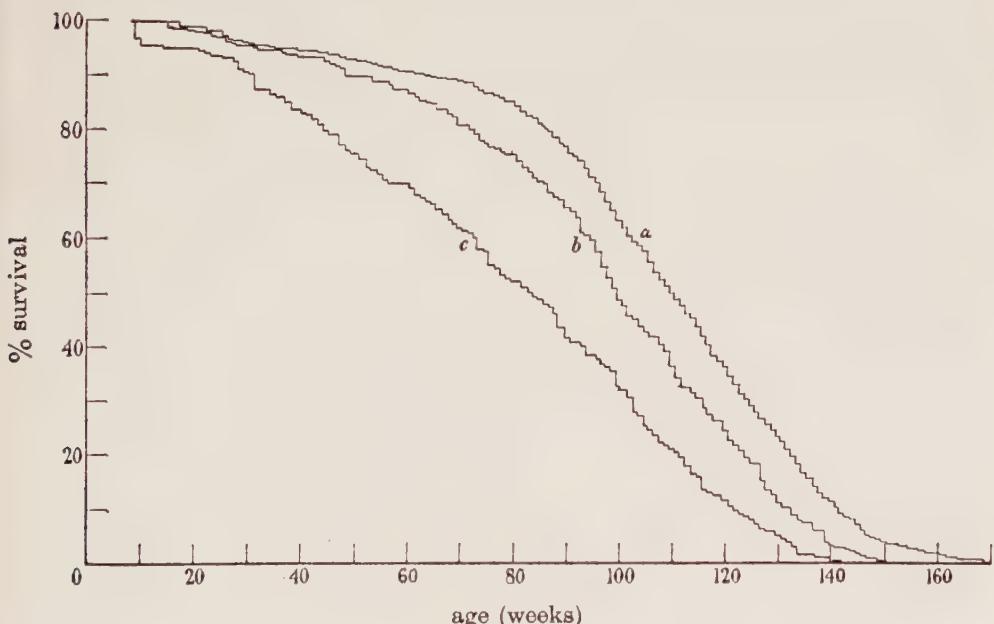


Figure 3-17. Survival curves of male SAS/4 mice: a, control group; b, 198 r group; c, 457 r group (From Lindop and Rotblat 1961), reproduced by permission of the *Proceedings of the Royal Society*.

kemia, lung tumors, and liver tumors; in females, leukemia and neoplasms of the lung and ovary accounted for 45 percent of deaths. The principal non-neoplastic conditions included infections and degenerative diseases of the lung, the kidney and the liver, as well as intestinal hemorrhage, obstruction, or infection.

Plots of age distribution of deaths from leukemia (Fig. 3-15) and from renal disease (Fig. 3-16) in male mice exposed to 198 r and 457 r show that the curves for the irradiated groups are advanced to earlier ages according to dose, and are nearly parallel with that of the control. Survival curves based on deaths from all causes are also displaced to the left and the advancement is dose-related (Fig. 3-17). All diseases developed at earlier ages but not at the same rate. Exposure to 50 r had no effect on survival rate.

Upton, *et al.* (1960) observed the delayed effects of atom-bomb radiation on 6- to 12-week-old (*C57L X A/He*)F₁ mice of both sexes. The mice were exposed to 223, 368, 578 and 697 r of gamma rays by placement at various distances from the hypocenter. Incidence of disease was based on the condition judged to be the primary cause of death. Neoplasms were more common than other conditions and were chiefly lymphomas, lung tumors, and hepatomas. Infection was the principal non-neoplastic disease and included pneumonia, dermatitis, abscesses and enteritis. The incidence of spontaneous nephrosclerosis was low.

The graphs in Figure 3-18 show that the development of neoplasms is

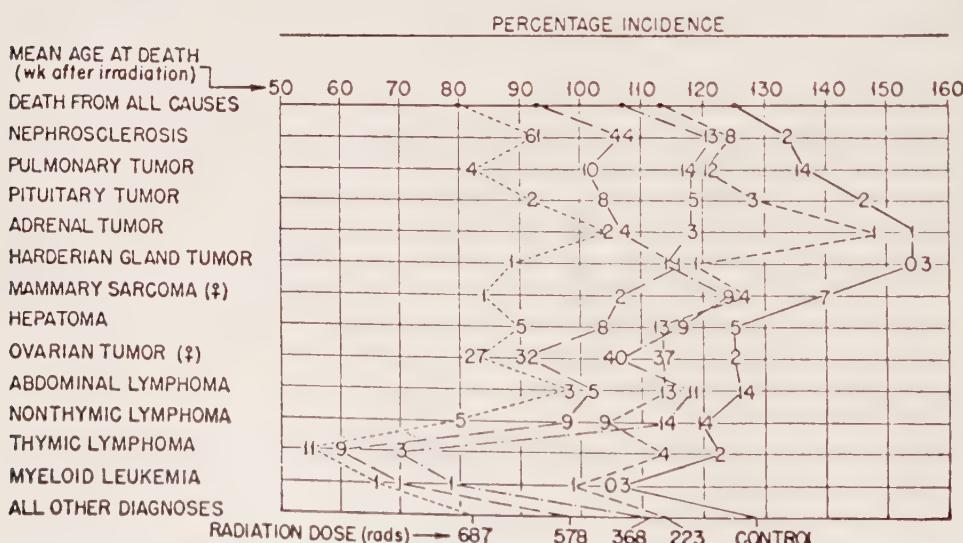


Figure 3-18. Mean age at death and incidence of various diseases in (*L X A*)F₁ mice exposed to gamma rays from an atomic bomb (From Upton (1960), reproduced by permission of S. Karger, Basel).

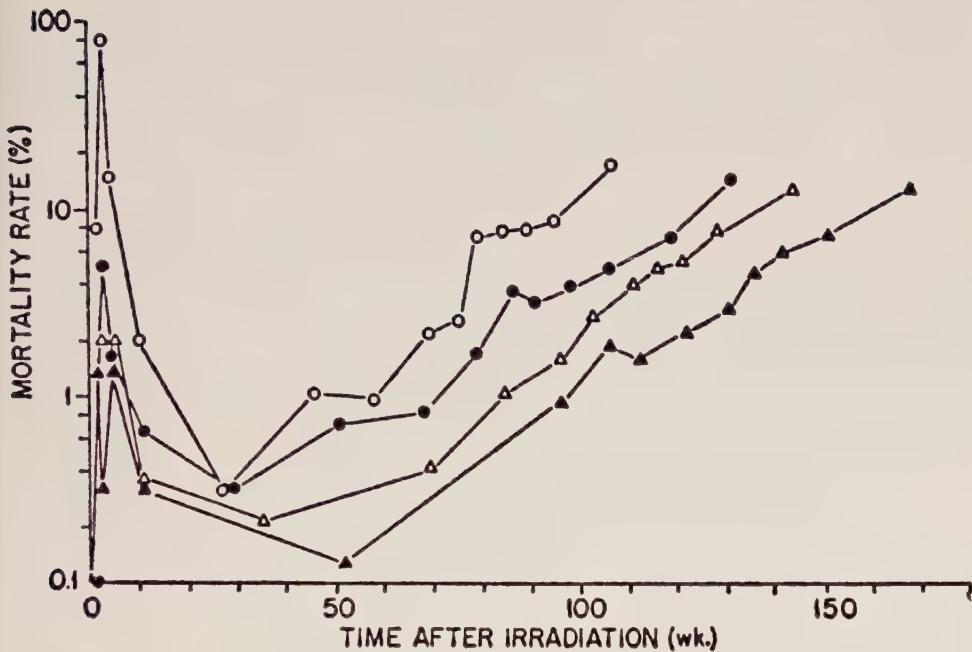


Figure 3-19. Mortality rate in female (L X A)F₁ mice exposed to gamma rays at 6-12 weeks of age. ○ 687 r, ● 578 r, △ 368 r, and ▲ nonirradiated control (From Upton (1960), reproduced by permission of S. Karger, Basel).

advanced to earlier ages in proportion to radiation dose and the mean age at death from all causes is correspondingly shortened. It is also seen that each type of tumor develops at a different time, but for a given dose of radiation the time of development follows the pattern set by the nonirradiated controls. In contrast with the wide variation in tumor incidence at different levels of irradiation, there is a progressive increase in nephrosclerosis with increasing intensity of exposure. A similar dose-related effect on nephrosclerosis was observed by Guttman and Kohn in rats (1960) in BALB/c mice (1963), and by Dunn (1967) in the latter strain as well as in CAF₁ mice. With minor variations the histologic changes described by Gorer (1940), Gude and Upton (1962), Dunn (1967), and Tucker and Baker (1967) are comparable. In the early stage of the disease there is thickening of the basement membranes of the glomerular capillaries, and deposition of PAS positive material in the mesangium. Later, the glomeruli show hyalinization and fibrosis, and the tubules are greatly dilated or atrophied. In many respects the lesions are similar to the changes seen in nephrosis in the rat.

The life-shortening effect of graded doses of gamma radiation are shown graphically in Figure 3-19. With the exception of the highest dose, the Gompertz curves for life expectancy are roughly parallel. At exposure

to 687 r, the curve of mortality is not only advanced to an earlier age but is also increased in slope (Upton, 1960). This may be explained by the exceedingly high incidence of nephrosclerosis.

Conclusions

The delayed effects of a single whole-body exposure of young mice to gamma radiation are similar to the cumulative effects of long-term exposure to small doses. Lifespan is shortened and time of development of lethal disease is advanced in proportion to intensity of radiation. Thus, it is concluded that the age changes leading to the onset of lesions are accelerated. Whether these changes represent premature natural aging or result from irreparable irradiation injury is not known.

RELATIONSHIP OF PATHOLOGY AND AGING

Little is known about the etiology of the diseases of senescence or the nature of the aging process, and thus the relationship between the two remains obscure. In general it may be stated that tissues become more susceptible to disease with increasing age, and that the forces accelerating or retarding the time of onset of lesions determine the lifespan.

The age changes in tissues can be influenced by both intrinsic and extrinsic factors. For example, the characteristic pathology of each species and even strain suggests intrinsic tissue differences. The latter may also be involved in the wide variety of constitutional diseases of genetically controlled mice. Effects of extrinsic factors such as level of food intake, sex, metabolic rate, and ionizing radiation are described in the present chapter.

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CHAPTER 4

HYPOPHYSECTOMY AND AGING IN THE RAT

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SUMMARY

HYPOPHYSECTOMY IN THE YOUNG RAT retards a number of physiological age changes in tail tendon collagen, the skeleton, the kidney and the ovary. It also inhibits the development of pathological changes in old age in the kidney, and the incidence of tumors is much lower. Despite the anti-aging action of hypophysectomy, the lifespan of the rat is markedly reduced after this operation. However, the lifespan of the hypophysectomized male rat can be restored almost to normal by periodic treatment with physiological doses of cortisone (1 mg per week). These studies indicate that in the rat, the pituitary gland secretes both aging and life-maintaining factors.

INTRODUCTION

In the hypopituitarism of Simmonds' disease there appears to be an acceleration of aging as described by Herman in Chapter 9.

In 1926 Smith developed a technique for hypophysectomy in the rat. Smith (1930) noticed that many rats hypophysectomized when 4½ months old developed a shaggy coat and a cachetic appearance, which seemed similar to the condition of human patients with Simmonds' disease. However, when objective measures of aging were used, Olsen and Everitt (1965) and Verzár and Spichtin (1966) showed that the aging of collagen was retarded in the hypophysectomized rat. This chapter reviews the literature on the effects of hypophysectomy on aging processes in a number of organs and tissues in the rat.

COLLAGEN

The long-term effect of hypophysectomy performed in young rats is to retard the aging process in rat tail tendon (Fig. 4-1). Two years after hypophysectomy the collagen fibers of tail tendon have the same collagen age as those from a one-year-old control rat. This subject is discussed in more detail by Everitt and Delbridge in Chapter 11.

SKELETAL AND BODY GROWTH

Following hypophysectomy (at 28 to 60 days) there is almost complete cessation of skeletal and body growth in the rat. The body weight of the

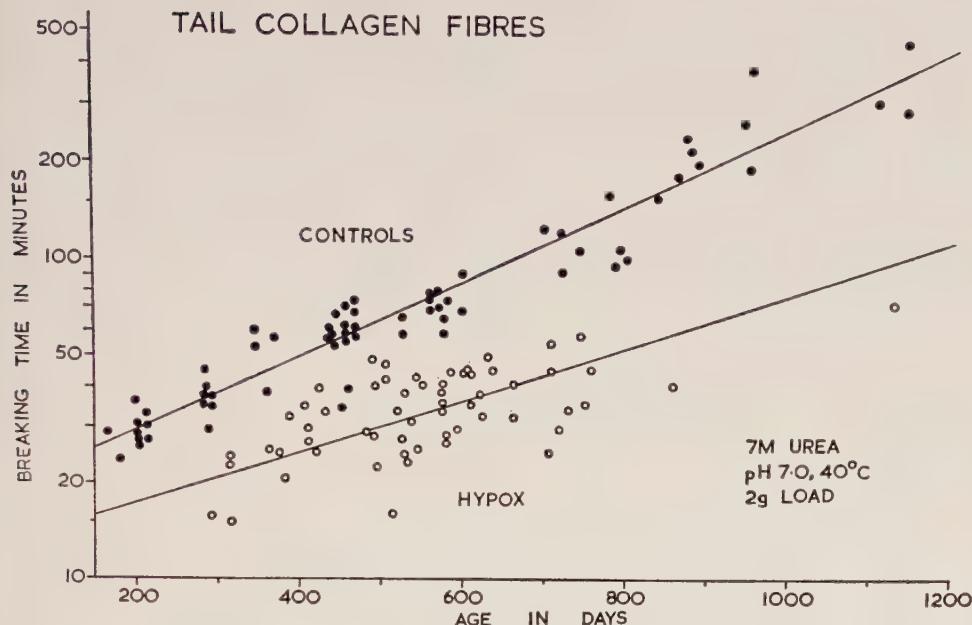


Figure 4-1. The effect of hypophysectomy in the young rat at 50 days in retarding the aging of collagen fibers in tail tendon. Collagen fibers from a 2-year-old male hypophysectomized rat have a similar collagen age (breaking time in 7 M urea at 40°C under a load of 2 g) to those of a 1-year-old intact rat (from Everitt, *et al.*, 1968) reproduced by permission of the *Journal of Gerontology*.

hypophysectomized rat does not show any significant increase with age (Fig. 4-2). There is however, a terminal fall in weight as the hypophysectomized rat approaches death, although somewhat smaller than in the control rat (Everitt, 1957).

Skeletal maturation is markedly retarded after hypophysectomy. The skeletal age of female rats hypophysectomized at age 1 month was estimated by Asling, *et al.* (1954) to advance only one month over the next 12-month period. Skeletal age increased by only 7 days between the chronological ages of 4 and 13 months.

When hypophysectomies are performed at a very early age (6 days) premature death occurs due to respiratory paralysis. Skull growth has practically ceased in these rats, but the brain continues to grow normally and hence a fatal compression of the brain eventually occurs (Walker, *et al.*, 1950).

METABOLISM

The minimal oxygen consumption of the rat declines with age. Probably 75 percent of this decline is due to a pituitary factor which decreases the re-

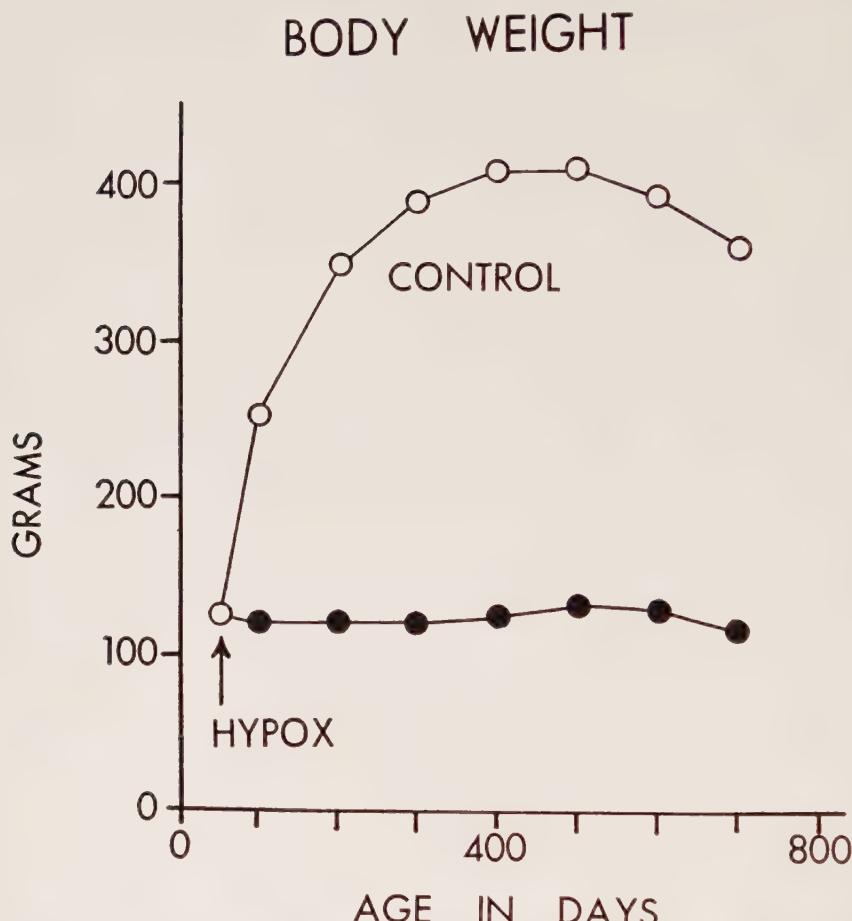


Figure 4-2. The effect of hypophysectomy in abolishing growth in the male rat. Body weights were recorded on 23 intact and 18 hypophysectomized rats living more than 700 days.

sponsiveness of peripheral tissues to thyroid hormones (Denckla, 1974). Immature rats are three times more responsive to thyroxine than adults. Hypophysectomy partly restores in adult rats the responsiveness to thyroxine found in immature rats, and arrests the normal age-associated decrease in responsiveness to thyroxine (Denckla, 1974).

KIDNEY

Hypophysectomy abolishes the progressive rise in protein excretion which occurs with increasing age in the rat (Everitt and Duvall, 1965) as shown in Figure 4-3. This proteinuria has been associated with the development of chronic renal disease (Berg, 1965). Hypophysectomy also retards

histological age changes such as the thickening of basement membranes in proximal tubules and glomeruli, thickening of Bowman's capsule, dilatation and atrophy of tubules and dilatation of glomeruli. Histologically the kidney of the old hypophysectomized rat resembles that of a young intact rat. Hypophysectomy halves the rate of thickening of the basement membrane lining the proximal tubule (Fig. 4-4).

In the old rat, the kidney enlarges in order to compensate for the loss of nephrons (Kennedy, 1957). This change appears to be similar to the renal hypertrophy induced experimentally by unilateral nephrectomy (Kennedy, 1957). The work of Astarabadi (1962) and others indicates that hypoph-

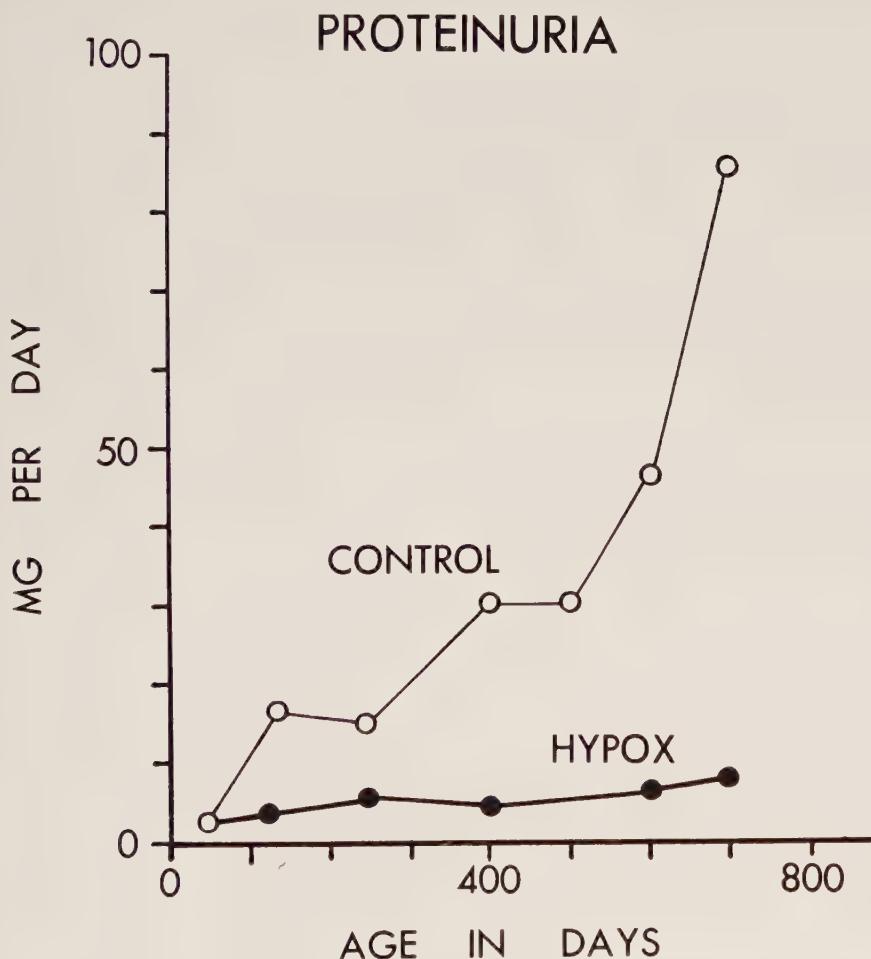


Figure 4-3. The effect of hypophysectomy in preventing the development of proteinuria in the male rat. Protein excretions were recorded on 23 control and 18 hypophysectomized rats living more than 700 days.

sectomy prevents compensatory renal hypertrophy following unilateral nephrectomy in the rat, although this has been questioned by Ross and Goldman (1970). Spontaneous renal hypertrophy is not seen in old hypophysectomized rats (Fig. 4-5).

HEART

Heart rate falls progressively with age in the rat (Everitt, 1958b; Everitt and Cavanagh, 1965). The acute effect of hypophysectomy is to reduce heart rate from 390 beats per minute in intact male rats to 240 beats in hypophysectomized male rats (Everitt and Cavanagh, 1965). Heart rate declined slowly with age between 150 and 800 days in intact controls, but not in hypophysectomized rats (Fig. 4-6). Thus, hypophysectomy may reduce the natural decline with age in heart rate.

Ventricular hypertrophy is a common finding in the old rat (Berg and

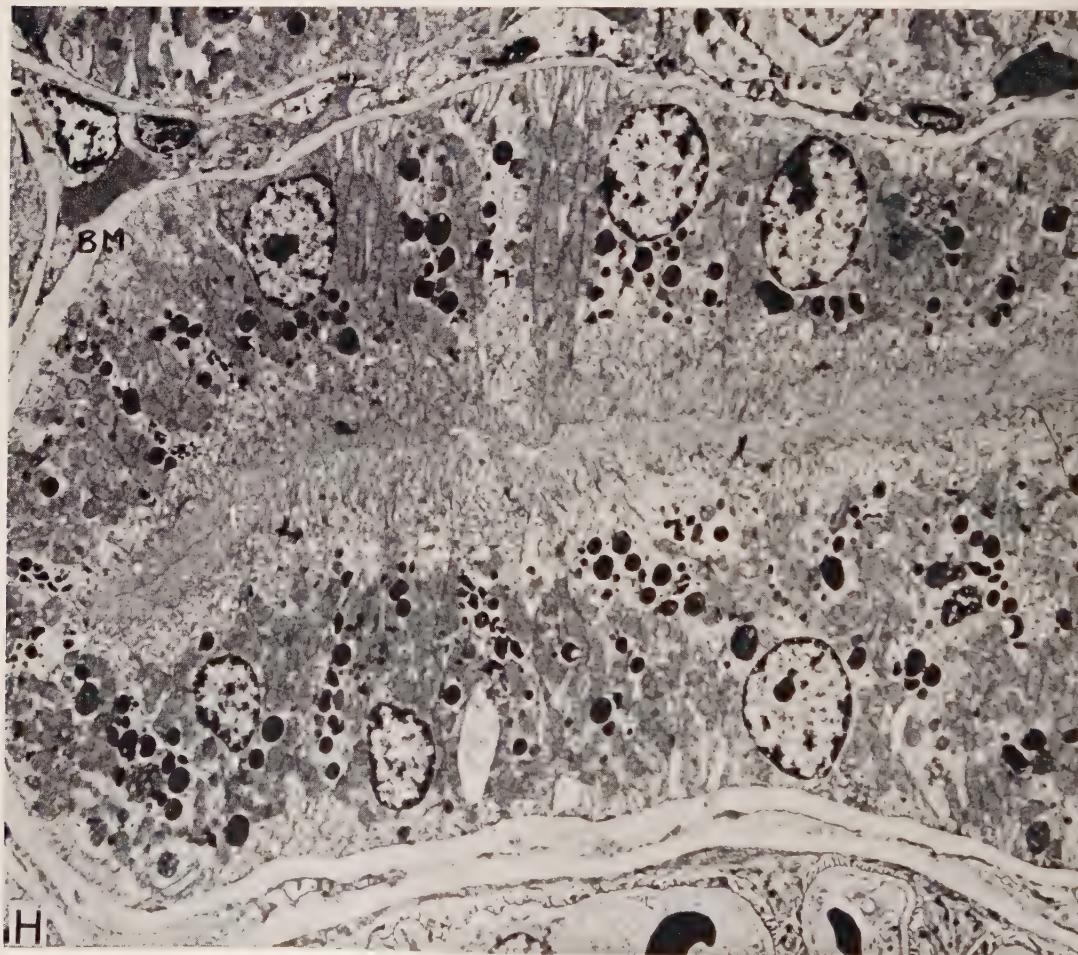




Figure 4-4. Hypophysectomy at 50 days retards the thickening of the basement membrane (BM) of the proximal tubule in the kidney of the rat. In old age at 800 days basement membrane thickness in the hypophysectomized rat (H) is half that of the control (C) ($\times 4,000$). Dr. Cedric Shorey and Miss Judy Major kindly supplied these micrographs.

Harmison, 1955; Everitt, 1958b). Autopsy data (Fig. 4-7) on our old hypophysectomized male rats (800 days and older) revealed a significantly smaller rise in ventricular weight than in the intact controls. Therefore the age-related rise in ventricular weight is partly dependent on the pituitary.

ARTERIES

The effect of hypophysectomy on arterial aging is largely unknown. Preliminary studies suggest that hypophysectomy may inhibit a number of age

KIDNEY WEIGHT

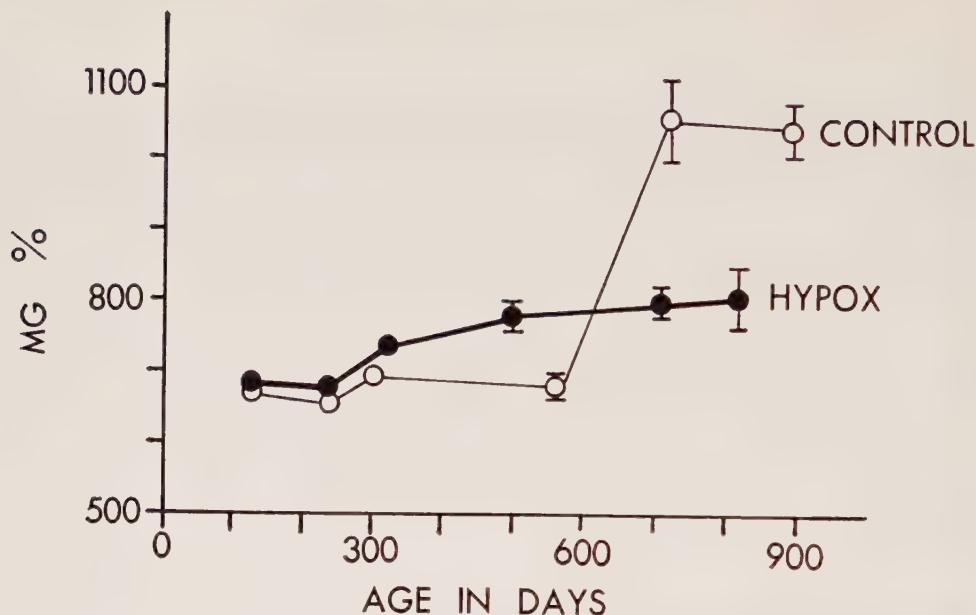


Figure 4-5. The effect of hypophysectomy in preventing renal hypertrophy in the old male rat. There were 10 rats in each age group. Kidney weights are mean \pm S.E.

HEART RATE

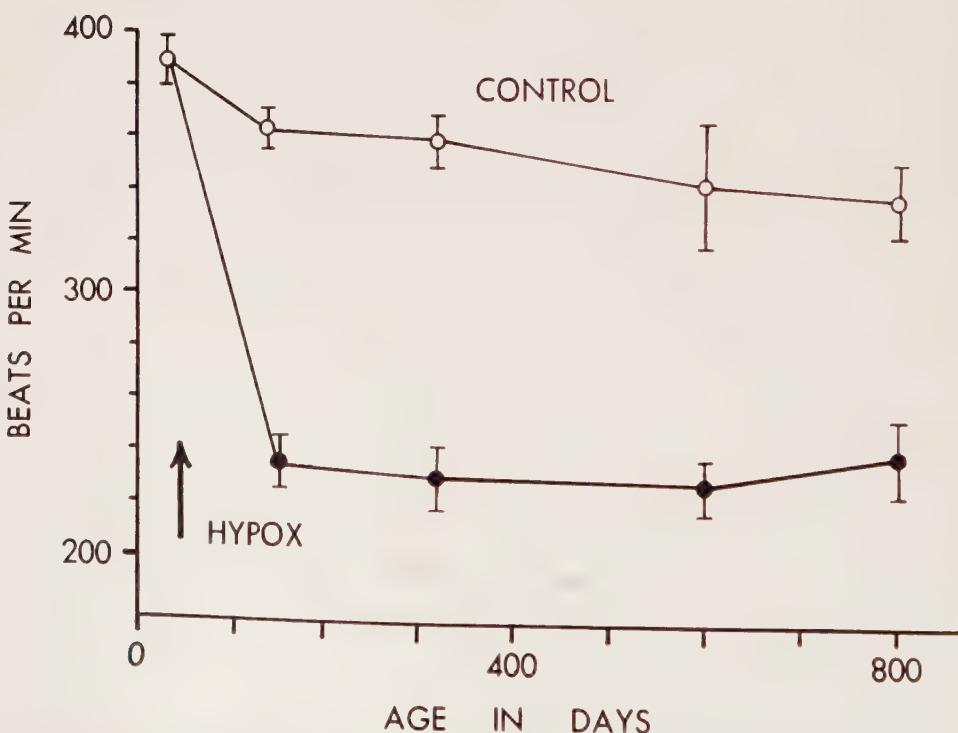


Figure 4-6. The decrease with age in the heart rate (mean \pm S.E.) of 20 intact male rats, measured under pentobarbital anesthesia. After a dramatic fall following hypophysectomy, heart rate remained constant in 15 rats between ages 150 and 800 days.

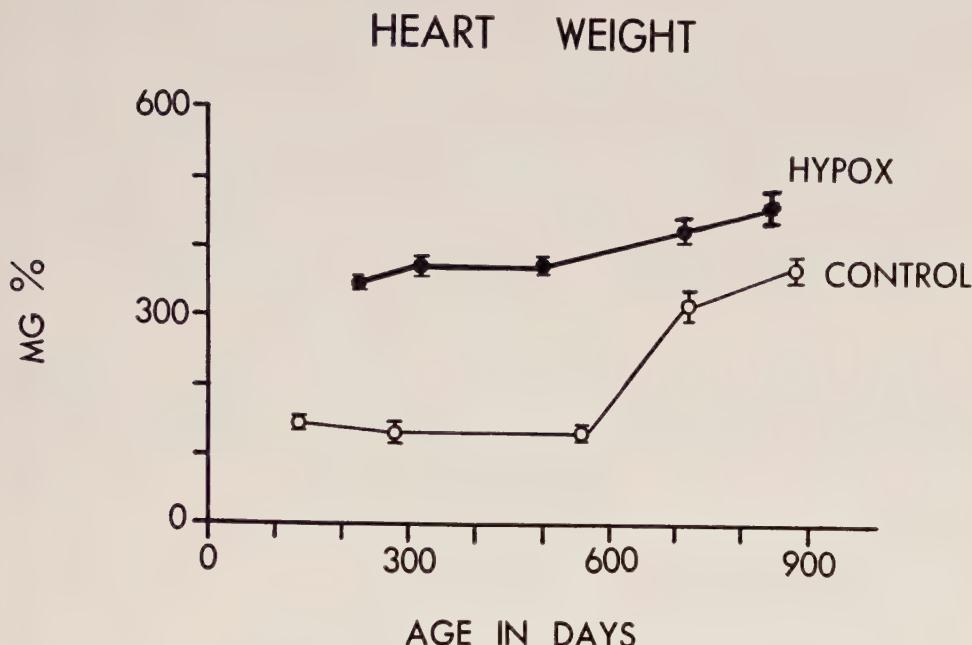


Figure 4-7. The inhibitory action of hypophysectomy on the development of cardiac hypertrophy in the old male rat. There were 10 rats in each age group. Heart weights are mean \pm S.E.

changes in the aorta of rats, such as the loss of distensibility and the degeneration of the elastic lamina (Everitt, Chapter 14).

Data on the experimental induction of atherosclerosis in the hypophysectomized rat are inconclusive. Patek, *et al.* (1963) reported an accelerated onset of atherosclerosis in the aorta of hypophysectomized rats, while Krol, *et al.* (1968) found that development was inhibited.

Arterial blood pressure has been shown to increase in old age in the rat (Berg and Harmison, 1955), just as it does in man. The long-term effect of hypophysectomy on the development of hypertension in the rat has not yet been studied. However, it is known that the short-term effect of hypophysectomy is to reduce the blood pressure of the rat due to a decreased cardiac output (Beznák, 1959). Also experimental DOCA hypertension develops more slowly in the hypophysectomized rat and the associated cardiac and renal lesions may be absent or more moderate than in the intact rat (Hall, *et al.*, 1964). The effect of hypophyseal hormones on blood pressure is discussed by Kovacs and Horvath in Chapter 13.

WATER BALANCE

In the senile rat (900 days old) both water consumption and urine production increase (Everitt, 1958a; Osborn, *et al.*, 1962; Foley, *et al.*, 1964).

This disturbance in water (and also electrolyte) metabolism involves a reduction in neurohypophyseal function (Friedman, *et al.*, 1956; Turkington and Everitt, Chap. 7).

Hypophysectomy produces a deficiency of the antidiuretic hormone which is secreted by the neurohypophysis. As a result, hypophysectomized rats excrete significantly more urine at all ages than controls (Everitt and Cavanagh, 1965). However, there was no significant deviation from the normal age change in either water consumption or urine production up to 700 days (the age of the oldest hypophysectomized rats studied).

BLOOD HEMOGLOBIN

It is well known that hypophysectomy produces a fall in the hemoglobin level of the rat (Crafts and Meineke, 1959; Bozzini, 1965; Piliero, 1969).

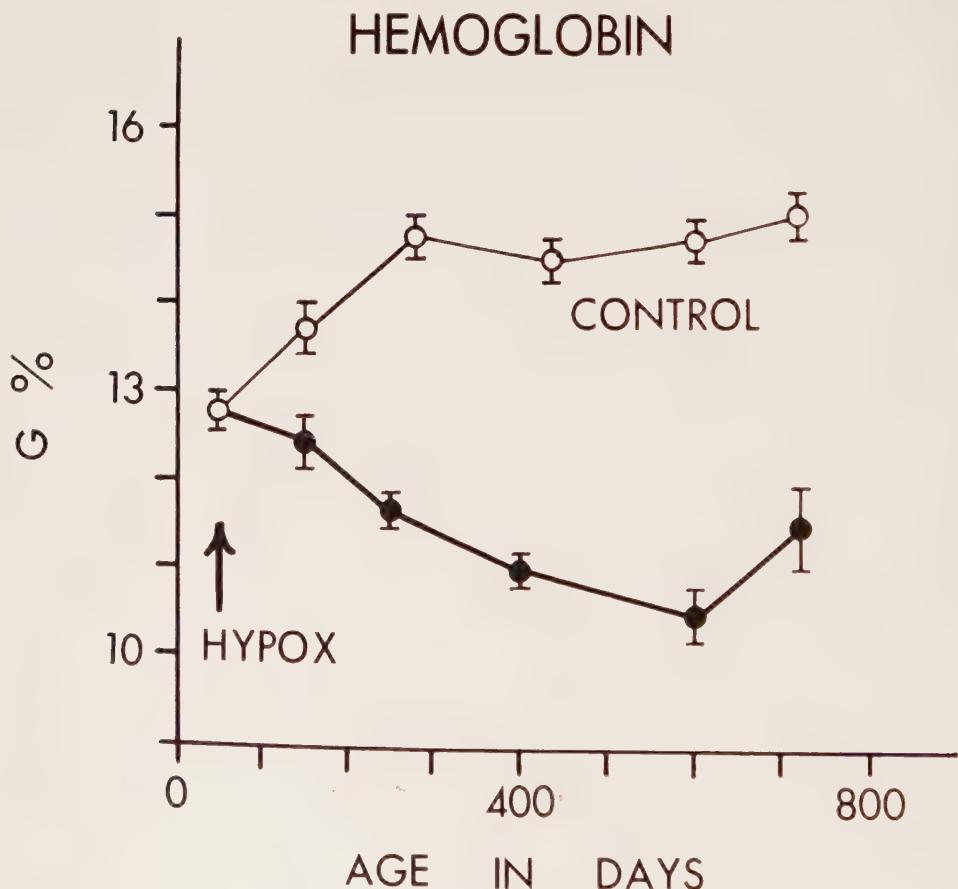


Figure 4-8. The progressive fall throughout life in the blood hemoglobin content of the hypophysectomized male rat. Hemoglobins are mean \pm S.E. (partly from Everitt and Cavanagh (1965), reproduced by permission of S. Karger, Basel).

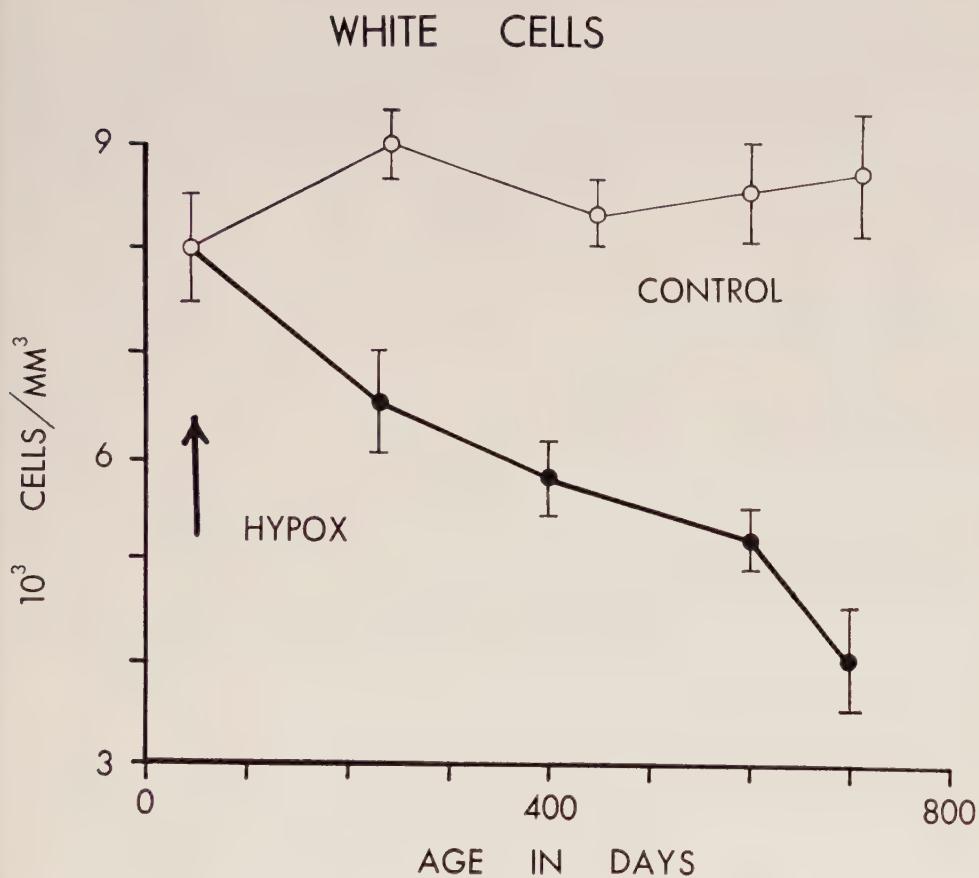


Figure 4-9. The progressive fall throughout life in the blood white cell count of the hypophysectomized male rat (partly from Everitt and Cavanagh (1965), reproduced by permission of S. Karger, Basel).

In a longitudinal study, Everitt and Cavanagh (1965) showed that hemoglobin values continued to fall throughout life in the hypophysectomized rat (Fig. 4-8) while the values in intact rats remained constant after maturity.

The anemia of the hypophysectomized rat may be physiological. It may result from the reduced oxygen requirement (low metabolic rate) of the hypophysectomized rat (Crafts and Meineke, 1957; Bozzini, *et al.*, 1968) and hence may not influence the life expectancy of the rat.

The lifespan (survival time) of red blood cells is prolonged significantly in hypophysectomized rats (Landau and Bristol, 1971). This may be associated with the reduced turnover of red cell iron in the hypophysectomized rat (Bozzini, 1965).

BLOOD WHITE CELL COUNT

Everitt and Cavanagh (1965) found that the total white cell count fell progressively throughout life in the hypophysectomized rat (Fig. 4-9). The count did not change with age in intact mature rats. The low white cell count does not account for the lowered resistance to infection in hypophysectomized rats, since the count is also low in FR rats (Table 4-I).

OVARY

Jones and Krohn (1961) showed that hypophysectomy in the mouse significantly retards, but does not prevent, the normal progressive loss of oocytes from the ovary (Fig. 4-10). Ovarian grafts from middle aged (300-400 days) hypophysectomized mice, when transplanted into young ovariectomized mice, were able to produce embryos which developed into normal mice. One ovarian graft from a CBA mouse "produced" its last litter at 502 days. In the normal CBA mouse, oocytes have disappeared completely at the age of 430 days. This experiment clearly demonstrated that hypophysectomy is able to retard aging of the ovary in the mouse. In an earlier study on the rat Ingram (1953) reported a slower disappearance of oocytes after hypophysectomy, but his data were not statistically significant.

TUMORS

Destruction of the pituitary by deuteron irradiation suppressed the appearance of spontaneous tumors in pituitary-dependent endocrine glands of the male rat, 27 months after irradiation (Van Dyke, *et al.*, 1959). The incidence of other spontaneous tumors may also be reduced by surgical removal of the pituitary gland. Gross examination of 24 old (700 to 948 days; one 1,127 days) hypophysectomized male rats in our laboratory failed to reveal any tumors. In a randomly chosen control series of 24 male rats of almost identical ages, 7 tumors (2 testicular; 3 abdominal nonendocrine; 1 mammary; 1 skin tumor) were seen.

Experimental tumorigenesis is often suppressed by hypophysectomy. Chronic injections of pituitary growth hormone over a period of 2 years produce tumors in many different organs in the intact rat, but not in the hypophysectomized animal (Moon, *et al.*, 1951). Hypophysectomy in the rat inhibits carcinogenesis induced by methylcholanthrene at the site of intramuscular injection (Moon and Simpson, 1955), and abolishes carcinogenesis induced by 3 methyl 4-dimethylaminoazobenzene in the liver (Dodge, *et al.*, 1961).

FOOD INTAKE

Hypophysectomy in the rat sharply reduces food intake to about one third of the normal adult intake (Fig. 4-11). This value is maintained throughout life, except for an acute fall before death.

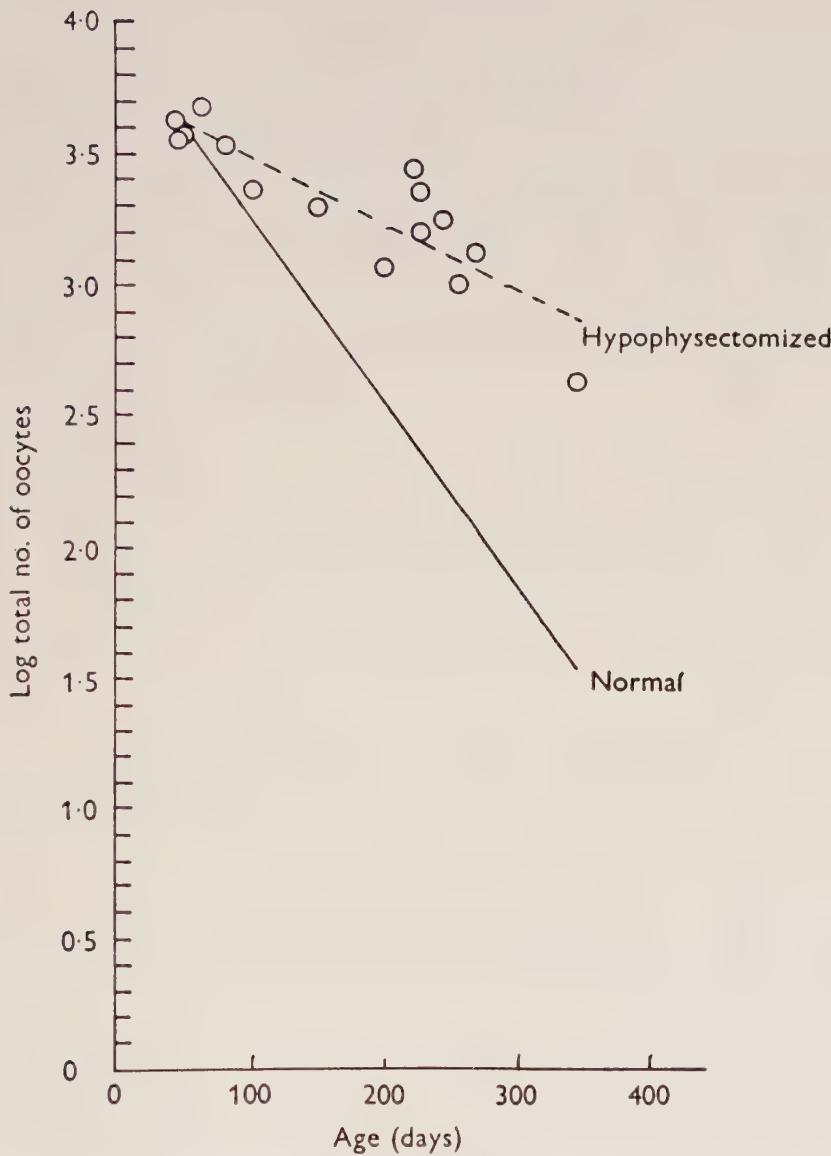


Figure 4-10. The relationship between age and the total number of oocytes in hypophysectomized CBA strain female mice compared with normal mice (from Jones and Krohn (1961), reproduced by permission of the authors and the *Journal of Endocrinology*).

Food restriction, like hypophysectomy, retards many aging processes. In Table 4-1 a comparison of a number of physiological parameters is made in old hypophysectomized and old restricted rats. Differences in the physiology of these two groups could explain the differences in their life durations.

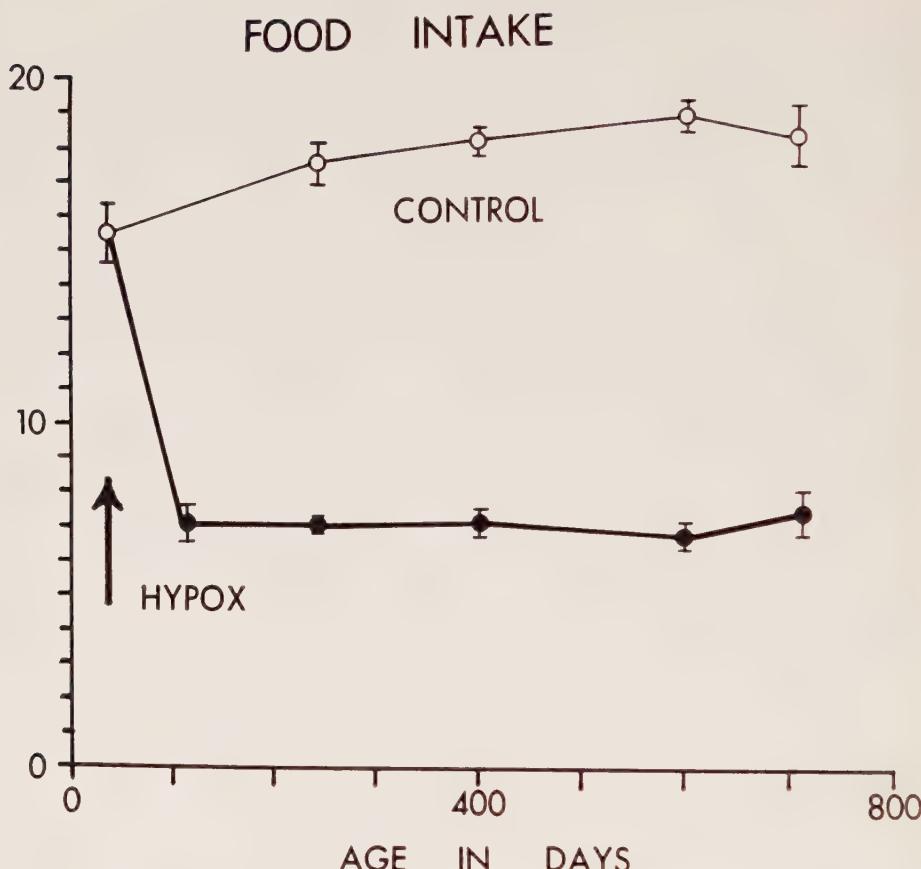


Figure 4-11. The effect of hypophysectomy on age changes in food intake in male rats living more than 700 days (partly from Everitt and Cavanagh (1965), reproduced by permission of S. Karger, Basel).

A low food intake in the intact animal (food restricted) reduces all parameters except blood hemoglobin, when compared with the old control (Table 4-I). In the hypophysectomized rat, a low food intake is also associated with depression of many body functions (body weight, protein excretion, hemoglobin, white cell count and heart rate). However, due to the lack of the antidiuretic hormone in the hypophysectomized rat, the daily water intake and urine excretion are increased by comparison with the food restricted rat. On the other hand hemoglobin and heart rate are much lower than in the food restricted rat. The anemia and the very low heart rate may thus be associated with the shortened lifespan of the hypophysectomized rat.

LIFE DURATION

Hypophysectomized (HYP) rats have a shortened lifespan when compared with intact control rats (Fig. 4-12). An intact pituitary gland is essential for a normal lifespan. Hormone replacement with cortisone (1 mg per week subcutaneously) significantly increased the life duration of the hypophysectomized rat. Cortisone treated hypophysectomized rats (HYP, CORT) had a mean life duration which was not significantly different from intact controls. Autopsies on two cortisone-treated hypophysectomized rats living more than 1,000 days revealed no gross pathology in one animal dying at 1,312 days, and a small testicular and an abdominal tumor in a second rat dying at 1,206 days.

Good results have been reported with long-term cortisone treatment of human hypopituitarism (Sheehan and Summers, 1954). Prednisolone prolongs the life of a short-lived strain of mouse (Bellamy, 1968).

Why do untreated hypophysectomized rats have a shortened lifespan? Lack of ACTH and consequently the adrenocortical hormones would seem to be of importance. The mode of action of cortisone in prolonging the

TABLE 4-I

PHYSIOLOGICAL PARAMETERS IN THE OLD HYPOPHYSECTOMIZED MALE RAT COMPARED WITH FOOD RESTRICTED AND OTHER GROUPS

Parameter	Old Hypox	Old Hypox + Cortisone	Old Food Restricted	Old Control	Young Control
Age (days)	608	600	599	613	314
No. of rats	10	8	10	10	10
Food intake (g/day)	6.1 ± 0.57*	6.3 ± 0.75	7	19.9 ± 1.2	20.6 ± 1.1
Body weight (g)	141 ± 5.0	136 ± 7.3	152 ± 4.4	505 ± 8.8	472 ± 11
Water intake (ml/day)	27.8 ± 4.4	35.2 ± 4.0	17.4 ± 1.2	30.4 ± 3.2	29.4 ± 1.5
Urine output (ml/day)	12.1 ± 1.3	26.5 ± 3.1	6.1 ± 0.6	14.9 ± 3.4	12.2 ± 2.0
Protein excretion (mg/day) ..	12.8 ± 1.2	4.9 ± 1.5	6.8 ± 1.4	60.6 ± 11.1	19.5 ± 3.3
Hemoglobin (g/100 ml)	10.1 ± 0.3	12.7 ± 0.4	13.2 ± 0.2	13.9 ± 0.8	13.5 ± 0.3
White cell count (10^3 cells/ μ l)	6.7 ± 0.2	5.3 ± 0.5	5.5 ± 0.5	10.0 ± 1.0	9.9 ± 0.6
Heart Rate (beats/min)	208 ± 11	271 ± 18	276 ± 21	342 ± 31	369 ± 23
Collagen breaking time (min) ..	60 ± 3.3	71 ± 4.1	82 ± 2.6	139 ± 2.3	49 ± 1.4

* Standard error of the mean.

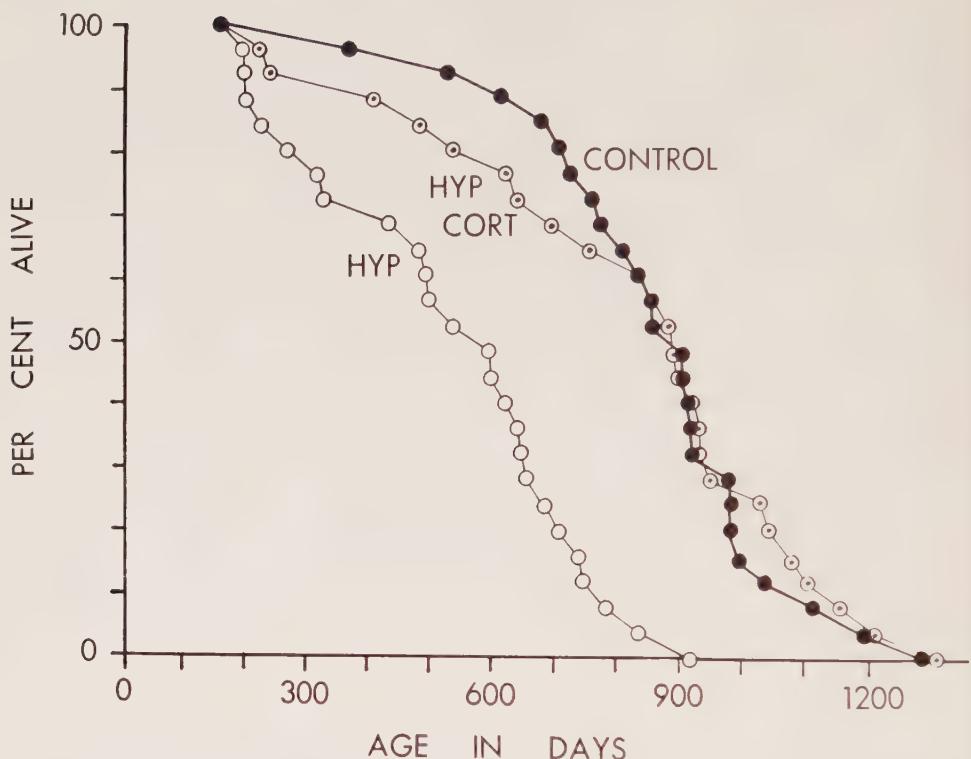


Figure 4-12. The life shortening action of hypophysectomy (HYP) in the male rat. Mortality was significantly reduced by the weekly subcutaneous injection of 1 mg of cortisone acetate (HYP. CORT) throughout life. The controls in this study were exceptionally long-lived; the mean life duration is usually 750-800 days.

life of the hypophysectomized rat is not clear. It may act by raising the hemoglobin level to normal, by restoring hemodynamic parameters to normal, by increasing the resistance to infectious diseases, by maintaining a normal blood sugar level during starvation, or by a combination of these factors.

The low blood hemoglobin level of the hypophysectomized rat may be restored almost to normal by cortisone treatment (Table 4-1). A low hemoglobin level is a major physiological difference between the hypophysectomized rat (which is short lived) and the food-restricted rat (which is long lived). On the other hand, if the anemia of hypophysectomy is a physiological response to the low metabolic rate, as suggested by Bozzini, *et al.* (1968), then it may not be a significant determinant of life duration.

Cortisone treatment increases blood pressure, cardiac output and renal blood flow in hypophysectomized rats (Kovács, *et al.*, 1965). Undoubtedly these hemodynamic changes will influence the survival of the hypophysectomized rat.

The natural resistance to toxins and bacterial infections is dependent on normal adrenocortical function. Consequently this resistance is depressed after either adrenalectomy (Perla and Marmorston, 1933) or hypophysectomy (Perla and Rosen, 1935; Perla, 1938). More recent work on this subject has been reviewed by Beisel and Rappoport (1969) and Selye (1971). In the hypophysectomized rat, the loss of this protection would undoubtedly increase its mortality on exposure to pathogens. Adrenocortical extracts were shown to increase the natural resistance of the hypophysectomized rat to histamine poisoning (Perla, 1935; Perla and Rosen, 1935).

Very low blood sugar levels (27.3 ± 2.4 mg% ; 6 rats) were found in our hypophysectomized rats when near death. Adrenocortical extracts and steroids like cortisone are able to prevent hypoglycemia in starved adrenalectomized rats (Long, *et al.*, 1940). The maintenance of a normal blood sugar level in starvation may be essential for the survival of the hypophysectomized rat when exposed to a minor intercurrent infection which stops it from eating. Under the same circumstances an intact rat with a functional pituitary gland would increase its production of ACTH and glucocorticoids and so maintain its blood sugar by stimulating gluconeogenesis. Long, *et al.* (1940) showed that gluconeogenesis is depressed in the hypophysectomized animal. It may be possible to maintain a normal blood sugar if the sick rat will drink glucose water. Boros-Farkas and Verzár (1967) found that 5% glucose as drinking water prolongs the life of the hypophysectomized rat.

CONCLUSIONS

The bulk of evidence supports the view that hypophysectomy has an anti-aging action on many tissues and organs in the rat. Hypophysectomy retards a considerable number of physiological and pathological age changes in the rat.

Despite its anti-aging effect, hypophysectomy shortens the lifespan. It is postulated that ACTH acting through the mediation of the adrenal cortex is the principal life-maintaining factor secreted by the pituitary gland. However an oversecretion of ACTH and the glucocorticoids, such as that occurring in the stressed animal, leads to an acceleration of aging. This topic is discussed in Chapters 16, 17 and 29.

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CHAPTER 5

AGE CHANGES IN HYPOTHALAMIC-PITUITARY FUNCTION

ANTONIO PECILE and ROSARIA BOSSA

SUMMARY

UNTIL NOW STUDIES of the hypothalamic control of age changes in anterior pituitary function have been concerned almost entirely with the early stages of life and with the onset of puberty. However both animal and human data on changes in old age are now accumulating. The hypothalamic control of ACTH secretion in man shows no evidence of a decline in function with aging. Nevertheless an aging change in the hypothalamic control of growth hormone secretion might be assumed from the observed reduction of growth hormone releasing activity in hypothalamic extracts of old animals. A decreased efficiency of the negative feed-back system which operates in the hypothalamic control of luteinizing hormone and of follicle stimulating hormone secretion in old age has been reported both in the rat and in man; this is in addition to the well known reduction of ovarian activity. In senile animals a hypersecretion of prolactin has been demonstrated, a fact which would indicate that in old animals the neurohumoral inhibitory control exerted by the hypothalamus becomes less effective.

It is too early for definitive conclusions, but there is little doubt that changes in the hypothalamus contribute to the control of pituitary function in each age period.

INTRODUCTION

The participation of the hypothalamus in endocrine regulation has been illustrated by relatively recent work and at present there is little doubt that the hypothalamus is an endocrine organ. In addition to the morphological findings which clarified the possibility of a connection between the hypothalamus and the pituitary through the portal system (Harris, *et al.*, 1966) evidence has accumulated demonstrating the existence of specific factors originating in hypothalamic structures which are able to control selectively the release and/or the synthesis of each hormone produced by the anterior pituitary gland.

Changes of pituitary function in the different life periods have been repeatedly considered particularly with the aim of studying the maturation

of the gland during the first stages of development, the alterations corresponding to the pubertal period and the possible modifications which appear with aging (Jost, 1966a; Werlff ten Bosch, 1966; Verzár, 1966).

Changes in hypothalamic function responsible for variations with age in pituitary function have received less attention but deserve systematic investigation.

What will be attempted in this chapter is 1. to present examples of pituitary hormonal changes with age and 2. to provide a survey of the most significant data on the possible participation of hypothalamic-neuroendocrine processes in these pituitary age changes.

AGE VARIATIONS IN ANTERIOR PITUITARY FUNCTION AND ITS HYPOTHALAMIC CONTROL

A few examples of changes in pituitary function at different ages will precede the discussion of the corresponding hypothalamic changes.

Growth Hormone (GH)

Growth promoting activity was detected in the pituitary glands of fetal pigs at the stage of 9 to 11 cm (Smith and Dortzbach, 1929), of fetal rats by the 19th day (Contopoulos and Simpson, 1957) and of the mouse fetus after the 18th day of gestation (Enemar, 1961). Although the presence of growth hormone in the gland does not demonstrate that it is actually secreted into the blood, it is consistent with the assumption that the hormone is secreted. The existence of augmented secretion of growth hormone at times of most rapid growth during life is an attractive concept. Newborn infants have elevated levels of GH; GH levels during infancy while lower than in the immediate neonatal period are higher than later in life. Under similar conditions (two or three hours after a meal) the level of plasma radio immunoassayable GH is throughout childhood and adolescence higher than in adults (Greenwood, *et al.*, 1964). The reasons for that may be independent of any change in the secretory capacity of the pituitary. Anyway it is clear that something changes with age and that variation in hypothalamic function may be at least one of the alternatives. Focusing particularly on the old age period it has been shown that the pituitary GH content does not vary with age in man (Gershberg, 1957) but Bowman (1961) found that, although the concentration of GH in the anterior lobe of the rat shows little variation, the ratio of dry (or wet) anterior pituitary weight to body weight exhibited a decrease with age. Thus, with a rather similar concentration of growth hormone in the pituitary at all the ages, the decreasing pituitary-weight/body-weight ratios would indicate that in the anterior pituitary gland there is less total growth hormone per gram of body weight as the rat ages.

Hypothalamic control of growth hormone (GH) secretion

The dependence of the pituitary on hypothalamic stimulation for the secretion of growth hormone has been recently demonstrated. Growth hormone releasing (GRF) activity has been found in hypothalamic extracts of the stalk median eminence *in vitro* (Franz, *et al.*, 1962) and *in vivo* (Pecile, *et al.*, 1965).

In addition to the stimulating activity on pituitary GH secretion by hypothalamic factor(s), the existence of central nervous mechanisms whose action is to inhibit the secretion of GH is indicated by the work of Krulich, *et al.* (1967).

Hypothalamic regulation of GH secretion seems of minor importance during fetal life. The absence of the pituitary in the human fetus as well as spontaneous or experimental apituitarism in mammalian and nonmammalian vertebrates is compatible with continued near-normal somatic growth up to the time of birth or hatching (Seckel, 1960; Pecile and Müller, 1966).

A remarkable growth hormone releasing activity was found in hypothalamic stalk median eminence (SME) extracts of neonatal rats. The application of electric shock to rats of age 1, 5 and 10 days induced a clear-cut decrease in pituitary growth hormone content. In the shocked animals GRF activity in the hypothalamic SME extracts was significantly reduced. These results indicate that in the very early stages of development hypothalamic mechanisms participating in growth hormone release are available, and that a response to stressful stimuli may be expected in infant rats as well as in adult rats (Pecile, *et al.*, 1969). GH secretion is influenced by the age of the individual. Higher levels of GH were obtained in the plasma of children than in that of adults (Hunter and Greenwood, 1964; Hunter and Rigal, 1966). According to Chalkley and Jackson (1966) GH levels were higher in children under one year of age than in older ones.

There is also evidence that the changes in GH secretion occurring at different ages of an individual might be controlled by the hypothalamus. Pecile, *et al.* (1965) have demonstrated that the GH-releasing activity of hypothalamic extracts is significantly higher in young rats (30 days) than in old adults (2 years).

Gonadotropic Hormones

In the fetus, gonadotropic hormones have been detected in the pituitary gland of the pig (Smith and Dortzbach, 1929), horse (Hellbaum, 1935) and man (Siegmund and Mahnert, 1928) but only during the period of growth of established sexual structures. They were not detected at all in the rat fetus (Contopoulos and Simpson, 1957). In the adenohypophysis

of the rabbit fetus, PAS positive material becomes more and more abundant between day 19 and 22 or 23 approximately (Jost and Gonse, 1953).

In the human fetus, gonadotropic activity was found in fetal blood but since the placenta produces gonadotropins it remains uncertain whether this activity originated from the fetal pituitary or from the placenta (Bruner, 1951). As reported by Werff ten Bosch (1966) in his excellent review on anterior pituitary function in infancy and puberty, changes take place in the amount of gonadotropic hormones in the hypophysis of the infant which may reflect alterations in the secretion rate of these hormones during infancy. The pituitary gland of the infantile female rat gradually increases in gonadotropic potency during the first two weeks of post-natal life and then increases more rapidly to reach a peak at about 21 days; thereafter a steady fall in hormone content sets in (Hoogstra and Paesi, 1955; Kragt and Ganong, 1967). Rather similar changes take place in the gland of the neonatal male rat except that the sharp rise to a peak in the hormone content occurs later, at about 27 to 30 days of age (Hoogstra and Paesi, 1955).

In human material, gonadotropic activity is practically absent from the glands of infants (Bahn, *et al.*, 1953a) although Ryan (1962) found that the pituitaries of infants contained about one fifth of the concentration of LH present in the glands of young women.

Pituitary glands from adult individuals seem to contain more gonadotropin than glands taken from immature individuals. While no differences seem apparent between the gonadotropin content of the hypophyses of young, mature and old animals (Solomon and Shock, 1950; Duncan, *et al.*, 1952; Blumenthal, 1954; Ceresa and Lacroix, 1951; Korenchevsky, 1961), in the human pituitaries a remarkable increase in FSH content in the post-menopausal female was ascertained (Bahn, *et al.*, 1953b).

In old age the anterior pituitary does not lose its capacity to produce gonadotropins. On the contrary, in old age when the ovary is not functioning the production of gonadotropin increases at the time of the menopause in females and probably also in certain males (Verzár, 1966).

Hypothalamic Control of Gonadotropin Secretion

The hypothalamic control of gonadotropin secretion is now well established. A specific hypothalamic releasing factor has been proposed for each gonadotropic pituitary hormone and specific areas in the hypothalamus have been recognized as responsible for the brain control of the pituitary through the releasers. An exception to the general rule is prolactin because the influence of the hypothalamus on the secretion of this hormone is ascertained to be inhibitory. However a prolactin releasing factor was also proposed by Kragt and Meites (1965) and Mess and Martini (1969).

A hypothalamic factor responsible for LH release (LRF) has been found in the hypothalamus of newborn rats and rabbits and 1 to 2 month old calves (Gallardo and Campbell, 1965; Campbell and Gallardo, 1965). LRF was detected in the hypothalamus of immature rats of both sexes (Ramirez and McCann, 1963). Arrau, *et al.* (1965) have also found this releasing factor in the hypothalamus of children.

In general terms the main finding of the present work (Campbell and Gallardo, 1966) is that LRF is present at a very early age.

From the results of Corbin and Daniels (1967) it appears that changes occur in the concentration of pituitary FSH and in stalk median eminence FSH-RF during the reproductive development of the female rat. At the time of puberty there is a precipitous drop in stalk median eminence (SME) gonadotropin releasing factors and in pituitary gonadotropin activity (Ramirez and Sawyer, 1965a and b, 1966; Kragt and Ganong, 1967). The induction of puberty seems therefore neuroendocrinologically characterized by conspicuous variations in hypothalamic FSH-RF and LRF and in pituitary FSH and LH.

Alterations in the hypothalamic control of LH and FSH secretion with aging have been observed both in the rat (Aschheim, Ch. 19) and in man (Dilman, Ch. 32). This is in addition to the well-known reduction of ovarian activity. The reason for hypothalamic variations independent of the alteration in the feed-back system due to the changed steroid hormone production are not yet clarified. The problem of the hypothalamic control of prolactin secretion through the prolactin inhibiting factor is also of much interest because of variations consequent to aging. Of relevance in this respect are the results of Aschheim and Pasteels (1963) who demonstrated a hypersecretion of prolactin in senile rats. They suggested that with aging the neorohumoral inhibitory control exerted by the hypothalamus becomes less effective in senile animals, and this fact induces the hyperactivity and proliferation of prolactin producing cells of the pituitary.

Thyroid Stimulating Hormone (TSH)

Thyroid stimulating activity was detected in the pituitary glands from pig fetuses near term (Rumpf and Smith, 1926) and from 19-day-old rat fetuses (Contopoulos and Simpson, 1957).

The TSH content of the human pituitary gland is highest during the first decade and then falls (Blumenthal, 1954). In view of the rapid changes in the growth rate around birth and at puberty corresponding alterations in the thyroid hormone requirements are to be expected and thus variations in the TSH secretion rate.

It is not known, however, through which homeostatic mechanisms adaptation may be achieved. One possible mechanism is a change in the thyroid

responsiveness to TSH but the hypothalamic control of TSH release should also be considered.

An increase of TSH activity in the pituitary of aged rabbits and also aged humans is reported by some authors (Marine, *et al.*, 1935; Severinghaus, 1937; Levey, 1963).

Hypothalamic control of TSH secretion

The hypothalamic control of TSH secretion is through a specific hypothalamic releasing factor (TRF). The hypothalamic area involved in the synthesis of TRF includes the major part of the anterior hypothalamus and is far larger than that related to CRF (Matsuda, *et al.*, 1963; Mess and Martini, 1969).

During the fetal period it appears that embryonic pituitary tissue is able to secrete TSH in amounts sufficient to promote thyroid function even in the absence of an appropriate hypothalamic stimulation. It seems very probable that hypothalamic connections are needed only a few days after birth, i.e. after the time when the portal circulation of the pituitary develops under normal physiological circumstances. The recent experiments of Jost, *et al.* (1970) have demonstrated that although fetal hypophyseal thyroid stimulating activity is largely independent of the hypothalamus some reduction of thyroid hormone is likely both in rats and in man in the absence of the hypothalamus. Such conclusions are based on the observation that 1. whereas the ^{131}I uptake in one hour is the same in entire and encephalectomized fetuses and is similarly reduced by propylthiouracil, after 24 hours the uptake tends to be lower in encephalectomized fetuses; 2. the amount of circulating thyroxine is only two thirds of the control value on day 21 in fetuses which had been encephalectomized on day 17.

Adrenocorticotropic Hormone (ACTH)

Adrenocortical stimulating activity was detected qualitatively in the pituitaries of human fetuses of ages varying from 16 to 22 weeks (Taylor, *et al.*, 1953; Ghilain and Schwers, 1957). In the rat fetus maximal pituitary stimulation of the adrenal cortex was found during a period covering days 18 to 20 of pregnancy, which in the rat lasts 21 days (Cohen, 1955, 1963). During the day before birth the growth rate of the fetal adrenal gland is decreased and pituitary stimulation is low (Jost, 1966b).

In a report of Skebel'skaya (1968) it is suggested that in the rat during embryonal development a feed-back relationship typical of adult organisms is established between the pituitary gland and the adrenals.

In man there is no apparent decline of ACTH production in old age. Blumenthal (1955) concluded that pituitary glands of humans between 8 and 71 years of age when transplanted subcutaneously into guinea pigs

produce similar increases in adrenal mitoses. In a recent study in elderly subjects (70-94 years) Jensen and Blichert-Toft (1971) concluded that there is no evidence for assuming a hypofunction of the pituitary and adrenal glands under basal conditions during the aging process.

Hypothalamic Control of ACTH Secretion

The hypothalamic control of ACTH secretion first suggested by the observations of De Groot and Harris is now a well established fact (Fortier, 1966). A specific hypothalamic factor called corticotropin releasing factor (CRF) is recognized as responsible for the release of pituitary ACTH. A hypothalamic inhibitory factor acting on ACTH secretion has also been proposed (Mangili, *et al.*, 1967).

The maturation of the hypothalamic control of pituitary ACTH secretion during fetal and neonatal life has been considered on the basis of experiments on the rat to occur rather slowly, so that practically no adequate control could be envisaged until a few days after birth (Adolph, 1957; Erskine, 1957; Schapiro, 1960; Milcovic and Milcovic, 1966). However this conclusion has been repeatedly questioned by many authors (Levine, 1965; Zarrow, *et al.*, 1966; Palkovitz and Mitro, 1968; Bartova, 1968) who found CRF in the rat hypothalamus as early as the first two weeks of life and demonstrated an efficient feed-back mechanism between ACTH and corticosteroids at birth. Of special interest in this context is the observation of Jost (1966b) who demonstrated that in encephalectomized fetuses (which while possessing their pituitaries do not have their hypothalami) the adrenal glands are atrophic. This indicates the existence of an essential relationship between the hypothalamus and the pituitary during fetal life which is consistent with the assumption that the hypothalamus might already be of importance for normal release of ACTH by the fetal pituitary.

No data are available to the authors' knowledge regarding observed changes of hypothalamic control of ACTH secretion during infancy and puberty.

In relation to old age Green and Friedman (1968) studied hypothalamic-pituitary-adrenal function in the elderly. In a group of healthy elderly subjects up to 91 years old (mean age 81) they have confirmed the normal range of values for diurnal plasma cortisol rhythm, response to hypoglycemic stress, corticotropin stimulation and dexamethazone suppression. These old people do not show any evidence of decline in the function and reserve of their hypothalamic-pituitary-adrenal axis.

CONCLUSIONS

The main conclusion of this chapter is that age changes in hypothalamic function are largely unknown. The most convincing studies shed light on

the participation of hypothalamic structures and secretions in pituitary function during the very early stages of life. Some important data are available which stress the variations in the content of the hypothalamic areas of the releasers for gonadotropins during the critical period of the onset of puberty. The large majority of other observations needs further extensive confirmation. On the other hand it must be repeated that reasoning in terms of the content of each factor in the hypothalamus or in the pituitary and even considering the blood levels may be unsatisfactory. A marked request for the output of a hormone or neurohumor may induce a depletion of its usual stores and so cause a low level at the site of origin, which may be the indication of an active function; on the contrary high contents of a hormone in the pituitary or of a neurohumor in the hypothalamus may suggest a reduced activity. It is too early for definitive conclusions. We are only beginning to collect the essential data which will clarify the hypothalamic control of pituitary function in each stage of life.

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CHAPTER 6

ASSESSMENT OF HYPOTHALAMIC PITUITARY FUNCTION IN OLD AGE

L. LAZARUS and C. J. EASTMAN

SUMMARY

THE ASSESSMENT of hypothalamic pituitary function in the elderly requires knowledge of the factors regulating pituitary hormone secretion and sophisticated techniques for measurement of pituitary and target gland hormones in response to provocative and inhibitory stimuli.

A dynamic approach, based upon the application of sensitive and precise radioimmunoassay techniques for measurement of changes in pituitary and target gland hormones in peripheral serum in response to pharmacological and physiological stimuli, has been detailed in this chapter. Tests that directly or indirectly assess growth hormone, thyrotropin, gonadotropins, adrenocorticotropin, prolactin, their target gland hormones and the interaction of the latter with the tropic hormones have been applied in the elderly to assess the functional integrity of the hypothalamic pituitary axis.

INTRODUCTION

The release of pituitary hormones is dependent upon the dynamic balance between many factors, including the circulating level of the pituitary hormone, the circulating level of the target gland hormone, the hypothalamic releasing hormone, the biogenic amine content of the hypothalamus and the cerebral input to the hypothalamus.

The control system for pituitary hormone release is summarized diagrammatically in Figure 6-1. Ideally, assessment of hypothalamic pituitary function requires techniques for the measurement of 1, 2, 3 and 4 and of the responses of 3 to change in each of the other parameters. At the present moment our techniques are not sufficiently finite to permit this assessment and the following discussion is based upon current knowledge (late 1973). The problems associated with this assessment in old age are several and depend upon such changes as the post-menopausal state and the concomitant disease states of many elderly patients who are unable to tolerate stressful procedures.

The hormones of the adenohypophysis include growth hormone (GH), prolactin (Prl), corticotropin (ACTH), thyrotropin (TSH), luteinizing

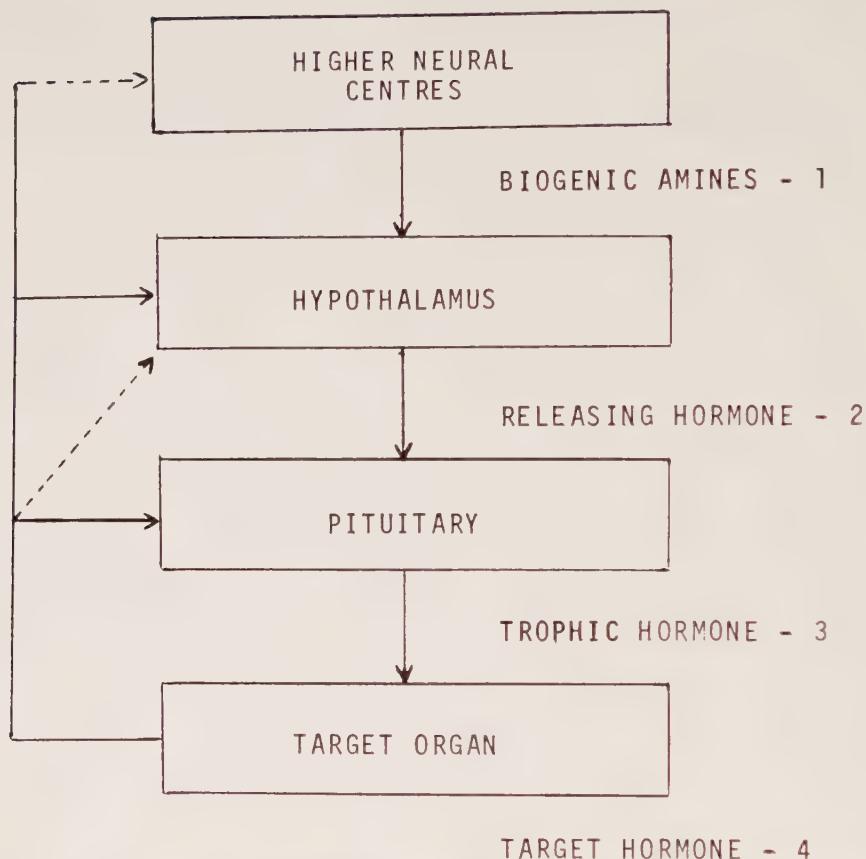


Figure 6-1. Diagrammatic representation of the control pathway of hypothalamic-pituitary function.

hormone (LH) and follicle stimulating hormone (FSH). Full assessment requires a separate study of the status of each pituitary hormone.

GROWTH HORMONE

Growth hormone (GH) is secreted throughout life and is detectable in peripheral blood from birth to old age by commonly employed radioimmunoassay methods (Lazarus, 1967). Because GH is secreted in regular and irregular bursts throughout a 24 hour period, there is great variability in plasma levels which are often difficult to interpret. Plasma immunoreactive GH levels are usually at their lowest in the early morning upon awakening and, excluding infants, samples taken at this time do not differ significantly with age (Lazarus, 1967). Studies of GH secretion rates and secretory patterns have revealed secretion rates of $91 \mu\text{g}$ 24 hours in prepubertal children and $690 \mu\text{g}$ 24 hours in adolescents, $385 \mu\text{g}$ 24 hours in young adults

and markedly decreased secretion in older patients (Finkelstein, Roffwarg, Boyar, Kream and Hellman, 1972). In 3 out of 5 elderly patients studied by Finkelstein, *et al.* (1972) the secretion rate approached zero, thus demonstrating an age related change in GH secretion.

While patients with GH deficiency show persistently low basal plasma GH levels, the assessment of hypothalamic control of GH secretion requires measurement of the plasma GH responses to either physiological or pharmacological stimuli. In patients with elevated basal plasma GH levels, lack of suppression by oral glucose helps to establish the diagnosis of pathologic hypersecretion of GH. In elderly subjects Dilman (1971) reported a paradoxical rise in GH after glucose loading. This finding, however, has not been corroborated by Dudl, Ensinck, Palmer and Williams (1973) in a more extensive study of GH secretion in elderly normal subjects.

Physiological Stimuli to GH Secretion

Several physiological stimuli are known to cause elevations in plasma GH concentration due to stimulation of the hypothalamic-pituitary axis. Exercise (Sutton, Young, Lazarus, Hickie and Maksyvitis, 1969), sleep (Takahashi, Kipnis and Doughday, 1968), decreases in blood glucose (Roth, Glick, Yalow and Berson, 1963; Glick, Roth, Yalow and Berson, 1965) and ingestion of protein (Knopf, Conn, Floyd, Fajans, Rull, Guntsche and Thiffault, 1966) are well defined stimuli to pituitary GH release. While sleep and exercise-induced GH release reliably assess the integrity of the hypothalamic pituitary GH secretory mechanism, small changes in blood glucose and protein ingestion are less reliable.

Exercise Test

The magnitude of the GH response to exercise is proportional to the degree and severity of the exercise performed (Sutton, Young, Lazarus, Hickie and Maksyvitis, 1969). Submaximal exercise may not result in a rise in plasma GH and elderly subjects may be unable to exercise sufficiently strenuously to achieve a response. Due to the dangers of cardiac irregularities during exercise, all elderly subjects should have a full cardiac assessment prior to the test and should be monitored with a continuous ECG recording whilst exercising.

Sleep

Sleep is a potent physiological stimulus to GII release. GII is secreted in episodic bursts, intimately related to specific periods of sleep identified by slow waves on the EEG (Takahashi, Kipnis and Daughaday, 1968; Honda, Takahashi, K., Takahashi, S., Azume, Irie, Sakuma, Tsushima and Shizume, 1969; Sassin, Parker, Johnson, Rossman, Mace and Gotlim, 1969;

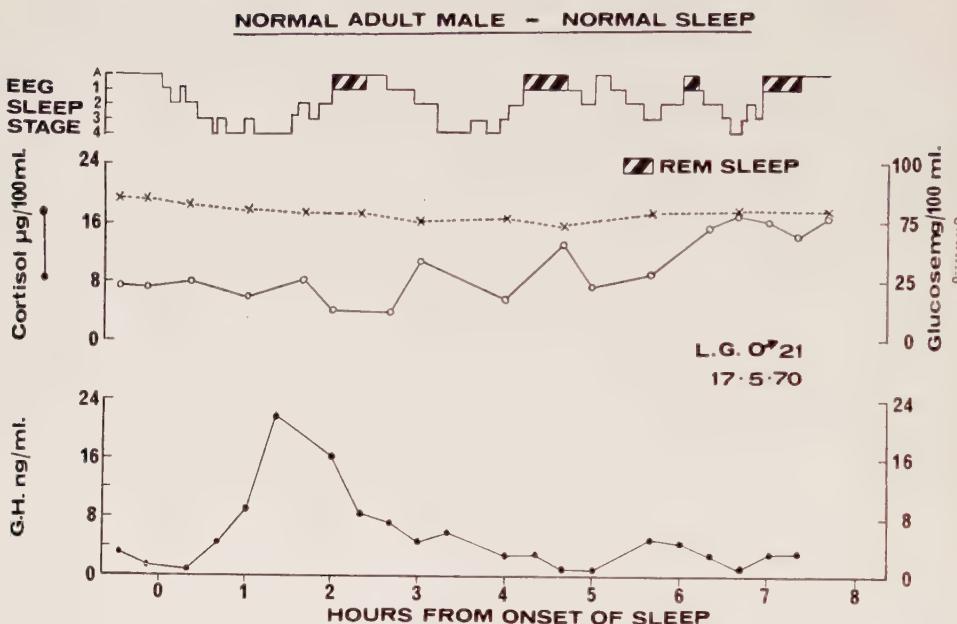


Figure 6-2. A typical sleep related rise in plasma GH concentration in a normal young adult male during polygraphically monitored normal sleep. Plasma GH is plotted in ng/ml where 1 ng is equivalent to $2 \mu\text{U}$ of the WHO IRP for immunoassay. Note the increase in plasma GH following the first descent into slow wave sleep—stages 3 and 4 on the EEG trace. Diminished or absent peaks occur with advancing age. Blood glucose concentration remains stable while plasma cortisol levels fluctuate throughout normal nocturnal sleep.

Parker, Sassin, Mace, Gotlin and Rossman, 1969; Eastman, Mitchell and Lazarus, 1971) as shown in Figure 6-2. There is a temporal organisation of sleep-induced rhythmic GH release which is established during infancy and persists as a consistent and reproducible phenomenon into adult life (Takahashi, *et al.*, 1968; Honda, *et al.*, 1969; Sassin, *et al.*, 1969; Parker, *et al.*, 1969; Eastman, *et al.*, 1971). Although sleep induced GH secretion has been studied extensively in children and in young to middle-aged adults, and the usefulness of this phenomenon as a physiologic test of hypothalamic pituitary function has been well demonstrated (Mace, Gotlin, Sassin, Parker and Rossman, 1973; Eastman and Lazarus, 1971; Underwood, Azumi, Voina and Van Wyk, 1971; Eastman and Lazarus, 1973), it is surprising that relatively little is known concerning GH release during sleep in the elderly. In a recent report Carlson, Gillan, Gordon and Snyder (1972) have shown diminished or absent GH peaks during sleep in elderly subjects which may be a causative factor in the decreased production of GH observed in the aged (Finkelstein, *et al.*, 1972). The mechanism of this change with age is not understood.

In our laboratory a normal response to sleep is a rise of greater than 10 ng/ml during the first episode of slow wave sleep. Because of the variability in responses of the elderly, failure to attain a level of 10 ng/ml in a patient over the age of 60 years is not diagnostic of hypopituitarism. The response must be interpreted in the light of other pituitary function studies.

Pharmacological Stimuli

In recent years a large number of unrelated pharmacological and stressful stimuli have been recommended for use in assessing GH secretion (see Glick 1969 for review). These stimuli include insulin-induced hypoglycemia, arginine infusion, bacterial pyrogens, glucagon, vasopressin and intravenous tetracosactrin. The pharmacological stimuli most commonly used are arginine infusion and insulin-induced hypoglycemia. The former appears to stimulate GH release via direct action on the pituitary and the latter via hypothalamic stimulation.

Insulin Induced Hypoglycemia

This test is designed to provoke GH release by producing hypoglycemia. The hypoglycemia threshold for GH release varies from one individual to

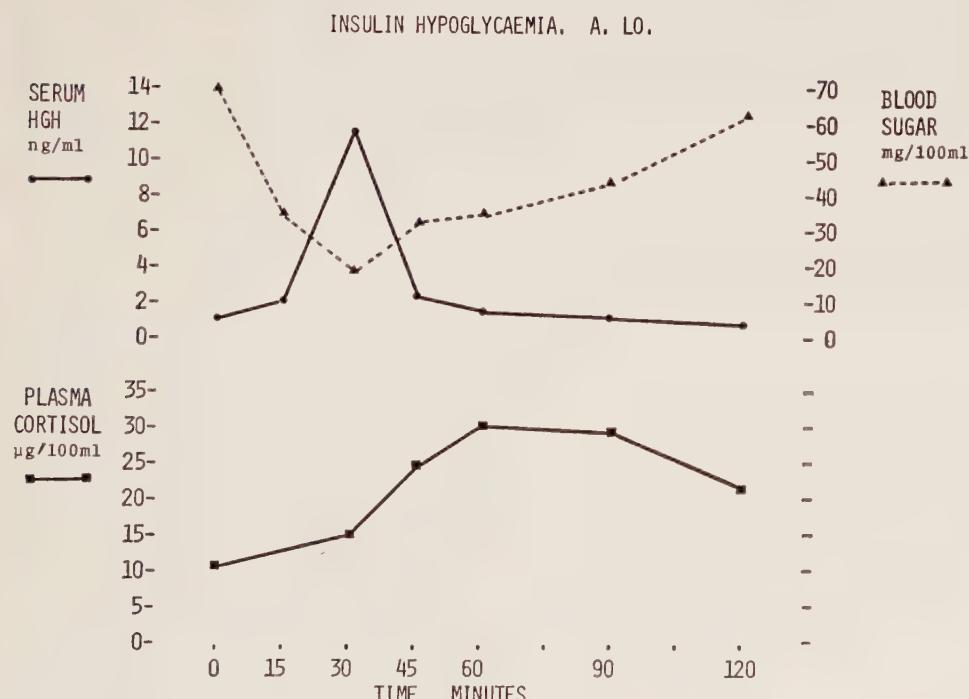
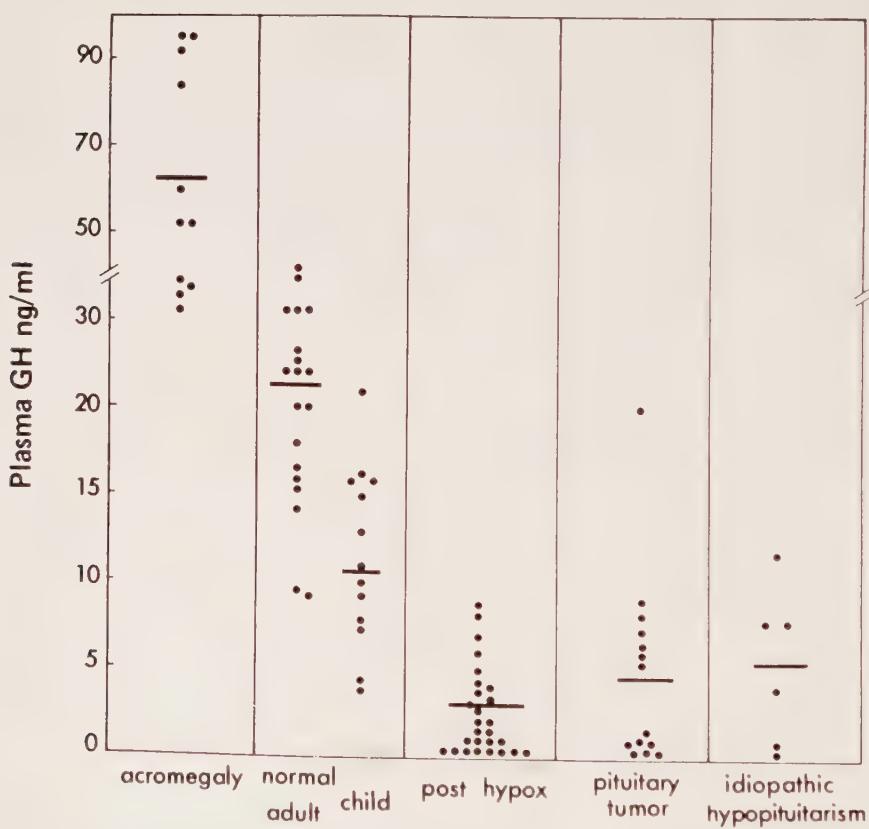


Figure 6-3. Typical GH and cortisol responses to insulin induced hypoglycemia in a normal adult male aged 67 years.

another, but a 50 percent fall in blood glucose is generally considered to be an adequate stimulus. The test is performed after an overnight fast by inserting an indwelling venous catheter into an arm vein and then injecting I.V. soluble insulin in a dose of 0.1U insulin/kg body weight. Venous samples are collected for measurement of blood glucose and plasma GH at 15 minute intervals for 2 hours; peak plasma GH levels are usually found from 30 to 60 minutes after the insulin injection. A typical response in a normal adult subject is shown in Figure 6-3 where it can be seen that a peak plasma GH level greater than 10 ng/ml follows the nadir of the blood glucose concentration. The response in adults is greater than the response in prepubertal children and we have not observed a significant decline with age. More detailed studies of the plasma GH response to insulin-induced hypoglycemia in elderly subjects by Cartlidge, Black, Hall



PEAK GH RESPONSES TO INSULIN INDUCED HYPOGLYCAEMIA

Figure 6-4. Peak plasma GH responses to insulin induced hypoglycemia in normal adults and children and in patients with hypothalamic and/or pituitary disease.

and Hall (1970) and by Sachar, Finkelstein and Hellman (1971) have failed to demonstrate a diminution in the peak GH response to this stimulus with age. Thus, measurement of the plasma GH response to insulin induced hypoglycemia remains a useful method for assessment of hypothalamic pituitary function from childhood to old age. The efficacy of this test in discriminating between normal subjects and patients with hypothalamic and pituitary disease is illustrated in Figure 6-4.

The test must be supervised since patients with hypopituitarism may exhibit profound and prolonged hypoglycemia. Symptoms of hypoglycemia may be masked, especially in elderly patients with cerebral artery disease, and there is always the danger of producing cardiac arrhythmias when organic heart disease is present.

Arginine Infusion

The arginine infusion test employs a different stimulus but it is performed and interpreted in a similar manner to the insulin hypoglycemia test. Arginine hydrochloride is infused via an indwelling venous catheter in a dose of 0.5 g/kg body weight over a period of 30 minutes. The peak plasma GH level achieved is similar to that observed in response to insulin hypoglycemia. A negative response to arginine occurs in approximately 20 percent of normal males, but this can be converted to a positive response after pretreatment with estrogens (Merimee, Rabinowitz, Riggs, Burgess, Rimoin and McCusick, 1967). Because of the variable incidence of negative responses in normal subjects to either arginine or insulin, Penny, Blizzard and Davis (1969) recommended the use of both stimuli in a sequential manner for the investigation of GH secretion in children. It is now our practice to use the sequential arginine infusion and insulin hypoglycemia test to investigate GH secretion in both adults and children (Fig. 6-5). We have not observed any diminution in the plasma GH response to arginine stimulation in elderly subjects which is in accordance with the recent report of Dudl, Ensinck, Palmer and Williams (1973).

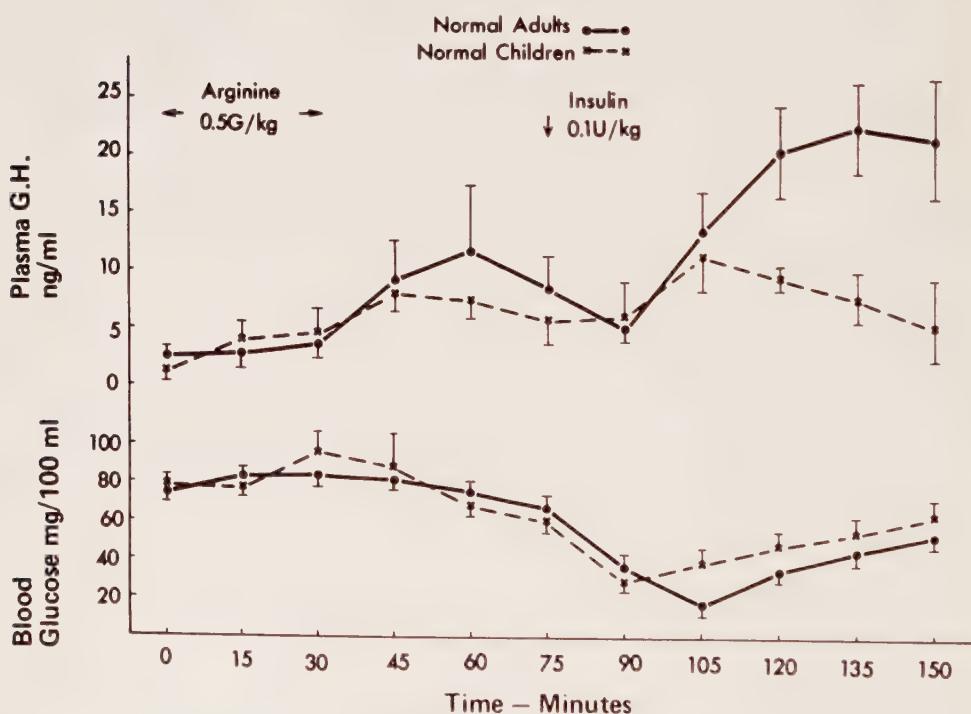
Hypothalamic Control of Growth Hormone Secretion

There is now substantial evidence that GH secretion by the pituitary is regulated by dual hypothalamic stimulatory and inhibitory neurosecretory mechanisms (Martin, 1973). The chemical nature of the GH releasing hormone has not been established. Kastin and co-workers (1973) described a decapeptide as the specific hypothalamic GH releasing hormone. However, the administration of this substance to normal men failed to elicit any significant change in plasma GH concentration.

Recently a tetradecapeptide with inhibitory activity has been iso-

lated from ovine hypothalami (Brazeau, *et al.*, 1973) and administration of this compound to man inhibits the normal GH response to pharmacological and physiological stimuli (Hall, *et al.*, 1973).

Noradrenaline, dopamine and serotonin, the putative neurotransmitters for hypothalamic control of pituitary GH release (Martin, 1973; Smythe and Lazarus, 1973) are present in high concentrations in the medial basal hypothalamus. The site at which the monoamines act to regulate GH secretion has not been clarified, but it appears that they modulate secretion of the releasing and inhibiting hypophysiotropic hormones which in turn control pituitary GH release. Neither noradrenaline nor dopamine cross the blood-brain barrier, but L-dopa, the carboxylated precursor of dopamine, enters the brain and is rapidly converted to dopamine. Following oral or parenteral administration of L-dopa to normal subjects there is a significant increase in plasma GH levels (Boyd, Lebovitz and Pfeiffer, 1970). Al-



SEQUENTIAL ARGININE INFUSION - INSULIN HYPOGLYCAEMIA

Figure 6-5. The mean \pm SEM plasma growth hormone and blood glucose responses to sequential arginine infusion and insulin induced hypoglycemia stimulation tests in 10 normal prepubertal children and 10 normal adults ages 18 to 67 years. Note the increase in plasma GH response to insulin induced hypoglycemia in sexually mature adults compared with prepubertal children. There is no significant decline in peak plasma GH response with age.

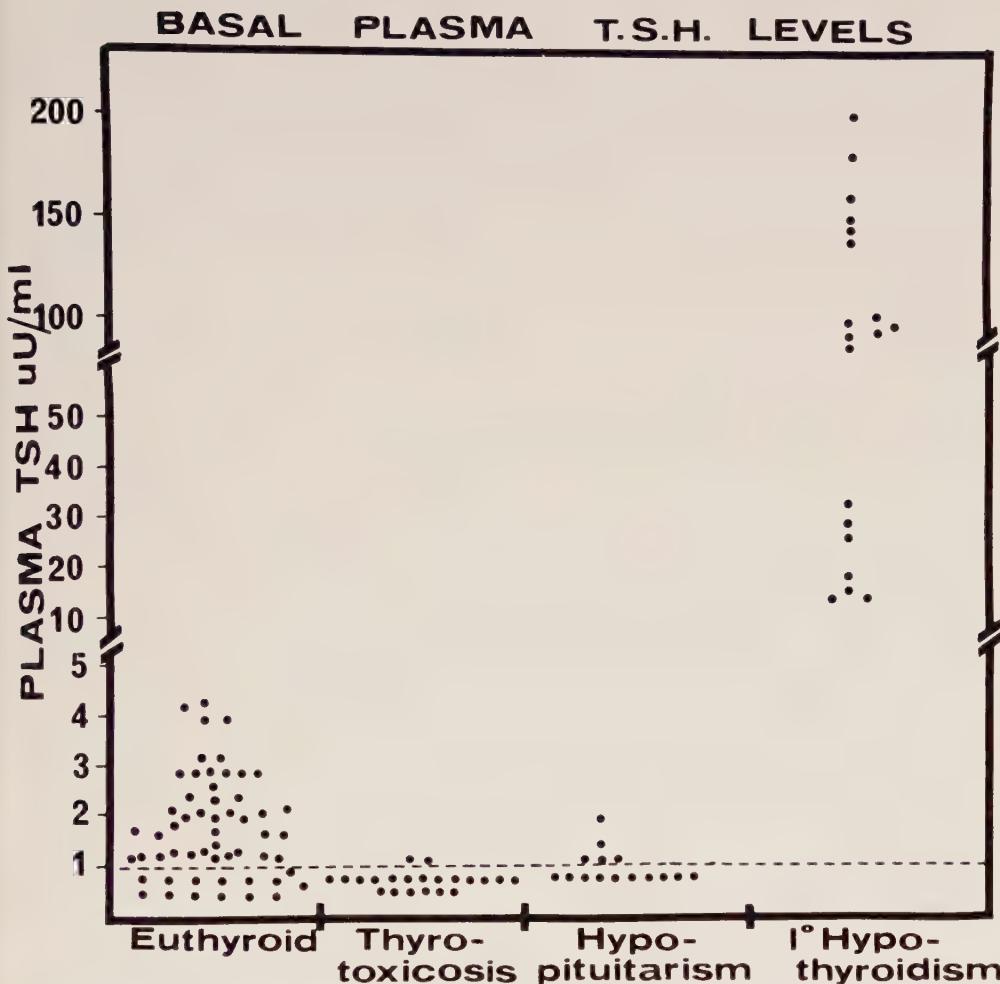


Figure 6-6. Plasma thyrotropin levels in normal adults aged 18 to 75 years compared with TSH levels found in patients with thyrotoxicosis, hypopituitarism and primary hypothyroidism.

though the administration of this substance to stimulate pituitary GH release has been proposed as a provocative test of pituitary function its clinical use has not yet been fully assessed. It is probable that methods for assessment of hypothalamic pituitary GH secretion in the future will utilize the specific releasing and inhibiting hormones and monoamine neurotransmitters for precise quantitation of GH secretion and localization of abnormalities in the control system.

THYROTROPIN (TSH)

In man, pituitary TSH secretion is the outcome of two interacting control systems, comprising neurogenic elements acting via the hypothalamic

neurohormone thyrotropin releasing hormone (TRH) and feedback control acting via the thyroid hormones, viz. triiodothyronine (T₃) and thyroxine (T₄) at the pituitary level and perhaps also at a hypothalamic site (Reichlin, Martin, Mitnick, Boshans, Grimm, Bollinger, Gordon and Malacarra, 1972). The assessment of basal pituitary thyroid function requires the direct measurement of circulating levels of TSH and direct or indirect measurement of circulating levels of thyroid hormones. TSH radioimmunoassays are now widely available and have replaced indirect methods for assessment of plasma TSH concentration.

Plasma TSH levels range from < 1.0 to 4.5 $\mu\text{U}/\text{ml}$ in young to middle aged adults (Fig. 6-6) but slightly higher levels may be found in elderly euthyroid adults (Mayberry, Gharib, Bilstad, and Sizemore, 1971). Lack of sensitivity of the TSH radioimmunoassay precludes measurement of the low concentration of plasma TSH in approximately 20 percent of euthyroid subjects. Because the pituitary thyrotrope is extremely sensitive to minor fluctuations in circulating thyroid hormone levels, plasma TSH concentrations in patients with primary hypothyroidism are invariably elevated and usually precede the onset of clinical and biochemical hypothyroidism (Utiger, 1971). Not only is an elevated TSH level useful in establishing a diagnosis of primary hypothyroidism, but it is also the simplest and most convenient method of differentiating primary from secondary hypothyroidism. The radioimmunoassay of TSH is of limited value however, in establishing a diagnosis of secondary hypothyroidism as plasma TSH estimations do not differentiate euthyroid patients from patients with mild hypothyroidism due to impaired TSH secretion of either pituitary or hypothalamic origin (Utiger, 1971).

Thyrotropin Releasing Hormone (TRH) Stimulation Test

Prior to the advent of synthetic TRH, there was no simple, effective method of augmenting TSH secretion, despite numerous attempts to devise stimulation tests using a wide variety of potential stimuli. It is now possible to assess the response of the pituitary thyroid axis to hypothalamic stimulation. The TSH response to intravenously administered, synthetic TRH is dose dependent up to 400 μg so that a standard dose of at least 400 μg should be used in all subjects (Haigler, Pittman, Hershman and Baugh, 1971). The normal response to both oral and intravenous TRH is shown in Figure 6-7. The response in old age is dependent upon sex, with maintenance of a young adult type response in females, but a marked decrease in response with age in males, the decline commencing between 40 and 60 with a further decline between 60 and 70 years (Snyder and Utiger, 1972).

By measuring serum T₃ levels, which show incremental increases of 40

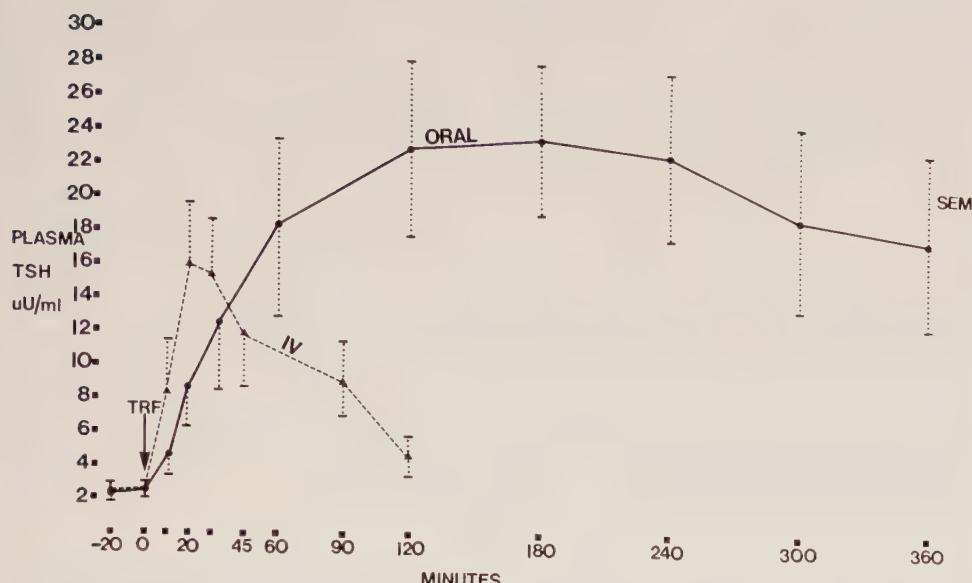


Figure 6-7: Illustrates the timing and magnitude of the normal TSH response to intravenously and orally administered synthetic TRH. Each point represents the mean \pm SEM for plasma TSH measured in 12 euthyroid volunteers aged 18 to 42 years. Intravenously administered TRH was given as a single injection in a dose of 400 μ g and orally administered TRH in a dose of 20 mg.

to 100 percent after intravenous TRH, the whole pituitary thyroid axis can be tested (Hollander, Mitsuma and Shenkman, 1972). The modest increases in serum T4 levels after intravenous TRH are inadequate for clinical testing. After oral TRH in a dose of 20 to 40 mg there is a sustained elevation in plasma TSH, T3 and T4 levels.

A single blood sample for measurement of TSH and T3 taken approximately 4 hours after oral TRH administration provides a reliable and simple screening test of the response of the pituitary thyroid axis to hypothalamic stimulation. It is our experience that this response is preserved into old age. However, no systematic studies have been performed to quantitate the TSH, T3 and T4 response to oral TRH in the elderly.

To Test Pituitary TSH Reserve

Whereas an absent or blunted response suggests, but is not diagnostic of, impaired pituitary TSH reserve, a normal TSH response to TRH excludes pituitary TSH deficiency (Haigler, *et al.*, 1971; Schalch, Gonzalez, Kastin, Schally and Lees, 1972). Euthyroid patients with pituitary tumors commonly exhibit normal TSH responses to TRH. Absent responses are seen in patients with hyperthyroidism (Ormston, Garry, Cryer, Besser and Hall,

1971), euthyroid Graves' disease (Lawton, Ekins and Nabarro, 1971) and in patients with autonomous thyroid nodules (unpublished observation).

Differentiation of Hypothalamic and Pituitary Hypothyroidism

A normal basal TSH level and a normal TSH response to TRH may occur in patients with secondary hypothyroidism due to hypothalamic disease (Castleman, Scully and McNeely, 1972; Patel and Burger, 1973). More often, patients with hypothalamic disease exhibit a delayed and sustained TSH response to TRH, similar to the response observed in patients with primary hypothyroidism (Schalch, *et al.*, 1972; Hall, Ormston, Besser, Cryer and McKendrick, 1972). The interpretation of the TSH responses to TRH in patients with secondary hypothyroidism is complicated further by the co-existence of both pituitary and hypothalamic disease in many cases (Patel and Burger, 1973).

GONADOTROPINS

The central nervous system, pituitary gland and gonads function as an integrated dynamic unit from early fetal life to old age. Gonadotropin secretion is regulated by the central nervous system and exteroceptive stimuli, in addition to direct feed-back control by gonadal steroids acting at hypothalamic and pituitary levels. The release of pituitary LH and FSH is stimulated by a common releasing hormone which has been isolated and recently synthesized (Schally, Arimura, Kastin, Matsuo, Baba, Redding, Nair, Debeljuk and White, 1971).

Gonadotropin secretion can be assessed either by direct radioimmunoassay measurement of LH and FSH in the basal state and in response to provocative or inhibitory influences, or through indirect tests reflecting the effect of these hormones on their target tissues. Although indirect tests such as the measurement of plasma testosterone are useful in the elderly male, ovarian senescence precludes the use of indirect tests in the post-menopausal female.

In the sexually mature premenopausal female gonadotropin levels in blood fluctuate in a rhythmic pattern related to ovarian secretion and ovarian function (Ross, Cargille, Lipsett, Rayford, Marshall, Strott and Rodbard, 1970). With advancing age ovarian function declines and responsiveness to gonadotropin stimulation decreases to the point where ovarian estrogens are secreted in inadequate quantities to suppress pituitary gonadotropin release. Thus in post-menopausal females serum LH and FSH levels are consistently elevated and are comparable with the levels measured in castrated adults. Similar doses of estrogen are required to suppress LH and FSH levels to eugonadal values in both castrated and post-menopausal women (Odell and Swerdloff, 1968). Although the threshold

of the hypothalamic pituitary axis to suppression by gonadal steroids may alter with age, recent studies in elderly post-menopausal women demonstrating suppression of gonadotropin responsiveness to clomiphene, presumably due to its weak estrogenic action, suggest that the sensitivity of the hypothalamic pituitary axis to feedback control is retained in old age (Odell and Swerdloff, 1968; Wise, Gross and Schalch, 1973).

In the sexually mature male the noncyclical pattern of gonadotropin secretion established during puberty is retained, albeit at different set points, throughout life. The concentration of LH in plasma is regulated by the circulating testosterone level, but can be modified by higher neural centres either by intrinsic regulatory mechanisms or in response to exteroceptive stimuli. The control of FSH secretion in the male is less clear. Pharmacological doses of either estrogen or androgen administered to sexually mature males cause suppression of FSH release (Odell, *et al.*, 1971; Lee, Jaffe, Midgley, Kohen and Niswender, 1972). Tubular substances emanating from germinal epithelium have been implicated in the regulation of FSH secretion, but further evidence is required to corroborate this theory (Franchimont, Millet, Vendrely, Lethawe, Legros and Netter, 1972).

In both males and females LH is secreted in a pulsatile manner, consisting of repetitive, abrupt discharges occurring with greatest frequency in the early hours of the morning, which are apparently unrelated to specific

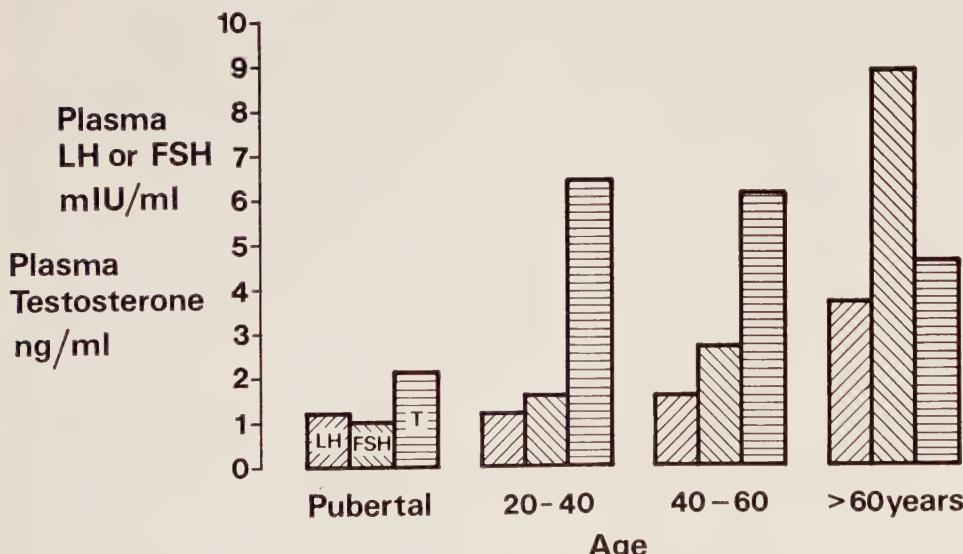


Figure 6-8. Plasma LH, FSH and testosterone levels in normal males from puberty to old age. Note the rise in plasma gonadotropin concentrations and decline in plasma testosterone concentration with age. (Data kindly supplied by H. W. G. Baker, J. M. Court, D. M. DeKretser, B. Hudson and C. Wang.)

stages of sleep (Nankin and Troen, 1972). Notwithstanding the episodic secretory pattern of LH, the serum LH concentration remains within a well defined range in adult males until the sixth decade, thence with advancing years there is a variable but significant decline in plasma testosterone levels (Vermeulen, Rubens and Verdonck, 1972). These findings have recently been confirmed by Baker, *et al.* (1973) who have also shown a good correlation between the decline in the plasma testosterone concentrations and a rise in the plasma LH concentration in male senescence (Fig. 6-8). As plasma FSH levels rise concomitantly with plasma LH levels, Baker, *et al.*, have concluded that senescent degeneration of both elements of the testis accompanies the aging process in males. Vermeulen, *et al.* (1972) have suggested that the great individual variation in testosterone levels in old age, ranging from as low as those found in females to levels considered to be relatively high for young men, might reflect the individual variation in the normal decline of male sexual vigor with age and in the rate of development of senescence. These factors must be considered in the assessment of hypothalamic pituitary function in the elderly.

The following scheme for assessment of gonadotropin secretion has been derived from a consideration of the control mechanisms of gonadotropin secretion in young adults and old age.

Basal Function

As circulating gonadotropin levels are elevated in post menopausal females, hypothalamic-pituitary disease is readily detected by measurement of basal serum LH and FSH concentrations. In the male, normal serum LH and FSH levels taken in association with a normal plasma testosterone level exclude any significant abnormality in the hypothalamic pituitary gonadal axis. Hypogonadism due to primary testicular disease or degeneration is characterized by elevated circulating gonadotropin levels and subnormal plasma testosterone levels. In this situation the degree of impairment in testosterone secretion can be assessed by measurement of the plasma testosterone response to gonadotropin stimulation in the form of administered human chorionic gonadotropin (Lipsett, Wilson, Kirschner, Korenman, Fishman, Sarfaty and Bardin, 1966). By contrast secondary hypogonadism due to primary hypothalamic and or pituitary disease is characterized by low normal or subnormal plasma LH and FSH levels. Further investigation is required using the following tests to assess the degree of impairment of gonadotropin secretion and to differentiate hypothalamic from hypopituitary hypogonadism.

Clomiphene Stimulation

Orally administered clomiphene citrate (CLOMID) stimulates the hypothalamic pituitary axis to release FSH and LH (Ross, *et al.*, 1970). In

man the Leydig cell responds to increased gonadotropin stimulation by an increase in testosterone production (Bardin, Ross, Rifkind, Cargille and Lipsett, 1969). The mechanism of clomiphene action is not precisely defined, but it would appear to act via the hypothalamus either by removal of hypothalamic inhibition through its anti-estrogenic action or by direct stimulation of the hypothalamus. Bardin, *et al.* (1969) first proposed the use of clomiphene stimulation in the investigation of hypogonadism. The test consists of the oral administration of 100 mg clomiphene for 5 to 7 days with serial measurements of serum FSH, LH and testosterone prior to clomiphene and 7 and 10 days after commencement of clomiphene. Whereas normal subjects exhibit a 100 percent rise in serum FSH and LH in response to clomiphene, patients with hypothalamic or hypopituitary hypogonadism exhibit smaller increments or no change in serum FSH and serum LH levels. The response to clomiphene is retained in old age (Wise, *et al.*, 1973), so that this test remains a useful diagnostic test in the elderly.

Luteinizing Hormone Releasing Hormone (LHRH) Stimulation Test

The recent elucidation of the structure and synthesis of the hypothalamic decapeptide luteinizing hormone releasing hormone (LHRH) (Schally, *et al.*, 1971) has now provided an important tool for the investigation of pituitary gonadotropin reserve (Kastin, Gual and Schally, 1972b). Synthetic LHRH administered to normal males or females evokes a significant and prompt increase in serum LH and FSH levels (Fig. 6-9).

The reproducibility of this response in humans suggests that both LH and FSH share a common releasing hormone (Schally, *et al.*, 1971; Kastin, *et al.*, 1972b). It is now well established that the gonadotropin response to synthetic LHRH is modulated at a pituitary level by circulating concentrations of gonadal steroids (Kastin, *et al.*, 1972b; Schally, Arimura and Kastin, 1973) so that the overall control of LH and FSH secretion may be mediated by an interaction of sex steroids with LHRH. It follows that interpretation of responses to administered synthetic LHRH requires knowledge of circulating gonadal steroid levels. To date, the use of the LHRH provocative stimulation test has not been fully evaluated. Nevertheless, preliminary reports point to the efficacy of this test in distinguishing between hypothalamic and pituitary lesions in patients with hypogonadotropic hypogonadism (Roth, Kelch, Kaplan and Grumbach, 1972; Naftolin and Harris, 1971). Preliminary results of studies performed in our laboratory are in agreement with these findings. Little is known concerning the gonadotropin responses to LHRH in the aged. It is our experience that elderly men have similar gonadotropin responses to younger men following synthetic LHRH administration. In elderly post-menopausal women the gonadotropin responses to LHRH are accentuated, presumably due to

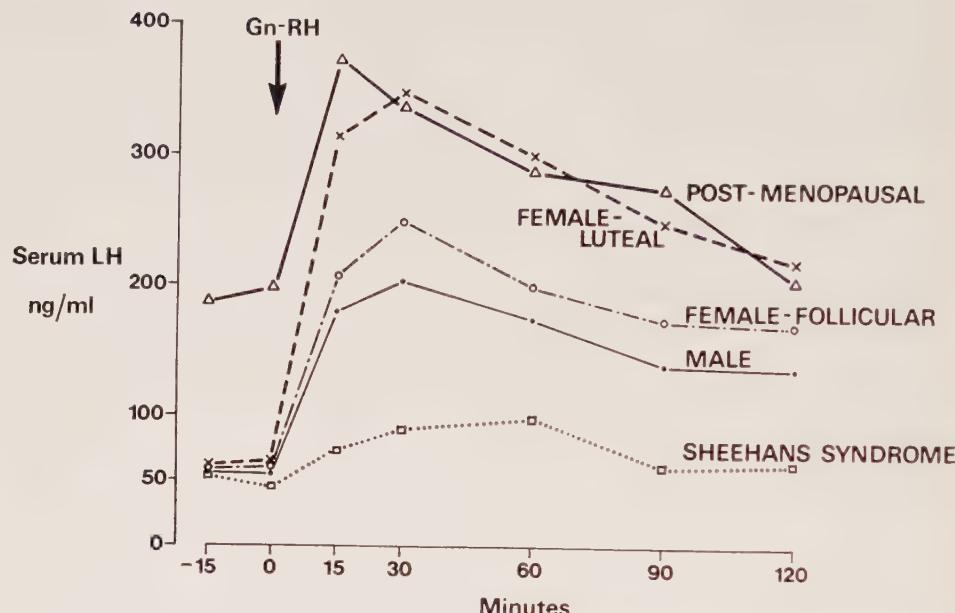


Figure 6-9. Illustrates the time course and magnitude of the rise in serum LH in response to a 25 μ g dose of synthetic luteinizing hormone releasing hormone (gonadotropin releasing hormone Gn-RH) administered intravenously to a normal male, a normal female during both the follicular and luteal phases of the menstrual cycle, a normal elderly post menopausal female and a hypopituitary patient with gonadotropin deficiency due to Sheehan's syndrome.

decreased circulating estrogen levels (Fig. 6-9). Elderly subjects with diminished pituitary gonadotropin reserve exhibit blunted or absent LH and FSH responses to synthetic LHRH. Further experience with the use of LHRH, especially in elderly subjects, is required to evaluate the clinical utility of this test in the assessment of hypothalamic pituitary function.

CORTICOTROPIN (ACTH)

ACTH secretion from the pituitary is under the control of the corticotropin releasing hormone from the hypothalamus. At the time of writing the hypothalamic hormone has not been isolated so that we do not have this available for clinical diagnostic use. The release of ACTH responds to feed-back alterations in plasma cortisol and to stress, and has a diurnal rhythm with maximal secretion in the early A.M. during sleep (Orth, Island and Liddle, 1967).

Ideally, studies of ACTH secretion should use direct measurement of ACTH in serum. This may be done using a radioimmunoassay which employs an antiserum directed against the N-terminal end of ACTH and the normal A.M. levels are 60 to 200 pg/ml. Due to the very low levels of

ACTH in serum it is necessary to extract the ACTH from plasma prior to measurement, thus making this radioimmunoassay a very tedious and expensive technique. In the presence of normal adrenocortical function a reliable index of ACTH secretion may be obtained by the measurement of plasma cortisol which is simple, precise and inexpensive. For these reasons it is common clinical practice to use the plasma cortisol measurement as an index of ACTH secretion, with the proviso that if the cortisol level is low or does not respond to pituitary-hypothalamic stimulation then it is necessary to perform an ACTH stimulation test to ascertain the functional state of the adrenal cortex itself (Lazarus, 1967).

The response of the hypothalamic-pituitary system to stress may be examined using insulin-induced hypoglycemia (described above) with measurement of the increment in plasma cortisol. A normal response is an increase of greater than $10 \mu\text{g}/100 \text{ ml}$ in the cortisol level following the induction of an adequate degree of hypoglycemia (Fig. 6-3). This test should not be performed in a patient who has coronary artery disease or cerebral arteriosclerosis. The cortisol response to insulin-induced hypoglycemia may be conveniently assessed simultaneously with the GH response during a combined arginine-infusion/insulin-hypoglycemia study. Elderly patients respond to insulin induced hypoglycemia with rises in plasma cortisol similar to the rises observed in young adults (Friedman, Green and Sharland, 1969; Cartlidge, Black, Hall and Hall, 1970).

In the absence of a positive cortisol response it is necessary to perform an ACTH stimulation test to assess the adequacy of adrenocortical reserve. This is performed by measuring the plasma cortisol response one hour following the I.M. injection of $250 \mu\text{g}$ of tetracosactrin. A positive response is an increase of greater than $10 \mu\text{g}/100 \text{ ml}$ in the plasma cortisol level 60 minutes following the tetracosactrin injection (Brownlie, Abernethy and Beaven, 1969). The responses in elderly subjects (Friedman, Green and Sharland, 1969) are similar to the responses we have measured in normal young adults.

Another test of stress response is the administration of bacterial pyrogens. This procedure, which utilizes the I.V. administration of highly purified nonantigenic bacterial antigens, is considered by most workers to act via the hypothalamus (Kohler, O'Malley, Rayford, Lipsett and Odell, 1967). This test may be associated with severe side-effects including muscle pains, chills, headache and vomiting and cannot be recommended for routine clinical use. A febrile response occurs, but some of the side-effects may be avoided by the administration of aspirin which does not interfere with the adrenal response. Indomethacin has been reported to block the cortisol response, suggesting that pyrogens act via the release of prostaglandins. The cortisol response reaches a maximum at 2 to 3 hours and a positive re-

sponse in our laboratory is an increase of greater than 10 $\mu\text{g}/100 \text{ ml}$ in the plasma cortisol concentration.

Vasopressin causes a significant release of ACTH and is thought to act directly on the pituitary in a manner similar to corticotropin releasing hormone (De Wied, Bohus, Ernst, De Jong, Nieuwenhuizen, Pieper and Yasumura, 1968). Synthetic lysine-vasopressin (LVP) is used in this test and may cause untoward side-effects, particularly via its pressor action (Tucci, Espiner, Tagger, Laufer and Thorn, 1968). These side-effects include cutaneous vasoconstriction with secondary facial pallor, nausea and an urge to defecate. More uncommon side-effects are vomiting, abdominal pain and occasionally, in women, uterine contractions. Coronary vasoconstriction may occur and for this reason LVP should never be given to patients with coronary artery disease or cerebral arteriosclerosis. The side-effects are related to the dose and route of injection of LVP and for this reason LVP should never be administered as a single I.V. dose. The dose of LVP is 10 I.U. by I.M. injection and blood samples for cortisol are taken at 0, 15, 30 and 60 minutes. The peak response usually occurs at 30 to 60 minutes and a positive response is an increase of greater than 10 $\mu\text{g}/100 \text{ ml}$ in the plasma cortisol level. The response to vasopressin in the elderly has not been fully assessed because of the significant risk of serious side effects in this population.

The diurnal rhythm of ACTH secretion is followed at a short time interval by a similar rhythm for cortisol and this rhythm is absent in Cushing's syndrome (Berson and Yalow, 1968). Recent studies of frequent sampling with an indwelling catheter have revealed, however, that the secretion of cortisol is episodic with a time interval of 60 to 90 minutes (Hellman, Nakada, Curti, Weitzman, Kream, Roffwarg, Ellman, Fukushima and Gallagher, 1970; Ceresa, Angeli, Bocuzzi and Perotti, 1970). For this reason the conventional practice of taking two plasma samples (one at 8 A.M. and one at 8 P.M.) to assess diurnal changes is unsatisfactory and if an assessment is necessary it requires frequent sampling throughout a 24-hour period.

The negative feed-back control of ACTH release may be assessed by the measurement of plasma cortisol after the administration of dexamethasone (Nugent, Nichols and Tyler, 1965). This test is of some importance in Cushing's disease where the essential lesion is a high set point for suppression of hypothalamic release of corticotropin releasing hormone. The procedure is to administer 1.0 mg of dexamethasone at 11 P.M. and to measure the plasma cortisol next morning at 8 A.M. In a normal subject the A.M. cortisol is suppressed to less than 5.0 $\mu\text{g}/100 \text{ ml}$. Failure of suppression indicates either a high set point for hypothalamic release of corticotropin releasing hormone, an extra-pituitary source of ACTH, or an autonomous

adrenal tumor with suppressed pituitary ACTH secretion. There is no significant difference between geriatric patients and young adults in the response to dexamethasone suppression (Friedman, Green and Sharland, 1969).

The feed-back response to a decrease in plasma cortisol levels may be examined by use of the enzyme blocking agent metyrapone (metopirone) (Lazarus, George and Stuart, 1963). This drug inhibits 11β -hydroxylase which is necessary for the final step in cortisol synthesis. As a result of this block the adrenal cortex produces mainly 11-deoxycortisol which is biologically inert and results in a compensatory increase of ACTH to maintain plasma cortisol levels. The block is never complete in a normal subject but the lack of compensatory ACTH release in a subject with hypothalamic-pituitary disease may result in acute steroid insufficiency and collapse. The response to metyrapone may be assessed by the measurement of plasma ACTH (Donald, Espiner and Beaven, 1972), plasma 11-deoxycortisol or urine excretion of 17 oxogenic steroids (17 OGS) (Lazarus, *et al.*, 1963). The latter is the simplest and is usually used clinically (Lazarus, 1967). The 24-hour urine excretion of 17 oxogenic steroids is assessed on 4 consecutive days. The first two days are basal and on the subsequent two days the subject is given 750 mg metyrapone orally every 6 hours. There is an increase in 17 OGS excretion (due to the production of large amounts of 11-deoxycortisol) and in a normal subject the 17 OGS on day 4 is usually twice the basal level. The minimal increase to be expected is 10 mg/24 hours (Lazarus, 1967). This test is potentially dangerous as mentioned above and also may cause dizziness, nausea and vomiting. It also involves hospitalization and prolonged urine collections and thus is not commonly used. As previously stated, a negative response requires the performance of an ACTH stimulation test to assess adrenocortical reserve. Consequently, it is apparent that measurement of plasma cortisol levels during a combined arginine-infusion/insulin-hypoglycemia test is the simplest and most effective way of assessing ACTH reserve in the elderly, and the results obtained with the use of this test in the elderly are similar to the results obtained in young to middle aged adults.

PROLACTIN

The hypothalamus controls prolactin secretion by the elaboration of a prolactin inhibiting factor (PIF) which exerts a tonic inhibitory effect on pituitary prolactin release (Meites, Lu, Wutke, Welsh, Nagasawa and Quadri, 1972). Removal of the inhibitory influence either by stimuli acting directly at a hypothalamic level or indirectly through higher neural centers, results in increased circulating prolactin levels. Hypothalamic catecholamines play an important role in regulating prolactin secretion,

serving as neurotransmitters to stimulate or inhibit release of PIF. Prolactin is present in human serum from birth to old age (Frantz, Kleinberg and Noel, 1972). Recently developed radioimmunoassays for serum prolactin have yielded results which are in close agreement with those obtained using well established bioassay methods (Frantz, *et al.*, 1972; Hwang, Guyda and Friesen, 1971). With the exception of the newborn, serum prolactin levels remain relatively constant throughout life and there is no significant difference between the basal serum prolactin concentrations in males and nonpregnant females.

In the evaluation of the hypothalamic-pituitary axis, basal serum prolactin estimations followed by stimulation and inhibition tests have proved particularly useful, especially in differentiating patients with hypothalamic disease from patients with pituitary disease.

Basal Function

The serum prolactin concentration in the basal state, is less than 30 ng/ml (Friesen human prolactin standard) (Hwang, *et al.*, 1971; Guyda and Friesen, 1973). Higher serum levels may be found during sleep, reflecting the nyctohemeral rhythmicity of prolactin release but the disturbed sleep patterns of the elderly may negate this response. Elevated serum prolactin levels are found after exercise, in response to psychogenic or physical stress and in association with chronic renal failure, and primary hypothyroidism (Frantz, *et al.*, 1972; Hwang, *et al.*, 1971; Guyda and Friesen, 1973; Lowenstein, Mariz, Peake and Daughaday, 1971). Hypothalamic disease or transection of the pituitary stalk in the presence of functioning pituitary tissue commonly results in elevated serum prolactin levels. Thus, an elevated basal serum prolactin concentration occurring in the absence of known stimuli and associated renal or thyroid disease, is suggestive of a hypothalamic disorder, and is an indication for further investigation. In addition we have recently investigated several patients with supposed nonfunctioning pituitary chromophobe adenomas in whom the serum prolactin levels have been extremely high (unpublished observations). Although some of these patients undoubtedly have secondary hypothalamic dysfunction these findings suggest that certain pituitary tumors secrete prolactin. Thus the diagnosis of a prolactin secreting tumour should be excluded in patients with elevated basal prolactin. This can be done with the use of stimulation and inhibition tests (*vide infra*). A low serum prolactin concentration is consistent with diminished pituitary prolactin reserve. Because of the great variability in serum prolactin levels in normal adults, a low serum prolactin level is not very helpful in establishing a diagnosis of hypopituitarism.

Stimulation Test

Insulin Induced Hypoglycemia

Hypoglycemia of sufficient magnitude is a potent stimulus to prolactin release as it is to that of growth hormone release. The measurement of serum prolactin during a standard insulin induced hypoglycemia test is a useful adjunct to the measurement of growth hormone in the assessment of hypothalamic pituitary function (Frantz, *et al.*, 1972).

Chlorpromazine (CPZ)

Phenothiazines and similar drugs which are capable of depleting the hypothalamus of catecholamines result in prompt and sustained rises in the serum prolactin concentration (Frantz, *et al.*, 1972; Turkington, 1971). This effect is mediated via suppression of hypothalamic PIF secretion. The intramuscular administration of chlorpromazine in a dose of 25 mg evokes a prompt increase in the serum prolactin concentration with peak levels being measured within 1 hour of administration. A normal prolactin response to CPZ is indicative of a normally functioning hypothalamic pituitary axis (Kleinberg, Noel and Frantz, 1971).

Thyrotropin Releasing Hormone (TRH)

TRH acts directly on the pituitary to increase prolactin secretion in addition to its effect on pituitary TSH secretion (L'Hermite, Van Haelst, Coppinschi, Leclerq, Goldstein, Bruno and Robyn, 1972; Jacobs, Snyder, Wilber, Utiger and Daughaday, 1971). The mechanism and significance of this effect is unknown. The prolactin and TSH response to TRH is preserved in old age. Subnormal or absent prolactin responses are observed in patients with significant impairment of pituitary prolactin reserve.

Suppression Tests

Levo Dopa

L-dopa, by increasing the secretion of prolactin inhibitory factor, inhibits pituitary prolactin release (Frohman, 1972). This effect is mediated presumably by dopaminergic fibres in the hypothalamus which control PIF secretion. The oral administration of L-dopa in a dose of 250 to 500 mg results in a significant decrease in the serum prolactin concentration in normal subjects. The inability to suppress prolactin release by L-dopa is suggestive of severe hypothalamic disease or an autonomously functioning pituitary tumor (Frantz, *et al.*, 1972; Malarkey, Jacobs and Daughaday, 1971).

Brom-ergocryptine

Brom-ergocryptine, an ergot derivative suppresses pituitary prolactin release by a direct inhibitory action on the pituitary cell (Yanai and Nagasawa, 1971; Besser, Parke, Edwards, Forsyth and McNeilly, 1972; Del Pozo, Burn, Re, Varga and Friesen, 1972).

CONCLUSIONS

The availability of radioimmunoassay techniques for measurement of circulating pituitary and target gland hormone concentrations in response to provocative and inhibitory stimuli permits full assessment of hypothalamic pituitary function in the elderly. Despite the progressive decrease in weight of the pituitary with age the application of dynamic tests of pituitary function has not revealed any significant impairment of pituitary tropic hormone reserve with age. With the possible exception of GH, pituitary hormone secretion continues, albeit at higher levels for LH, FSH and to a lesser extent TSH, throughout old age. The results of hypothalamic pituitary function studies employing provocative and inhibitory stimuli suggest that normal stimulatory and inhibitory mechanisms are retained in the elderly and have not provided any evidence for age related defects in feedback or neural control of hypothalamic pituitary function.

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CHAPTER 7

THE NEUROHYPOPHYSIS AND AGING WITH SPECIAL REFERENCE TO THE ANTIDIURETIC HORMONE

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SUMMARY

HERE IS CONVINCING EVIDENCE of a diminished secretion of antidiuretic hormone (ADH) by the neurohypophysis of the old male Wistar rat. The quantity of neurosecretory material (believed to be neurohypophyseal hormones) present in the neurohypophysis is reduced in the old rat.

In the muscles and plasma of the old rat there is a redistribution of water, sodium and potassium, similar to that seen in diabetes insipidus where there is a deficiency of ADH. These shifts are reversed by ADH. Further evidence of ADH deficiency is to be seen in the increased excretion of water in the old rat.

Bioassays of antidiuretic hormone reveal significant falls in the urinary excretion and plasma level of ADH in the old rat. The response of the neurohypophysis of the old rat to dehydration is reduced and delayed in its secretion of ADH. There is no evidence of any loss of sensitivity of the renal tubules to ADH in the old rat. Thus it appears that the neurohypophysis has an impaired ability to secrete ADH in the old Wistar rat.

INTRODUCTION

For some time it has been suspected that the neurohypophysis, like most other organs, undergoes a functional decline in the old rat. Until now this belief has been based on indirect evidence such as age changes in fluid and electrolyte balance and in the amount of neurosecretory material stored in the pituitary. Recent studies from this laboratory provide more direct evidence of a decline in the secretion of antidiuretic hormone in the old male rat.

AGE CHANGES IN THE MORPHOLOGY OF THE NEUROHYPOPHYSIS

Although morphological age changes in the anterior pituitary are well documented (Cooper, 1925; Roessle and Roulet, 1932; Rasmussen, 1938; Spagnoli and Charipper, 1955; Korenchevsky, 1961; Verzár 1966), very little is known about the corresponding changes in the neurohypophysis.

The data of Rasmussen (1938) suggest that the weight of the human neurohypophysis may increase in old age, possibly due to the increased connective tissue content (Spagnoli and Charipper, 1955). The vascularity of the neurohypophysis is reported by Xuereb (1964) to diminish in elderly men and women.

Histologically the old neurohypophysis is characterized by an invasion of basophilic cells, thought by some workers to come from the intermediate lobe. According to Tesauro (1952) this invasion probably starts at about 10 years in man and ends at 40. The invasion of basophils has been observed by a number of workers using human material (Spark, 1935; Shanklin, 1953; Randall, 1962). In the 1930's it was thought that hypertension was due to hypersecretion of vasopressin believed to be secreted by the basophils. However, Spark (1935) showed that there was no correlation between the frequency or degree of basophilic invasion and the incidence of hypertension. This has been confirmed by other workers. On the other hand more recent work by Frolkis, *et al.* (1973) shows that small blood vessels become more sensitive to vasopressin in old age, and also the blood level of vasopressin may increase with age in man.

AGE CHANGES IN THE HORMONE CONTENT OF THE NEUROHYPOPHYSIS

The pituitary content of neurohypophyseal hormones (antidiuretic hormone and oxytocin) is often judged from the amount of neurosecretory material (NSM) stained with the chrome-alum hematoxylin stain of Gomori (1941).

Rodeck, *et al.* (1960), using the Gomori stain to detect NSM, showed that there was no significant difference in the NSM content of the posterior lobes of young (3-6 months) and old (12-24 months) rats. However, in senile rats (over 24 months) the amount of NSM is reduced. Other workers (Morrison and Staroscik, 1964; Dunihue, 1965) have also observed a decrease in amount of NSM present in the pituitaries of old or senile rats. To Dunihue (1965) this reduction suggested that there is a diminished synthesis and storage of neurohypophyseal hormones in old age.

In a study of human pituitaries, Currie, *et al.* (1960) failed to find any relationship between age and the amount of antidiuretic hormone and oxytocin in the neurohypophysis. Only a small group of subjects aged 46 to 72 years was used.

In cattle pituitaries Nikitin (1961) observed a decline in old age in antidiuretic and oxytocic activity.

It is important to realize that by itself the assay of the amount of hormone stored in the gland gives no information about the rate of manu-

facture or secretion. The amount of hormone stored in the gland is merely a balance between the amount being produced and the amount being released.

AGE CHANGES IN WATER AND ELECTROLYTE METABOLISM

The antidiuretic hormone (ADH) is a major regulator of fluid balance and its secretion is controlled principally by changes in the osmotic pressure of blood and by changes in the extracellular fluid volume. For this reason age changes in fluid and electrolyte balance may be related to age changes in the secretion of ADH.

Redistribution of Water and Electrolytes in Old Rats

The redistribution of water and electrolytes as an organism ages has been well documented (Friedman, *et al.*, 1960; Korenchevsky, 1961; Chu-Sek, *et al.*, 1968). There is a progressive shift of water from the intracellular to the extracellular phase (Berger, *et al.*, 1950). Such a shift may simply represent progressive cell death and as such would be irreversible (Lowry and Hastings, 1952; Andrew, *et al.*, 1959; Yengst, *et al.*, 1959).

Friedman, *et al.* (1963a and b) re-examined these electrolyte changes by measuring sodium, potassium and water distribution in the gastrocnemius muscle and plasma of young (5-7 months) and old (20-25 months) male Wistar rats. They found an increase in total sodium in the old rat, the gain being both intracellular and extracellular, but mainly extracellular. There is a corresponding decrease in total potassium, the loss being entirely cellular. These electrolyte shifts are associated with a shift of water from the intracellular to the extracellular phase. The shift of water along with cations in the old organism maintains the normal plasma Na^+ and K^+ concentrations. Similar observations on electrolyte and water shifts in the old organism have been recorded in man (Allen, *et al.*, 1960; Macgillivray, *et al.*, 1960; Shock, *et al.*, 1963).

The pattern of electrolyte and water distribution in the old rat bears a striking resemblance to many features of diabetes insipidus as described in man by Findley (1949). In the young rat with diabetes insipidus, induced experimentally by hypothalamic lesions, the water and electrolyte shifts are similar to those observed by Friedman, *et al.* (1963a and b) in the old rat. However, the changes seen in diabetes insipidus are more severe than those detected in old rats. Thus these studies provide indirect evidence of a decline in ADH secretion in the old rat.

Water Turnover and Age

In old rats the turnover of water (ratio of urine production: water intake) is increased (Everitt, 1958; Friedman, *et al.*, 1967). The increased

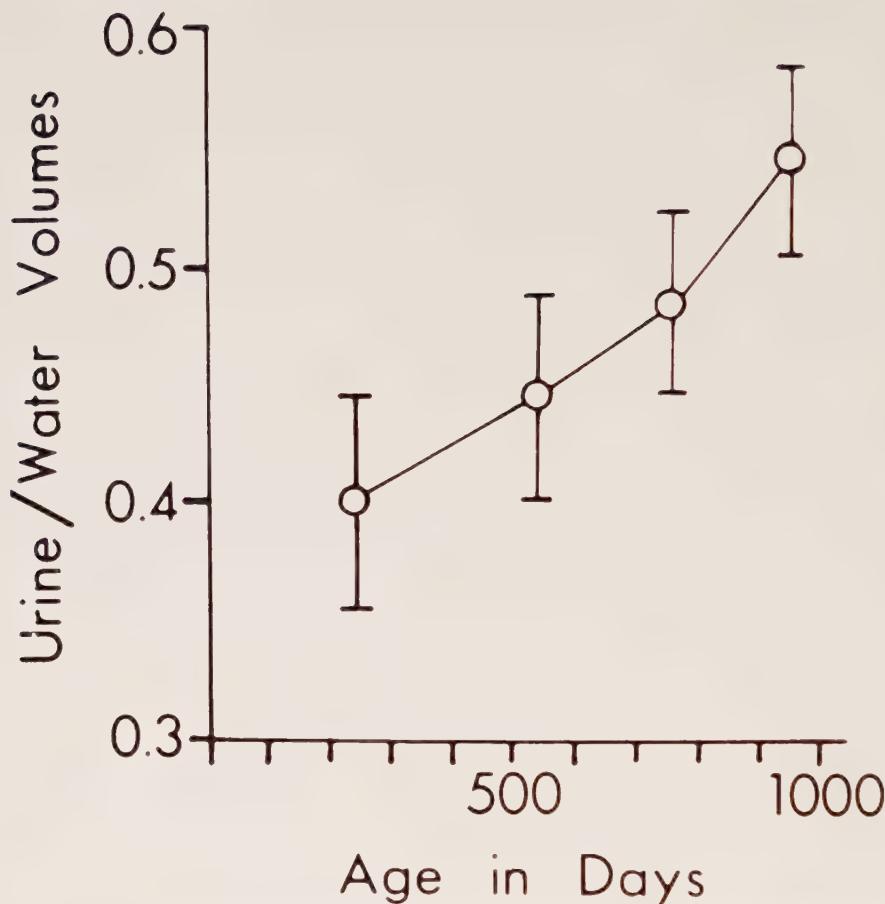


Figure 7-1. The effect of age on water turnover as measured by the urine volume: water intake volume ratio of the male Wistar rat. Means \pm SEM are plotted for 25 rats in each group.

water turnover in old rats is similar to, but less marked than, that seen in diabetes insipidus. In Figure 7-1 it can be seen that the water turnover rises with increasing age in the rat.

RENAL SENSITIVITY TO ADH AND AGE

The alterations in water metabolism and turnover seen in older rats may be due not only to neurohypophyseal hypofunction, but also to a diminished responsiveness of the kidneys of old rats to ADH. The human kidney has been shown to be less responsive to ADH in old age (Miller and Shock, 1953).

In our laboratory we tested the responses to ADH of young (250-300

days) and old (700-800 days) rats which were ethanol blocked and hydrated, so that a stable diuresis was achieved in both groups. All rats were healthy and matched for weight. The results are shown in Figure 7-2. It is evident that both young and old rats responded with comparable degrees of antidiuresis to the same doses of arginine-8-vasopressin.

Thus the renal tubules in the old rat are just as sensitive to ADH as in the young rat. Therefore the mild form of diabetes insipidus which is evident in most old and senile rats is probably not nephrogenic in origin.

Rats with extensive renal pathology, including chronic glomerulonephritis, acute tubular necrosis and other diseases which result in extensive renal fibrosis may show signs of ADH resistance. This effect would be superimposed on a neurohypophyseal hypofunction and thereby produce a more severe diabetes insipidus. The presence of these lesions in some old rats may explain the large variation in the degree of "diabetes insipidus" detected in old age.

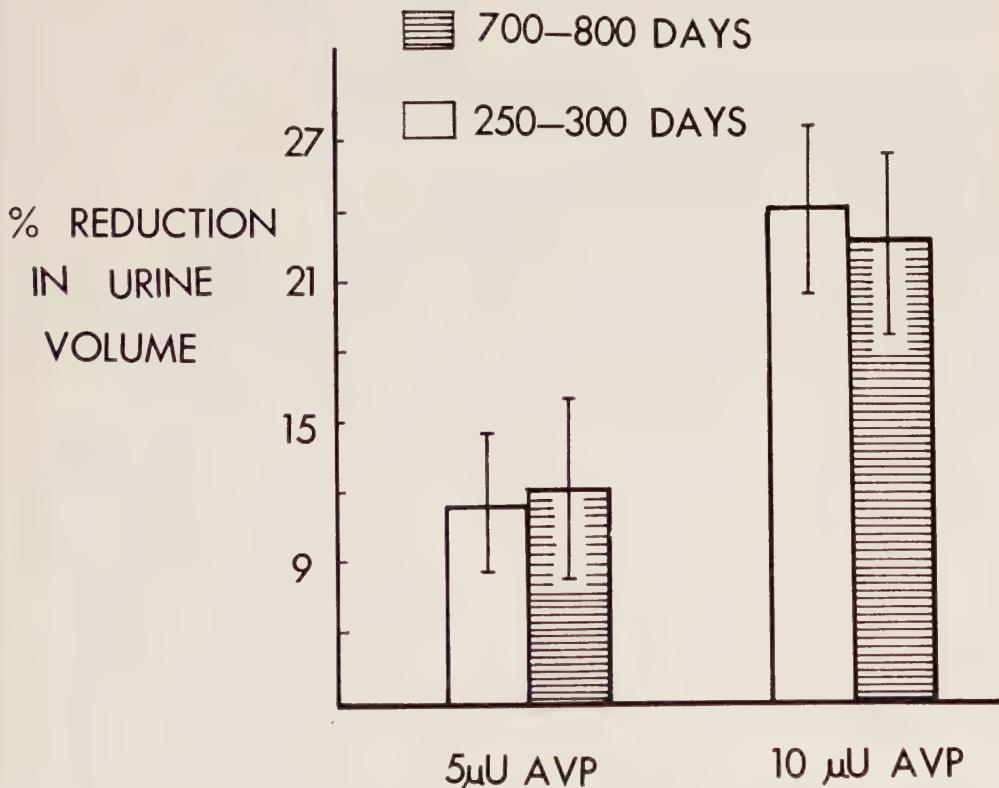


Figure 7-2. The effects of 2 doses of arginine-8-vasopressin on the inhibition of a diuresis in hydrated young and old rats. Means \pm SEM are plotted for 10 young and 6 old rats.

The predominant defect in water metabolism in the old Wistar rat evidently is of neurohypophyseal origin. In the absence of renal pathology there is no evidence of a decline in renal sensitivity to ADH.

SECRETION OF ADH IN THE AGING RAT

Two approaches have been used to measure the secretion rate of hormones. The isotope dilution technique (Peterson, 1959) and the metabolic clearance technique (Tait and Bursten, 1964). Neither of these methods has been applied to the study of age changes in the secretion of neurohypophyseal hormones. Only indirect measures of hormone secretion such as the daily excretion of ADH in urine or the plasma ADH level have been employed in studying age changes. Data on oxytocin secretion in old age are lacking.

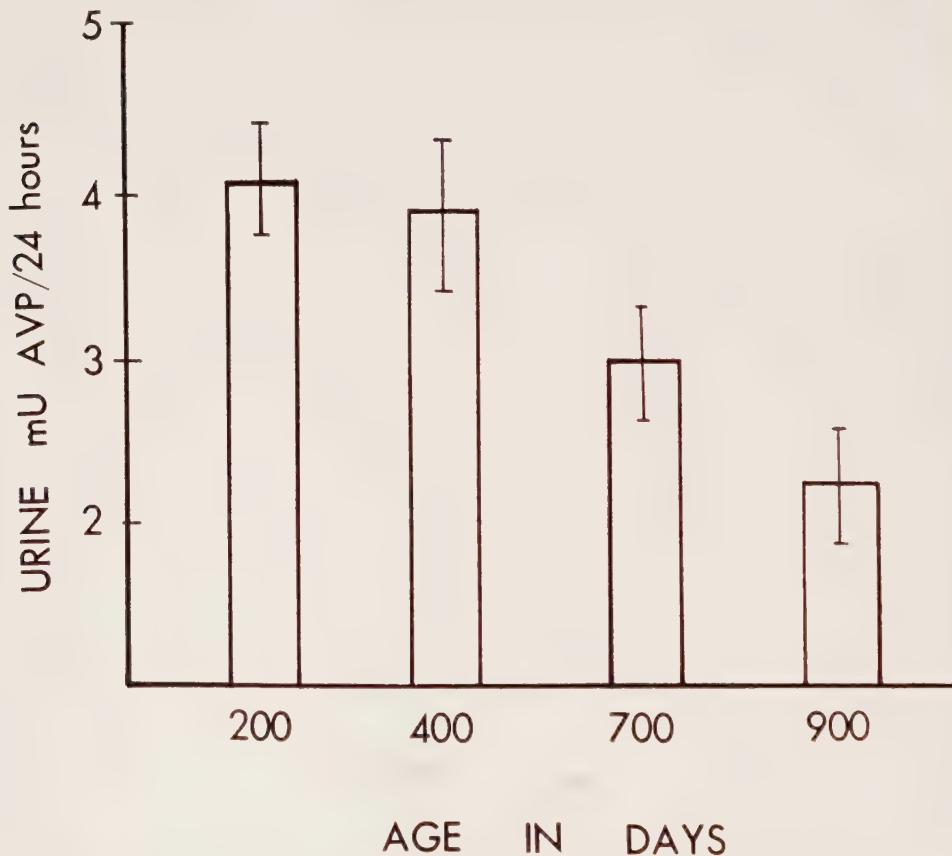


Figure 7-3. The decline with increasing age in the daily excretion of antidiuretic hormone in the male rat. Means \pm SEM are plotted for 10 rats aged 200, 400 and 700 days, and for 6 rats 900 days.

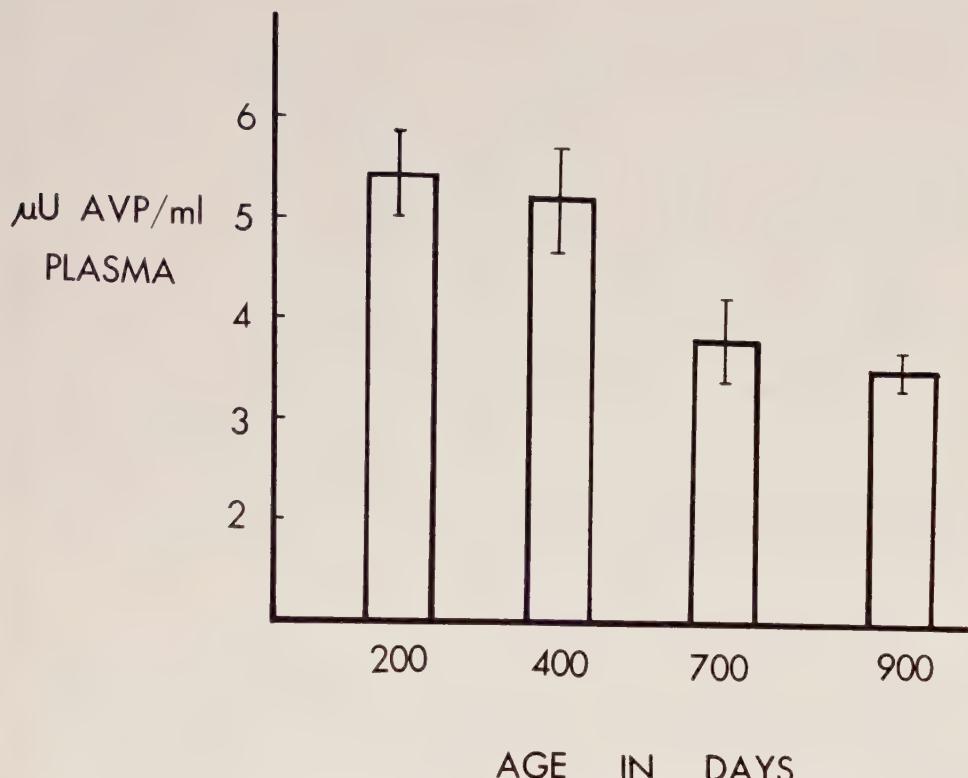


Figure 7-4. The fall in the plasma level of antidiuretic hormone in the old male rat. Means \pm SEM are plotted for 10 rats aged 200 days, 10 at 400, 8 at 700 and 6 at 900.

Urinary ADH Excretion and Age

The quantity of ADH excreted in urine was found by Miller and Moses (1971) to parallel very closely the changes in ADH content of the neurohypophysis. In other words the quantity of ADH excreted is believed to reflect the total amount of ADH secreted.

Urine was collected from rats housed in metabolism cages without subjecting them to stress, which is known to increase ADH secretion (Moran, *et al.*, 1964). ADH was extracted from urine with phenol (Jessup, *et al.*, 1955) and bioassayed on young male Wistar rats by determining the reduction in urine output according to a variation of the Guzek and Lesnik (1968) technique.

In Figure 7-3 it can be seen that the daily excretion of ADH falls significantly in old rats. This strongly suggests that the secretion of ADH declines with age in the male Wistar rat.

Plasma ADH Level and Age

The collection of blood samples usually stresses animals sufficiently to raise ADH secretion. Rapid decapitation is the most effective means of preventing ADH release into general circulation when collecting a blood sample from the trunk. ADH was estimated in the plasma using phenolic extraction and bioassay in young male rats by a variation of the technique of Guzek and Lesnik (1968).

In Figure 7-4 it can be seen that the plasma ADH levels in the two groups of old rats (700 and 900 days) are significantly lower than those measured in younger rats (200 and 400 days). This study did not exclude the possibility that the low plasma ADH levels were due to increased catabolism of ADH in old age. However, it is clear from the data in Figure 7-3 that the low ADH levels in old rats are not due to increased renal excretion of ADH. It seems reasonable to conclude that the reduced plasma ADH levels in the old rat are a direct result of a reduction in the amount of ADH secreted by the neurohypophysis.

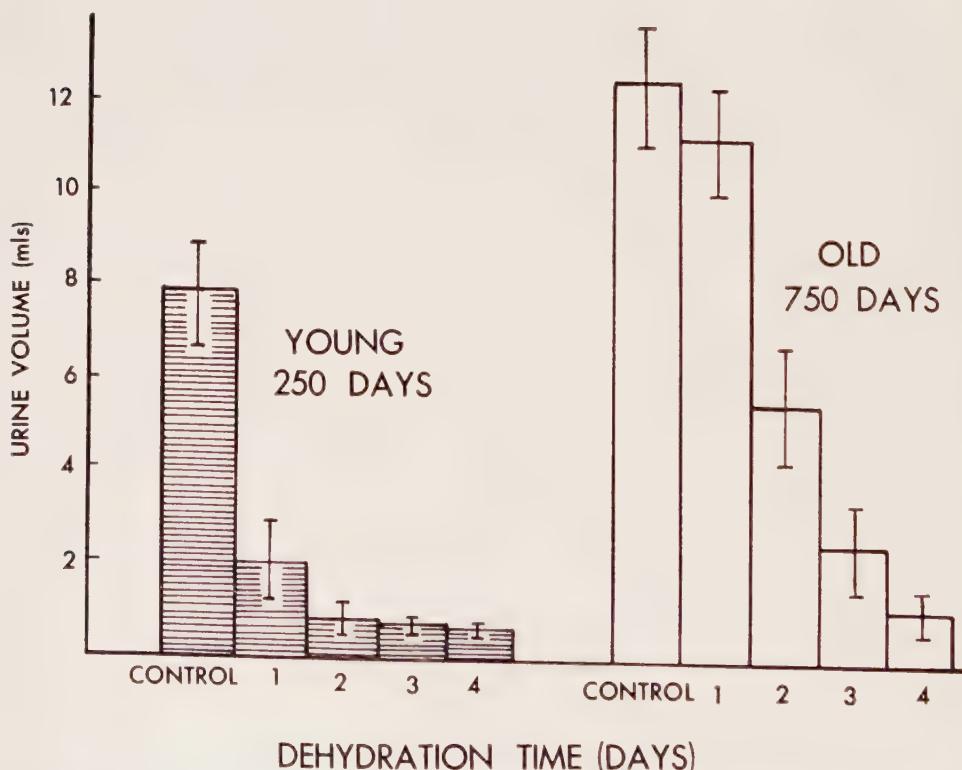


Figure 7-5. The daily urine volume in young and old rats during a control period and during 4 successive days of dehydration (1, 2, 3, 4). Means \pm SEM are plotted for 6 rats in each group.

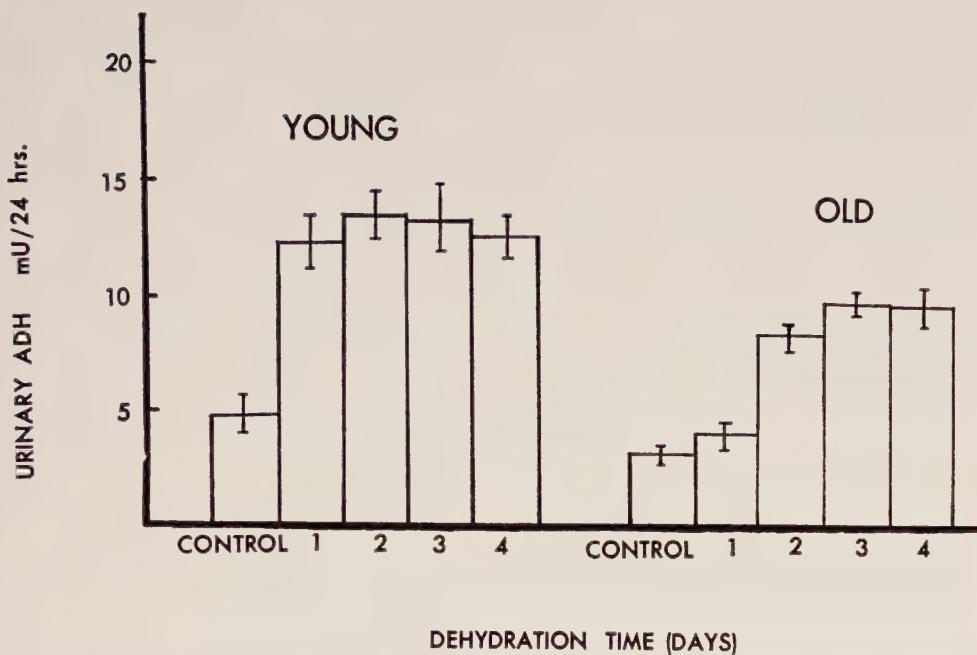


Figure 7-6. The daily urinary antidiuretic hormone excretion in young (250 days) and old rats (750 days) during a control period and during 4 successive days of dehydration (1, 2, 3, 4). Means \pm SEM are plotted for 6 rats in each group.

NEUROHYPOPHYSEAL RESPONSIVENESS AND AGE

The classical studies of Verney (1947) established that the effective osmotic pressure of plasma in the internal carotid artery played a major role in the control of ADH secretion. The responsiveness of the central osmoreceptor mechanism, in young (under 6 months) and old rats (over 20 months), to hypertonic saline injection was tested by Friedman, *et al.* (1956). The young rat showed an antidiuretic response but the old rat was refractory. Friedman and his coworkers interpreted these data as evidence for a decline in neurohypophyseal function with age.

In our laboratory we studied the responses of young (250 days) and old rats (750 days) to dehydration (water withheld for 4 days). The young and old rats were matched for weight. Measurements of daily urine volume (Fig. 7-5) and daily urinary ADH excretion (Fig. 7-6) were made. In young rats there was a prompt fall in urine output once water was withheld, but the response was much slower in the old rat (Fig. 7-5). Similarly the response as measured by ADH excretion in urine was much faster in young rats and reached significantly higher levels, when compared with the old rats (Fig. 7-6).

This difference in the pattern of the response to dehydration suggests

that the old rat releases ADH less rapidly, possibly because of an impaired sensitivity of the osmoreceptors. The young rat responds almost maximally on the first day whereas the old rat requires 3 to 4 days to achieve this maximal response. The level of ADH excretion after 4 days (and after 6 days in a few rats studied) was significantly less in the old than in the young rat. This suggests that the amount of ADH stored in the neurohypophysis in the old rat is less than that stored in the young. This conclusion correlates well with the finding of reduced neurosecretory material in

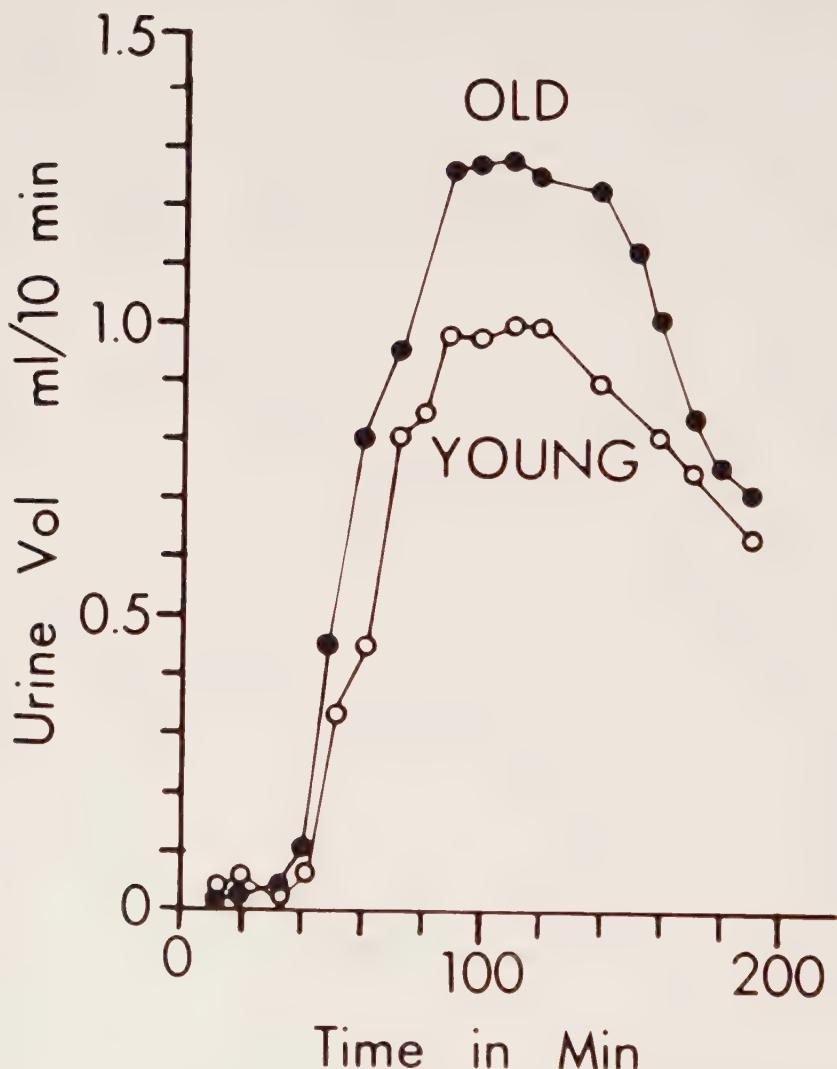


Figure 7-7. Water diuresis curves of young (300 days) and old rats (750 days) matched for weight, after the oral administration of 4.5 ml of 0.2% NaCl. There were 6 rats in each group.

the neurohypophysis of the old rat (Rodeck, *et al.*, 1960; Morrison and Staroscik, 1964; Dunihue, 1965).

In a second study we compared the response of young (300 days) and old rats (750 days) to water loading. Measurements of daily urine volume (Fig. 7-7) showed that old rats excreted a larger proportion of the administered water load than young rats. Similar results were reported by Friedman and Friedman (1957). This suggests that the neurohypophysis of the old rat is less responsive to the fall in osmotic pressure due to water loading than that of the young rat.

The present studies indicate 1) that the neurohypophysis of the old rat has an impaired ability to secrete ADH or possibly an impaired ability to sense the osmolality changes which normally elicit ADH secretion and 2) that ADH storage in the neurohypophysis is reduced.

EFFECTS OF REDUCED ADH SECRETION

The marked disturbance of water and electrolyte metabolism in the old rat is related to a deficiency of antidiuretic hormone (Friedman, *et al.*, 1960). In these old rats the ability to perform muscular work is markedly diminished. The decline in muscular work performance is associated with a distortion in the transcellular distribution of sodium, potassium and water in skeletal muscle. The administration of a modest amount of antidiuretic hormone (pitressin tannate) to old rats during a 4-week period improved the work performance of the gastrocnemius muscle to a significant extent (Friedman, *et al.*, 1963b).

Some of the neurophysiological and cardiovascular age changes may be explained on the basis of the reduced concentration gradients of sodium and potassium. Age changes such as the decline in the conduction velocity of nerves and the fall in the resting cardiac output might possibly be explained in this way.

Life duration may be shortened by the deficiency of ADH in the old rat. Friedman and Friedman (1963 and 1964) administered posterior pituitary extract to old rats for 6 months. They found an improvement in the condition of their rats and a significant reduction in mortality. There is evidence that this life-prolonging effect may be due to the oxytocin component of the posterior pituitary extract (Bodanszky and Engel, 1966). This work on life span has possible far-reaching and exciting prospects and there is need for a more extensive and probing trial of the life prolonging effect of oxytocin.

CONCLUSIONS

In the old male Wistar rat there is convincing evidence for a decline in neurohypophyseal function with regard to the secretion of antidiuretic hormone. There is no evidence of aging at the principal target organ for

ADH, since the renal response to ADH does not change with age. Whether the neurohypophyseal hypofunction is due to a reduced sensitivity of the osmoreceptors, or whether there is a defect in the synthesis of ADH or in the ADH release mechanism has not been established.

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CHAPTER 8

THE PATHOLOGY OF THE PITUITARY GLAND IN OLD AGE

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SUMMARY

ALTHOUGH IT IS GENERALLY THOUGHT that pathological changes in the pituitary gland are relatively unimportant in the elderly, it is shown that they may achieve considerable significance. Treatable tumors occur, and also reversible endocrine changes. Much is to be learnt from careful pathological investigation of pituitary glands, which may sometimes have no macroscopic abnormality.

Disturbances of the pituitary blood supply lead to vital changes in the clinical picture and may be associated with raised intracranial pressure or trauma. The incidence, investigation and treatment of pituitary tumors are described. Tumors of the pituitary gland occur in experimental animals and assist the study of those which occur in man.

INTRODUCTION

It is interesting that Harvey Cushing in his classical works on the pituitary gland omitted any reference to the aging process or to the specific features of pituitary abnormalities in the aged. Aschoff (1937) felt that the pituitary gland had an important role in senile changes but was not the dominant factor in them. The stimulation and restraints on the activities of the pituitary gland in the aged were more likely to be dominated by the nature of the body in which the gland found itself than the reverse. Although the gland undergoes characteristic aging changes, Aschoff (1937) considered that its endocrine and anatomical adjustments are part of the pattern of senescence, not in a causative role but as a participating one.

It is evident from a number of studies of the pathology of old age that the pituitary gland is not especially commonly affected by disease processes in the aged. Physiological changes in hormone secretion are discussed by Lazarus and Eastman in chapter 6 of this monograph. Changes in the morphology of the gland are outside the scope of this chapter and were reviewed previously by Korenchevsky (1961) and by Charipper, *et al.* (1961). Monroe (1951) in his clinical and pathological survey of almost eight thousand cases over 61 years of age found the pituitary gland dis-

eased in only 6 patients, all of whom had pituitary tumors. Howell and Piggott (1951, 1952, 1953a and b) in a series of studies of the morbid anatomy of old age do not mention the pituitary at all, and McKeown (1965), who studied 1,500 patients aged 70 or more at autopsy, believed that pituitary diseases are probably the least important of endocrine disturbances in the aged. Descriptions of pituitary pathology are to be found in works by Russell (1961) and Currie (1966).

To the present authors, the results of some previous large scale surveys of autopsy material are open to the criticism that a brief macroscopic look at the pituitary gland (let alone the hypothalamus) can give very little information of value. Pituitary glands may contain small lesions such as infarcts, adenomas or secondary carcinomas which are unsuspected clinically and not obvious macroscopically.

With the increasing clinical and research requirements for human pituitary hormones, pituitaries are collected at autopsies all over the world for processing by pharmaceutical manufacturing laboratories. This raises several points of interest. It may be that human pituitaries will not be included in large scale research studies in future, or will be included with only a macroscopic description. Abnormal glands containing unsuspected lesions, which may be secreting hormones, possibly abnormal ones, may be included in batches of glands collected for hormone extraction.

The pituitary may easily be damaged during its removal from the pituitary fossa at autopsy. The neural lobe is most easily damaged and to avoid this the utmost care is needed. Removal of the gland *in situ* in the fossa and dissection after fixation may be the best way of avoiding damage to the gland. After death, autolytic changes take place rapidly in the anterior lobe. Distortion of the shape of the cells and alteration of their staining properties are to be expected within 6 to 8 hours. Although the general distribution of the various types of cell is not disorganized by post-mortem changes the more subtle distinction between cells may easily be disturbed by autolysis.

In many instances the microscopic examination of the pituitary, particularly of the posterior lobe, is incomplete without study of the hypothalamus and pituitary stalk. Obviously the hypothalamus cannot be thoroughly studied as a routine procedure, but if it is preserved it can be sectioned after fixation in cases with pituitary gland pathology. Similarly any study of the pathology of the pituitary gland should include histological, and if possible biochemical, studies of those endocrinines which are target organs of pituitary tropic hormones. It should always be borne in mind by the histologist that the appearances and staining properties of pituitary cells merely reflect the balance between hormone synthesis, storage and release. A cell which is tinctorially a chromophobe may be pouring out hor-

mone and the appearance of the target glands and tissues generally may be a valuable indication of the secretory activity of a pituitary tumor. Conversely a state of hypofunction of the anterior pituitary may be more easily detected by the state of the target organs than by the histological appearance of the pituitary itself. This is particularly relevant in cases with disturbance of the hypothalamic control of anterior lobe function.

DISTURBANCES OF PITUITARY BLOOD SUPPLY

It is generally accepted that the blood supply to the anterior pituitary is almost entirely portal in nature. "Long portal vessels" drain capillary plexuses in the tuber cinereum and pituitary stalk while "short portal vessels" originate in the rich capillary plexuses of the neural lobe (Xuereb, Prichard and Daniel, 1954a and b; Daniel and Prichard, 1966). The artery

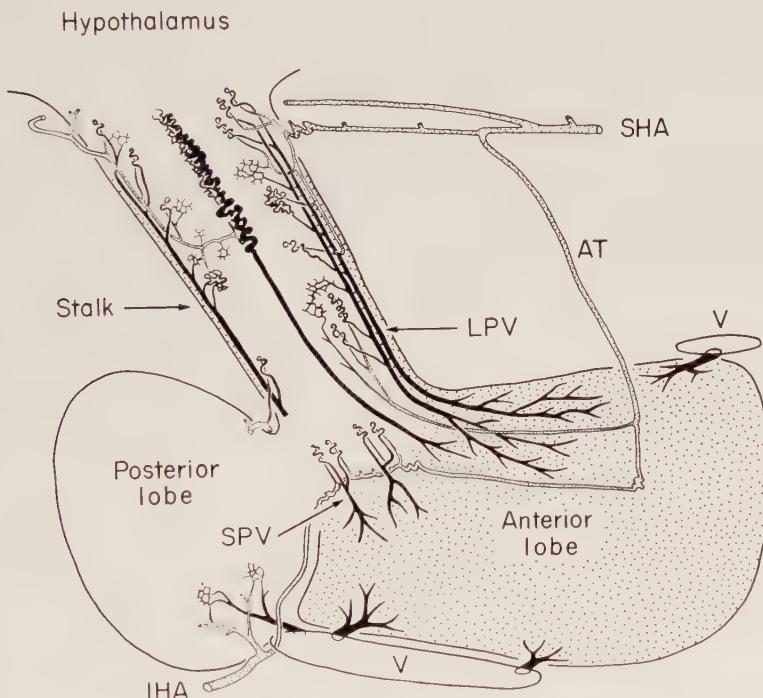


Figure 8-1. Diagram showing the main features of the blood supply to the pituitary gland. The anterior lobe (stippled) receives portal venous blood through the long portal vessels (LPV) and short portal vessels (SPV) which drain capillary plexuses in the stalk and posterior lobe respectively. The capillary beds of the stalk, typically convoluted and complex, are supplied by the superior hypophyseal artery (SHA) which lies above the diaphragma sellae. The posterior lobe is supplied by the inferior hypophyseal artery (IHA) which lies below the diaphragma sellae. Venous sinuses (V) lie adjacent to the gland. AT—artery of the trabecula. (From Daniel & Prichard, 1966, reproduced by permission of the *American Heart Journal*.)

supplying the tuber and stalk is the superior hypophysial artery, which is suprasellar, while the inferior hypophysial artery supplies the neural lobe and is below the diaphragma sellae (Fig. 8-1). Compromise of the blood supply to the primary capillary plexuses or of the portal vessels of the stalk will lead to deprivation of blood supply and subsequent infarction of the anterior lobe of the pituitary.

Spontaneous Infarction of the Anterior Pituitary

Small infarcts in the anterior lobe are a common finding in the routine study of pituitaries at autopsy. They are frequently unilateral and occupy only a small area of anterior lobe. In the majority of cases no local pathology is obvious except atherosclerotic disease of the arteries at the base

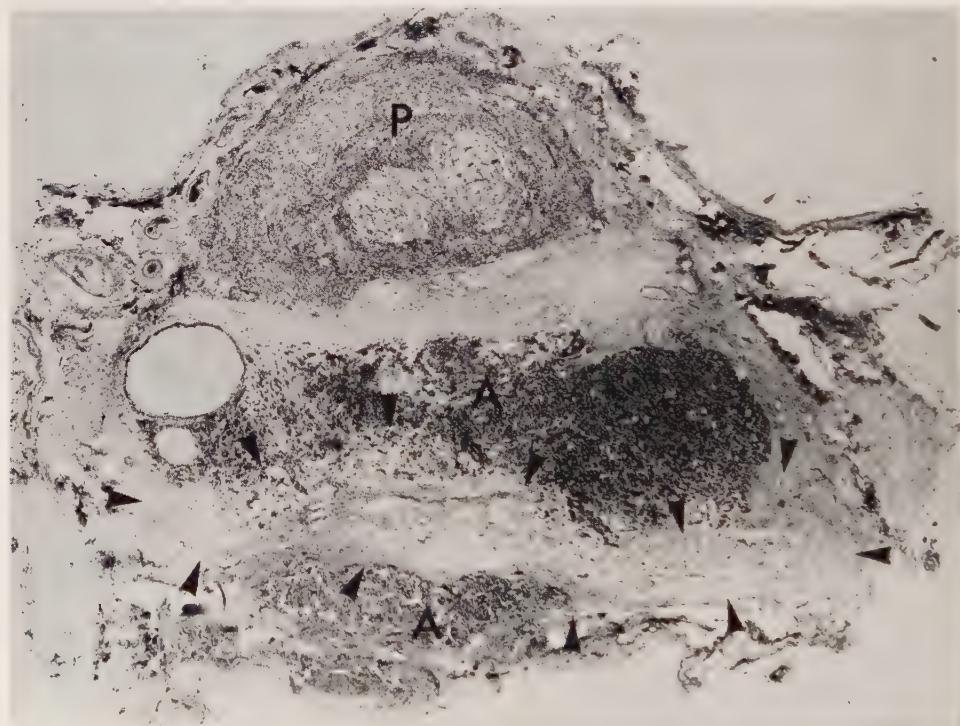


Figure 8-2. Horizontal section through the pituitary gland of a man aged 67. He had diabetes mellitus from the age of 40, with severe diabetic retinopathy. At the age of 59, his insulin requirements fell and his vision improved. After this he did not receive insulin. He died of myocardial failure. The anterior lobe of the pituitary gland shows extensive replacement by scar tissue (arrows), the end result of infarction. Some anterior pituitary glandular tissue (A) remains in the region adjacent to the posterior lobe as well as under the capsule. The posterior lobe (P) is shrunken and most of it is hypercellular. The central part of the posterior lobe is of normal cellularity and contained nerve fibers and neurosecretory material. (Hematoxylin & Van Gieson stain, magnification $\times 17$.)

of the brain. More rarely and of greater interest is the pituitary infarction occurring in association with diabetes mellitus. This association is emphasized by Warren, Lecompte and Legg (1966) who describe 32 infarcts of the anterior pituitary in 1036 autopsies of diabetic patients (3.1%). The infarcts in their series varied in size from 2 mm in diameter to almost the whole gland. In a larger number of nondiabetic autopsies the incidence of pituitary infarction found by Warren, *et al.* was only 0.9 percent. Only one of their diabetic patients had clinical evidence of hypopituitarism since most of the infarcts occurred shortly before death. Mallins (1968) considers hypopituitarism to be a common event in diabetes mellitus. A sudden reduction of insulin requirement with a danger of insulin overdose in diabetics is well known as the "Houssay phenomenon" from the experimental studies of Houssay and Biasotti (1930). Calvert and Caplin (1957) found that in well-documented cases in the literature there was no predilection for age or sex among the affected diabetics and noted the high fatality rate and the absence of clearly defined clinical features other than insulin sensitivity. Histological examination of the acutely infarcted gland shows necrosis of varying amounts of anterior lobe tissue but invariably a thin rim of cells beneath the capsule is spared. Some anterior lobe tissue adjacent to the posterior lobe also usually survives. The cells within the infarct retain their differential staining reactions, though weakly, for some days after nuclear staining is lost. With longer survival the infarct becomes replaced by scar tissue and increasing fibrosis leads to shrinkage and great distortion of the gland (Fig. 8-2). Occasionally diabetes insipidus has been recorded in association with the Houssay phenomenon. The posterior lobe may be partly scarred and smaller than usual as is found in some cases surviving for a long time after post-partum pituitary necrosis (Sheehan and Whitehead, 1963). The changes in the neural lobe are probably secondary to infarction of the stalk which may be due to obstruction of its venous outflow when long portal vessels are occluded.

Pituitary Necrosis

In a study of Simmonds' disease carried out by Soffer (1951) 12 percent of the patients studied were between the ages of 60 and 70, and none were over the age of 70. The author remarked that difficulties in diagnosis of this condition in old age might account for this finding.

Infarction of the anterior pituitary of varying degree is quite commonly seen in cases with raised intracranial pressure (Wolman, 1956). The mechanism of this infarction is probably compression of the vessels of the tuber cinereum and stalk when the hypothalamus is pushed downward by increased pressure above it.

Trauma to the pituitary stalk is likely to increase in frequency since

there is an increasing incidence of road traffic accidents, particularly with injury to elderly pedestrians. Acute massive infarction of the anterior lobe occurs after traumatic rupture of the pituitary stalk (Daniel, Prichard and Treip, 1959) and is due to the interruption of long portal vessels. Fractures of the skull base are usually also found. Trauma to the pituitary stalk should be suspected in cases with diabetes insipidus or with prolonged coma apparently out of proportion to the degree of injury. The pathology of pituitary lesions occurring after head injury has been reviewed by Daniel and Treip (1966).

PITUITARY TUMORS IN EXPERIMENTAL ANIMALS

Adenomas of the pituitary gland are commonly found in experimental animals and have been described in a wide variety of species. Most work in this field has been done with mice and rats and there is a very extensive literature on the subject. Spontaneous adenomas in aging animals represent a source of confusion in any study of tumor induction in the pituitary gland. Their incidence in rats is usually considerably greater than in mice, and the sex and strain of animal used are also important factors in determining the occurrence of spontaneous tumors. Upton and Furth (1955) found adenomas in 8 percent of female LAF₁ mice aged 3 years, an incidence four or five times higher than in males. In normal old mice used as controls by Upton, *et al.* (1960) the incidence of spontaneous tumors was 4.2 percent in females and less than 1 percent in males.

In rats the influence of advancing age on the frequency of spontaneous tumors was studied by Saxton and Graham (1944) who found that extremely few adenomas developed before 1 year of age. In one strain of animals (Yale) 60 percent of males and 30 percent of females aged 500 days or more had tumors, though in another strain (Sherman) less than 4 percent of males had tumors. In the Vanderbilt strain tumors occurred in 14 percent of males and 28 percent of females 17 months or more of age. Saxton and Graham described these tumors as "chromophobe adenoma-like lesions." In a more recent long-survival experiment Griesbach (1967) used rats of the Long-Evans strain and found that 94 percent of males and 43 percent of females 2 years old had pituitary adenomas. He noted that the majority of adenomas in the males consisted of gonadotroph cells and few were chromophobe in type. In the females no gonadotroph-cell tumors were seen and most were chromophobe in type.

It is of interest that most spontaneously-occurring adenomas, as with the majority of chromophobe adenomas in man, do not seem to be associated with changes in other endocrine glands except as a result of destruction of functioning pituitary tissue by the tumor itself. Tumors occurring as a result of experimental procedures, however, are more often functional. It

seems that such induced tumors are the result of a disturbance of the normal relationship between target organ, its specific trophic cell in the pituitary and the mechanism of the control of pituitary cell function by the hypothalamus.

Tumors of the anterior pituitary have been induced in mice and rats by the chronic administration of estrogen, by ionizing irradiation, gonadectomy, administration of ^{131}I , chronic administration of goitrogen and of iodine-deficient diets. Tumors were found in mice exposed to ionizing irradiation (atom-bomb irradiation) at an earlier age and with an increased incidence than in old control animals (Upton, *et al.*, 1960). The tumors were more common in females (as in controls) and were twice as common in the mice exposed to neutrons compared with those exposed to gamma rays. Clifton (1959) reviewed the experimental induction of tumors in rats and mice and noted that those adenomas arising after prolonged exposure to estrogens are usually "mammatrophic." Transplantation experiments show that these estrogen-induced mammatrophic tumors will survive only when high estrogen levels prevail in recipient animals (i.e. the tumors are "dependent"). Repeated transplantation results in the tumor becoming "autonomous" and growing in the absence of high estrogen levels. On the other hand mammatrophic tumors arising spontaneously or after ionizing irradiation are autonomous from the outset. These mammatrophic tumors consist of sparsely-granulated acidophil cells and are often called chromophobe adenomas. It seems likely that they arise from the sparsely-granulated acidophils which secrete prolactin in rats and mice (Bielschowsky, 1954). Tumors composed of thyrotrophic cells rarely occur spontaneously (Griesbach, 1967) and rarely after total body irradiation (Clifton, 1959), but are readily induced by any procedure which impairs thyroxine formation by the thyroid such as surgical or radio-thyroidectomy, administration of goitrogens or iodine deficient diets (Bielschowsky, 1953). Tumors secreting adrenocorticotrophic hormone and melanocyte stimulating hormone have also been found in mice after irradiation and consist of chromophobe cells. There is some doubt as to whether specific somatotropic hormone-producing tumors occur.

Some aspects of experimental pituitary tumor pathology can readily be related to normal physiological mechanisms such as the specific pituitary cell hyperplasias which occur in response to specific stimuli. These include the hyperplasia of gonadotrophs after gonadectomy, the thyrotrophs after thyroidectomy and the prolactin-secreting cells after the administration of estrogen. Other aspects of pituitary physiology do not seem to be related to tumor development, though at first sight it would seem that they should. For example cellular proliferation in the young female rat is greater than in the male, while in the sexually mature female, pituitary cells are sub-

jected to periodic stimulation to proliferate by estrogen (Hunt, 1947; Crane and Loomes, 1967). Further, the pituitary of the mature female rat is larger than that of the male (Weinbren and Fitschen, 1959); yet the incidence of pituitary adenomas in old female rats is usually less than in males. The rate of cell proliferation falls progressively with age (Hunt, 1943; Crane and Loomes, 1967) although the incidence of tumors rises steadily with increasing age in male rats. It is also difficult to correlate the findings in experimental animals with pathology in man, since we find that most pituitary adenomas in man are apparently nonsecreting and the commonest functional tumor of the gland is the acidophil adenoma—a tumor scarcely known in experimental animals.

PITUITARY TUMORS IN MAN

The general principles with regard to the diagnosis and treatment of pituitary tumors apply to all age groups and were reviewed by Schurr (1966). There are, however, certain special features which particularly affect the history when these neoplasms are found in the elderly. A review of 20 pituitary tumors diagnosed in patients over the age of 60 and seen at the Neurosurgical unit of the Guy's, Maudsley and King's College Hospitals, London, showed that there were 15 chromophobe adenomas, 3 craniopharyngiomas, and 2 acidophil adenomas. There were 9 males and 11 females. This formed a group of approximately 8 percent of the total number of pituitary tumors in a period of nearly 20 years. Although pituitary disturbances are rare in the aged, it can be seen that they are by no means to be ignored. It is well known that pituitary tumors may cause symptoms on account of interference with normal hypophysial function, or with the function of neighboring structures, as a result of enlargement of the tumor, or on account of the effects of raised intracranial pressure. In the elderly, the presence of hypopituitary features such as loss of sexual function, may be masked by the natural aging process. Disturbances of vision may be attributed to age, or they may be obscured by coincident abnormalities such as cataracts, glaucoma, retinal detachment, or even injuries that may have occurred during a long lifetime. Signs of raised intracranial pressure are a late feature and are only likely to arise in the largest tumors. Attention was drawn by Russell and Pennybacker (1961) to the incidence of mental changes in no less than half of a large group of middle-aged and elderly patients who were suffering from craniopharyngiomas. This is a significant and important point, for craniopharyngiomas may be large and they obstruct the circulation of the cerebrospinal fluid. This additional obstruction in a brain which has already been affected by age and arteriosclerosis, will bring about generalized dementia or defects of memory which would not be seen in younger people (Williams and Pennybacker, 1954). Three of their patients had actually been admitted

to mental hospitals and there was profound impairment of recent memory, apathy, and somnolence in another 8 out of their 24 cases. The presence of mental changes may make it even more difficult to elicit abnormalities of vision or important points of the history, and are a particular reason why an X-ray of the skull should be included in the routine investigation of dementia in the aged. In our own group of elderly pituitary patients these mental changes were very much less common, and it is significant that they were seen in the craniopharyngiomas without exception. It is therefore more likely that an elderly patient suffering from a craniopharyngioma will be affected by mental changes than one suffering from an adenoma of the pituitary gland itself.

Referring again to our own pituitary tumors in patients who were more than 60 years old at the time of diagnosis, it was striking that the history was less than a year in duration in a little over half the cases, and more than 6 years in duration in just over a quarter. There was a history of epilepsy or of attacks of unconsciousness in more than a third of the patients (7 out of 20). This is a much higher incidence than would normally be expected from this group of neoplasms at an earlier age. It may be related to a higher incidence of extension of the tumors into cerebral tissue, but the discrepancy in this respect is not enough to account for the difference. Nearly all the patients presented with disturbances of vision, and these again showed a different pattern from the younger patients. Thirteen out of the 20 patients had visual failure in which one eye was severely affected and the other very much less so. In 5, both eyes were more or less equally affected. Bitemporal hemianopia occurred in only 9 out of 20 patients, and 13 had central scotomas. This incidence of central scotomas in pituitary tumors is considerably higher than is normally expected; it is somewhat rare in younger patients, and usually indicates a posterior chiasmal lesion (Wilson and Falconer, 1968). Only a quarter of the patients presented with headache, and a complaint of a feature that might have been related to hypopituitarism was only found in two patients. Thus it can be seen that the usual criteria for the diagnosis of a pituitary tumor may not be present, or that they may be distorted, and that in this age group it is very easy to miss these lesions. Costello (1936) in a study of a thousand pituitary glands from patients who were not known to be suffering from pituitary disease, found adenomas in no less than 22.5 percent, and these occurred with greater frequency in old age.

NEURORADIOLOGY OF PITUITARY PATHOLOGY IN THE AGED

Pituitary Fossa

The same enlarging process produced in the pituitary fossa by tumors of the gland occurs in the elderly as in the young. The pituitary fossa is

enlarged by erosion of the dorsum sellae, deepening of the floor, and erosion of the tuberculum and anterior clinoid processes (Fig. 8-3). This enlargement may frequently be asymmetrical and may cause the floor to appear to be duplicated in the lateral projection. Enlargement into the sphenoidal air sinus tends to be greater with acidophil adenomas than with chromophobe adenomas, and it should be remembered that craniopharyngiomas may be almost entirely suprasellar and therefore associated with little or no enlargement of the fossa at all.

If a pituitary tumor, whether it be an adenoma or a craniopharyngioma, reaches sufficient size to obliterate the foramen of Monro, or to form a mass within the skull of dimensions which are great enough to produce obstruction to the cerebrospinal fluid circulation, the effect of generalized



Figure 8-3. Lateral X-ray of the pituitary fossa of a 64-year-old female with a chromophobe adenoma. (Compare Fig. 8-4b.) The dorsum sellae is rarefied and displaced backwards. The fossa is enlarged and deepened. The sphenoidal sinus is encroached upon, and the anterior clinoid processes are "sharpened" from below. (Same case as Figure 8-6a, and 8-6b.)

raised intracranial pressure may be seen in the dorsum sellae. If the pituitary fossa is enlarged by a tumor, the displacement of the dorsum sellae and rarefaction may be due more to the tumor than to generalized pressure, but where the sella is of normal size, as it may be with a craniopharyngioma, the decalcification of the dorsum sellae must be distinguished from the normal aging process in that structure (Figs. 8-4a and b). As the skull gets older, the dorsum sellae becomes less visible owing to decalcification, but the essential difference between the decalcification of age and that due to raised intracranial pressure is that the outline is preserved in the former. In the case of a suprasellar craniopharyngioma, flattening of the top of the dorsum sellae may occasionally be seen.

Calcification within the pituitary fossa and in its neighborhood may be valuable in diagnosis, but in the elderly it is important to distinguish intrasellar calcification from calcification in the internal carotid arteries where they lie in the cavernous sinus (Fig. 8-4a). This portion of the internal carotid artery is most commonly seen to be calcified, lime salts being deposited in the fatty material of the atherosclerotic vessels. The calcification is usually linear when seen laterally, and if it is possible to detect it in the antero-posterior projection it may be seen as a circular outline. Calcification in the dura of the middle fossa adjacent to the squamous temporal bone may occasionally be visible in the lateral projection, but is not seen when viewed at right angles. Very occasionally, calcification may be found in chromophobe adenomas, either in the solid material or in the wall of a cyst. These tumors are usually large (Deery, 1929). Mention must be made of the rare occurrence of calcification many years after an operation on a pituitary adenoma. It is presumed that the hemorrhage left after an intracapsular removal of the tumor becomes organised and later calcifies. Two patients have been seen with spherical "calculi" entirely filling the pituitary fossa and extending slightly above it. Both had been operated upon many years before by the sphenoidal route. Craniopharyngiomas are the most common tumors to produce intrasellar or suprasellar calcification, which may consist of small flecks in the solid part of the tumor, or linear calcification within a cyst wall. Calcification was found in just over half the series of middle-aged and elderly patients with craniopharyngiomas, which was reported by Russell and Pennybacker (1961). Aneurysms sometimes present as intrasellar masses, and in these circumstances, calcification in the wall might occur, although the authors are not aware of an example. Calcification may be made more easily visible by means of coned views, stereoscopic films, or tomography. Destruction of the lesser wing of the sphenoid bone or optic foramen may mean that a pituitary adenoma has become invasive (Jefferson, 1955).



A



B

Figure 8-4a and b. Senile changes in the pituitary fossa in a 69-year-old female without specific pituitary pathology. (A) The dorsum sellae is rarefied but the outline is preserved and the fossa is not enlarged. The anterior clinoid processes are not distorted. Calcification of the carotid siphon overlies the pituitary fossa and must be distinguished from intrasellar calcification by anteroposterior views and tomography if necessary. (B) Normal pituitary fossa of a female of 22 years of age for comparison.

Pneumoencephalography of Pituitary Lesions

Pneumoencephalography is usually the most definitive radiological investigation for pituitary pathology (Fig. 8-5). Air introduced into the subarachnoid space by lumbar puncture will outline the basal cisterns and enable any suprasellar extension of the mass to be seen. Adjustment of the position of the patient will also enable the outline of the third and lateral ventricles to be seen. A suprasellar mass will distort the anterior part of the third ventricle and may occlude the interventricular foramen (Monro) causing enlargement of the lateral ventricles. A mass of tumor arising in the pituitary fossa and extending to the frontal lobes or the middle fossa will distort the outline of the anterior or temporal horns, respectively. Clarification of the outline of the chiasmatic cistern and the third ventricle may be obtained by tomography, or by the use of positive contrast material which can be put into the lateral ventricle through a burr hole

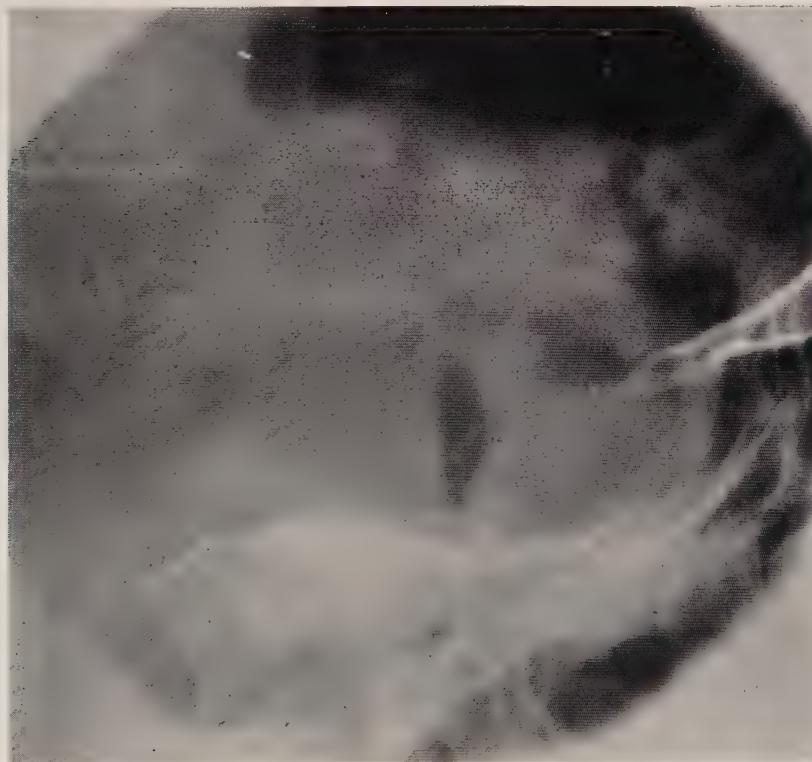
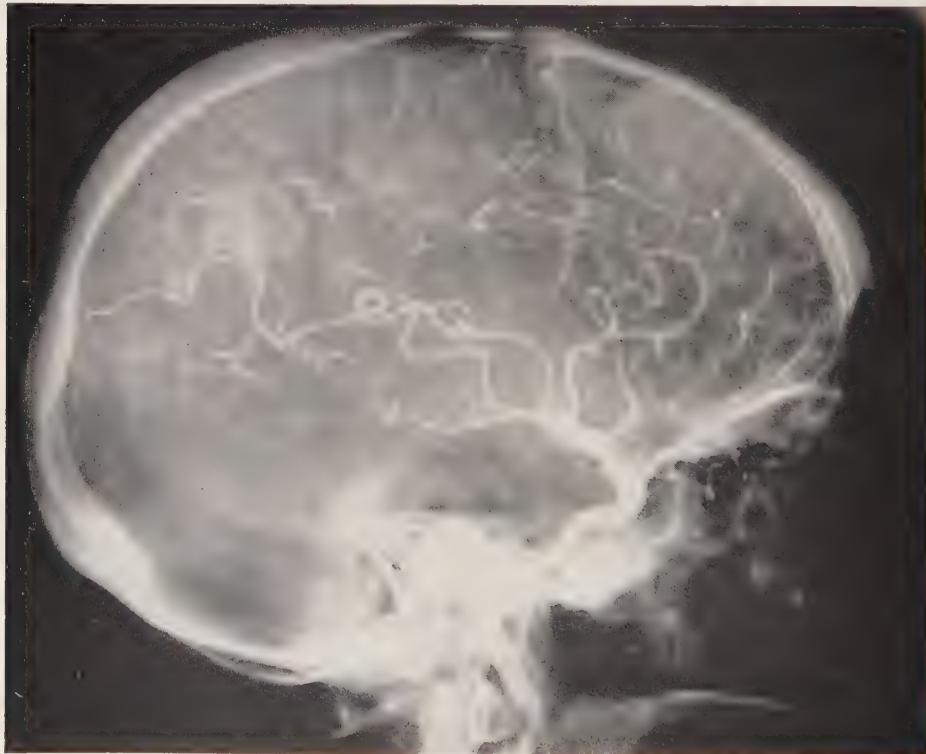


Figure 8-5. Lateral pneumoencephalogram of a 60-year-old female with a chromophobe adenoma. Air in the chiasmatic cistern outlines the suprasellar extent of the tumor, and air in the third ventricle shows how the antero-inferior part of this structure is obliterated.

and passed through the interventricular foramen into the third ventricle under radiological control with image intensification. Lumbar air encephalography of pituitary tumors should not be done in the presence of raised intracranial pressure, unless a burr hole has been previously made in order that the ventricular pressure may be lowered if the necessity should arise.

Arteriography of Pituitary Lesions

The presence of a suprasellar tumor, whether it is arising from within the pituitary fossa or entirely suprasellar in origin, will cause the anterior cerebral vessels to be raised and the internal carotid arteries to be displaced laterally (Fig. 8-6). This distortion of the outline of the arterial tree has to be distinguished from changes due to atherosclerosis. Elongation of the internal carotid artery as a result of changes in its walls and loss of the elastica will cause the bifurcation of the artery to be elevated, and there may even be some apparent outward displacement. However, in these cases the anterior cerebral artery tends to dip downwards rather than to be raised to an even higher level. In the lateral projection the carotid siphon is opened out by the presence of a suprasellar tumor, but some degree of opening of the siphon can also be seen in atherosclerosis. However,



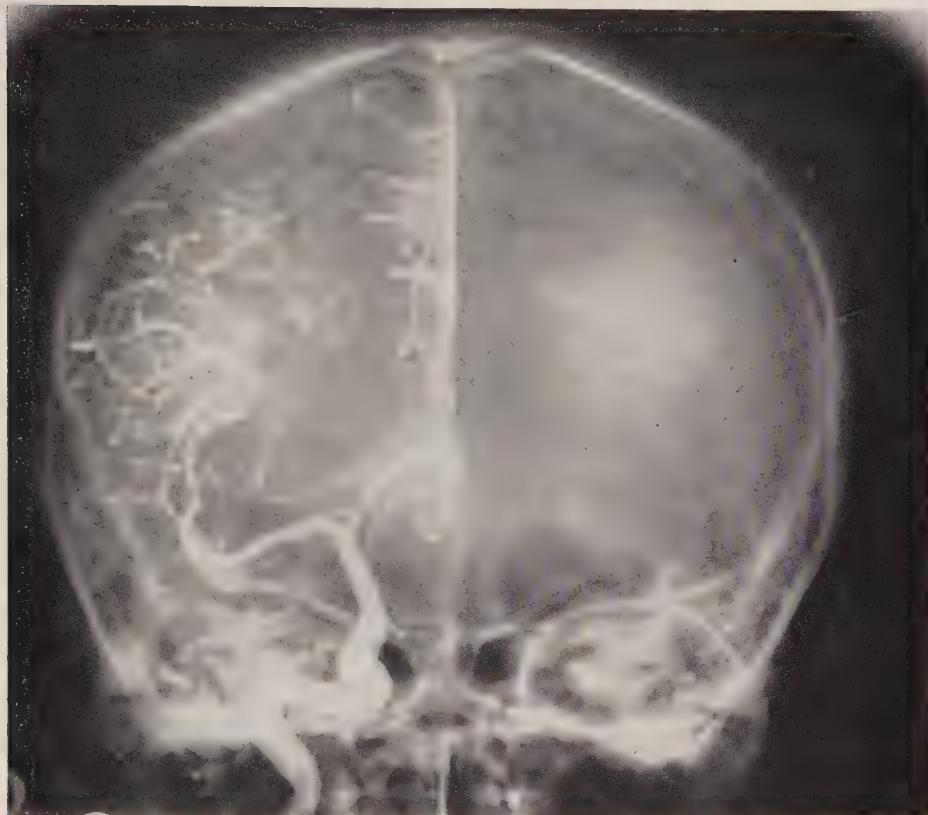


Figure 8-6a and b. Lateral arteriogram showing stretching of the carotid siphon by a pituitary adenoma. Anteroposterior arteriogram showing lateral displacement of the carotid siphon and elevation of the first part of the anterior cerebral artery by a chromophobe adenoma. (Arteriograms of case illustrated in Fig. 8-3.)

changes in the walls of the vessels produce an irregular narrowing which may be helpful in differentiating the conditions. Both changes may be present at the same time in the group of patients under consideration. It is possible for large craniopharyngiomas to lie entirely posterior to the carotid arteries and their branches. Such tumors usually occupy the third ventricle and are very likely to occlude the interventricular foramen. In this case they will obstruct the flow of cerebrospinal fluid and give rise to ventricular dilatation. The arterial displacement produced by ventricular dilatation consists of separation of the anterior and middle cerebral vessels in the antero-posterior projection, and widening of the arc of the anterior cerebral artery and downward displacement of the middle cerebral artery in the lateral projection. An extension of a pituitary tumor into the middle fossa may elevate the middle cerebral artery on that side, and

frontal extensions frequently elevate the first few centimeters of the anterior cerebral arteries beyond the anterior communicating artery. In the rare examples of an internal carotid aneurysm which occupies the pituitary fossa and gives rise to the clinical picture of an intrasellar lesion, arteriography may be expected to show the nature of the pathology and its extent.

CHROMOPHOB E ADENOMAS

Endocrine Symptoms

As has been mentioned, most of the typical features of hypopituitarism are mimicked by the normal changes with age. The presence of hypo-adrenocorticism should, however, be remembered. This is associated with fatigue and apathy, and may lead to acute adrenal insufficiency in times of stress. Water excretion may be impaired and may be compensated for by drinking less. If the water intake is forced, water intoxication may be produced. The plasma sodium may be low and there may be a disturbance of salt metabolism. Glucose tolerance is affected in about half the patients with pituitary chromophobe adenomas of all ages, and the insulin tolerance is reduced in a slightly greater number. This may be confused with disturbances of sugar metabolism due to other causes in older patients. Hypothyroidism is rarely a cause of symptoms before treatment although replacement therapy may be required afterwards. Abnormalities of metabolism may be produced as a result of pressure on the hypothalamus or interference with its blood supply.

Treatment

The treatment of chromophobe adenomas is by surgery or radiotherapy. If there is involvement of the visual pathways there should be no hesitation in recommending operation. In cases where there is a significant extrasellar extension, the frontal approach is preferable, as it allows accurate decompression of the optic pathways to be performed. If the operation is carried out under steroid cover there is little disturbance to the patient. The transsphenoidal approach has few advantages. In arteriosclerotic patients, the internal carotid artery can be more tortuous than usual and may approach the midline, so that it is vulnerable when operating upon the gland from below. There is also a risk of cerebrospinal rhinorrhea and meningitis, which is greater than with the transfrontal approach. Even in the elderly, it is wise to consider postoperative radiotherapy, as this undoubtedly reduces the recurrence of these tumors.

ACIDOPHIL ADENOMAS

These tumors occur less frequently in the elderly than in younger patients and only two were found in our own series. There are no special features which make the treatment of these patients any different from

their younger counterparts. Changes in the general appearance of the patient are less important in old age. One of our patients presented with visual failure and the other with diabetes mellitus. It should be remembered that the threat to life among patients suffering from acromegaly comes from the associated intractable diabetes mellitus and heart failure complicated by hypermetabolism. Again, it may be said that if vision is impaired the choice of treatment is operation, but if radiological studies show that there is no significant suprasellar extension of the tumor, then radiotherapy may be used. It has the disadvantage that a biopsy is not obtained.

BASOPHIL ADENOMAS

We did not encounter any basophil adenomas over the age of sixty and it must be unusual to encounter primary Cushing's disease at this age. However, it is perhaps worth mentioning that patients who have had an adrenalectomy for the treatment of an adrenal tumor or hyperplasia may later present with a pituitary adenoma, and that these tumors may be chromophobe or acidophil in nature. Furthermore, many of the tumors are of the invasive type described by Jefferson (1955) and they should therefore be treated as radically as possible.

CUSHING'S SYNDROME

It should be remembered that this syndrome may be produced from the medical use of steroids or corticotropin. Decreased glomerular filtration and renal tubular absorption, and electrolyte imbalance are easily induced by hormone therapy. Diabetic glucose tolerance curves may be produced more readily in iatrogenic Cushing's syndrome in old people than in young, because of the natural tendency for decrease in glucose tolerance in the aged. No cases of primary aldosteronism have been reported over the age of 70 years (McKeown, 1965).

CRANIOPHARYNGIOMAS

More than half of the 24 middle-aged and elderly patients with craniopharyngiomas who were described by Russell and Pennybacker (1961) were over the age of 60. All but one of these patients had a reduction of visual acuity or an abnormality of the visual fields. The majority of the field defects were bitemporal, but they were often very incongruous. Mild features of hypopituitarism such as increased sensitivity to cold, lassitude, and diminished beard growth, were seen in less than half the cases, but 13 of the 24 had no endocrine abnormalities other than those changes which might be attributed to age. It is interesting that 5 patients had anosmia and that epilepsy was found in 3 out of 24. Approximately half the patients showed flecks of calcification above the sella turcica in a lateral X-ray, and the pituitary fossa was enlarged in a third. Just over half the

patients had a raised cerebrospinal fluid protein. Although of developmental origin, craniopharyngiomas may remain silent until middle or later life, and it is assumed that the rate of growth is either very slow or intermittent. Some of the patients described by Russell and Pennybacker were observed for a period of 20 years without any change being noticed. A period of rapid growth may be followed by one of long inactivity, and this makes it difficult to assess the results of treatment. Malignant changes can occur (Wertheimer and Corradi, 1957), but they are so rare that they need not be taken into consideration. Simple aspiration of fluid from a cystic craniopharyngioma should be the first line of treatment in the elderly. If the tumor is cystic and mainly situated beneath the chiasm it is worth considering the possibility of a radical removal under steroid cover. However, if the tumor is solid, or the cyst is very big, radical removal is likely to be impossible. If the visual fields are affected, a frontal exploration should be carried out and an attempt made to decompress the optic nerves and chiasm, or to carry out a radical removal.

A big tumor obstructing the foramen of Monro, may be treated by the use of a ventriculo-atrial shunt, which has advantages over the ventriculocisternostomy of Torkildsen, but the valve should not have an opening pressure that is less than 6 cm of water. It is very doubtful if radiotherapy has a place in the treatment of craniopharyngiomas.

In the group of 20 patients over the age of 60 at the time of diagnosis and suffering from chromophobe adenomas, acidophil adenomas and craniopharyngiomas, 9 patients lived for 5 years or more in good general health. Another 6 patients had an excellent result but the follow up was less than 5 years at the time of writing. Very good postoperative vision was retained in one eye in half the patients, and in both eyes in a further 6 patients. A poor result was obtained in 2 patients, and 3 patients died while they were in hospital.

CARCINOMATOSIS AND THE PITUITARY

Although malignant neoplastic disease is frequently encountered in the elderly, primary carcinoma of the pituitary gland is very rare. Metastasis of carcinoma to the gland is, however, common and has some interest because of the correlation between the anatomy of the blood supply and the location of metastatic deposits. In man the evidence that the anterior pituitary receives mainly portal venous blood is indirect (Xuereb, Prichard and Daniel, 1954a and b; Daniel, Prichard and Schurr, 1958). In cases with disseminated carcinoma one would not expect to find metastatic deposits in the anterior lobe since solid masses of malignant cells should be filtered out of the blood stream in the primary capillary plexuses. A study of this question (Duchen, 1966) showed that in fact when the pituitary was in-

volved in cases with carcinomatosis, the metastatic deposits were found in the pituitary stalk or posterior lobe. The anterior lobe did not contain isolated metastases and only became infiltrated if the posterior lobe was involved first. Several cases have been seen in which destruction of the posterior lobe was associated with degeneration of supraoptic and paraventricular nuclei and clinical diabetes insipidus.

CONCLUSION

The clinical and pathological investigation of pituitary abnormalities in the elderly differs in no way from that of other age groups. The important point is to remember the possibility of their existence.

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CHAPTER 9

SENILE HYPOPHYSEAL SYNDROMES

E. HERMAN

SUMMARY

THE AUTHOR DESCRIBES a number of pathological syndromes associated with premature senility. There appear to be two types of pathological aging involving the pituitary, one associated with hypopituitarism as in Glinski-Simmonds' disease and the second with hyperpituitarism or hypercorticism as in Cushing's syndrome. In addition the author suggests that these pathological aging types may have their physiological equivalents in old subjects of thin stature (the leptosomic type) and those who are heavy and obese (the pyknic type).

INTRODUCTION

There are various theories which try to explain the process of senescence. For the most part there is no agreement as to the role of endocrine glands in aging. Changes in pituitary function are seen by a number of authors (Simmonds, 1914; Aschheim and Zondek, 1927; Pribram, 1927; Lucien, 1929; Aschoff, 1937; Everitt, 1966) as a cause of aging. There are two schools of thought, one that aging is accelerated by pituitary deficiency and the other by pituitary hyperfunction.

The object of this paper is to present a number of senile syndromes believed to be of pituitary origin. An attempt will be made to show that there are two types of aging which are related respectively to hypo- and hyper-pituitary function. This concept was discussed earlier (Herman, 1938 and 1967).

PATHOLOGICAL PITUITARY SYNDROMES MIMICKING PREMATURE SENESCENCE

In the following diseases senile changes have been associated with pathological changes in the pituitary: 1. Progeria, 2. Werner's syndrome, 3. Rothmund's disease, 4. Ateliosis, 5. Adultismus, 6. Glinski-Simmonds' disease, and 7. Cushing's syndrome.

One must differentiate between a premature senescence in a physiological framework (due to acceleration of the aging process caused by genetic factors) and a premature senescence resulting from disturbances of the endocrine glands, including the pituitary. There are whole families char-

acterized by the late appearance of the physical and mental features of senility, in contradistinction to those in which the signs and symptoms of aging appear disproportionately early in life.

Progeria

The term "progeria" comes from Greek words "pro" meaning early, and "geron" meaning old (aged, ancient).

The syndrome of progeria was first described by Hutchinson (1886), but the term "progeria" was coined in 1904 by Gilford, when he examined the boy observed several years earlier by Hutchinson. They both clearly depicted the characteristic features of the syndrome which manifests itself in children and young people and consists of dwarfism and a senile appearance (Fig. 9-1). That is why the French authors call this syndrome a senile dwarfism—"nanisme type senile."

True progeria should be differentiated from pituitary dwarfism in which some features of premature senescence may be observed.

SYMPTOMATOLOGY. After a period of normal somatic and mental development, the child presents features of premature senility, i.e. there is a mixture of childhood and senescence. Wilson (1944) states that this syndrome represents an intermixture between the clinical features of a child of five, and of a wizened, dwarfish old man. Some normal proportions of build are retained.

The head is small and dolichocephalic, the skin is wrinkled, thin, frail, inelastic and dry, the hairs are thin and fall out, the nails are atrophic, the milk-teeth persist for a long time, there is muscle atrophy, osteoporosis, fragility of bones, underdevelopment of genitals, sometimes a cataract, cardiac murmurs, premature atherosclerosis, frequently cerebral ictus. Most patients die before the 20th year of life. According to Reichel, *et al.* (1971) approximately 52 cases have been recorded in the literature.

ETIOLOGY. It is likely that this condition is due to congenital disturbances, which only manifest themselves after one or more years of apparently normal development. There are three reports of multiple affected siblings, suggesting an autosomal recessive mode of transmission (Mostafa and Gabr, 1954).

Breitman (1949) differentiates 3 types of congenital senility: the atrophic type, with cachexia; adiposity of eunuchoidal, female or asexual type; and atrophy of senile pituitary, gonads and the thyroid gland.

Pituitary dysfunction was first suggested by Gilford (1904) and abnormalities of the pituitary have been reported by a number of workers (Orriço and Strada, 1927; Talbot, *et al.*, 1945; Manschot, 1950; Thomson and Forfar, 1950; Atkins, 1954). However, the results of pituitary, adrenal and thyroid function tests have usually been reported as normal or inconsistent



Figure 9-1. Progeria in a patient aged 17 years (from Gilford (1904), by courtesy of *The Practitioner*.

(Reichel and Garcia-Bunuel, 1970). Nevertheless the studies of Villee, *et al.* (1969) suggest that progeria may represent not simply pituitary dysfunction but also a failure of the tissues to respond to growth hormone. They found that two boys with progeria had very low serum growth hormone levels and that these levels did not increase in response to insulin hypoglycemia. Treatment of these patients with human growth hormone

failed to achieve any significant retention of nitrogen nor any increase in height. Both patients were relatively resistant to insulin.

In these patients the skin is thin and wrinkled as in old age. Villee, *et al.* (1969) found that the solubility of skin was low, and that the shrinkage temperature was raised. These are indications of increased aging of collagen in skin. The abnormal changes in collagen could possibly explain the premature aging of the skin, blood vessels, myocardium and skeleton in progeria (Reichel, *et al.*, 1971). The age pigment, lipofuscin, was found to accumulate in the myocardium (Reichel and Garcia-Bunuel, 1970).

PATHOLOGY. There are only a few pathological observations of these cases. Reichel and Garcia-Bunuel (1970) reviewed the postmortem findings in 10 patients with progeria. Aortic or coronary atherosclerosis was described in 9 out of 10 patients. Reichel and Garcia-Bunuel reported myocardial fibrosis in their 2 cases. Elevations in serum cholesterol and lipoproteins have been observed in a number of cases (Keay, *et al.*, 1955) although not in all (Villee, *et al.*, 1969).

Werner's Syndrome (Adult Progeria)

In 1904 Werner described four siblings with cataracts and scleroderma. This syndrome is usually encountered in the third decade when development is already more or less complete. It resembles the infantile progeria, but is even more closely related to physiologic senescence, as it occurs in the adult patient. Persons affected by it are short, have a large head, a beak nose, a small mouth and chin (a so-called bird-like face), early baldness and or graying, pseudosclerodermic skin changes with trophic ulcerations, thinning of the distal parts of the extremities, a retarded development or absence of secondary sex characteristics, juvenile cataracts, osteoporosis and early atheromatous changes. Riley, *et al.* (1965) pointed out that generalized arteriosclerosis and coronary artery disease were found in all 9 of the published autopsies on patients with Werner's syndrome.

The cause of Werner's syndrome has been sought in endocrine disturbances of the hypophysis, the thyroid (exophthalmos), the parathyroids (abnormal calcium metabolism) and the gonads. There is a 20 percent incidence of overt diabetes in Werner's syndrome (Irwin and Ward, 1953). Attempts to relate pituitary dysfunction to Werner's syndrome were made by Kallos and Ruppe (1954) and Grant (1957). However, pituitary cytology was found to be normal by Perloff and Phelps (1958).

Because of its many resemblances to progeria in children, Werner's syndrome may be a later expression of progeria (Reichel, *et al.*, 1971). The disease is clearly inherited as a history of consanguinity and multiple affected siblings is common.

Rothmund's syndrome is similar to Werner's syndrome in respect to the early appearance of cataracts and scleroderma, its endocrine involvement and the heredo-familial etiology. Thannhauser (1945) clearly separated these two syndromes. Senile changes are less developed in Rothmund's syndrome.

Ateliosis (Congenital Hypopituitary Dwarfism)

In 1904 Gilford used this term to describe dwarfs of normal proportions. This term is formed from the Greek (*a*, no + *telos*, target) meaning that the target or mature state is not reached. It is characterized by an arrest of growth (dwarfism) and development (infantilism) with signs of premature senility, particularly in the skin where collagen solubility is diminished. Rimoin, *et al.* (1966) found that ateliotic dwarfism is due to a recessively inherited isolated deficiency of growth hormone; the secretion of TSH, FSH and ACTH appears to be normal.

A lack of growth hormone is found in both ateliosis and progeria. However, in ateliosis the senescent changes are minimal. Evidently the marked senility of progeria is due to more than a lack of growth hormone.

Adultismus

The term was introduced by Breitman (1949), the syndrome comprising states of an intermixture of arrested development with signs of late growth. In such people some organs grow between childhood and adolescence while others become proportionally smaller. According to Breitman (1949), there is an accelerated development (superevolutism) and not a monosymptomatic infantilism.

Glinski-Simmonds' Disease (Pituitary Cachexia, Sheehan's Syndrome)

Glinski (1911 and 1913) and Simmonds (1914) described a disease characterized by progressive loss of weight and premature senility. The disease is usually known as Simmonds' disease although Glinski reported it earlier (Robertson, 1951).

Patients affected by Glinski-Simmonds' disease present many features of premature aging. Signs of senility are excessive emaciation; cachexia; progressive muscular weakness; baldness and graying of the hair; dry, atrophic, inelastic and wrinkled skin (*gelodermia*); brittle nails; insufficiency and atrophy of sex and other endocrine glands. There is bradycardia, arterial hypotension, low body temperature, diminished metabolic rate, decreased excretion of 17-ketosteroids, 11-oxysteroids and of gonadotropins, hypoglycemia, flattening of the glucose tolerance curve, considerable insulin hypersensitivity, and sometimes a high level of blood cholesterol.

The patient with Glinski-Simmonds' disease looks old, and in physical

TABLE 9-I
COMPARISON OF PHYSICAL STATUS IN SIMMONDS' DISEASE AND OLD AGE

<i>Characteristic</i>	<i>Simmonds' Disease</i>	<i>Old Age</i>
Nutrition	Poor to good	Poor to good
Body weight	Slight to severe loss, averaging 44 lb.	Small loss, averaging 13 lb.
Body fat	Reduced subcutaneous and visceral fat	Reduced subcutaneous and visceral fat(?)
Muscles	Wasted and weakened	Atrophic
Skin	Pale, thin, wrinkled, dry and inelastic	Yellow, pigmented, thin, wrinkled, dry and inelastic with accentuated folds
Hair		
Head	Normal to gray, sparse	Whitened, sparse to bald
Axillary and pubic	Falls out (in 80 per cent)	Sparse
Heart size	Small	Small to enlarged
Heart rate	Slow	Slow to normal
Blood pressure	Hypotension	Hypertension (in 65 per cent of persons more than 70 years old)
Atherosclerosis	Rare	Frequent (in 90 per cent of persons more than 70 years old)
Female breast	Atrophic	Atrophic
Male and female genitalia	Atrophic	Atrophic
Menstruation	Amenorrhea (in 82 to 98 per cent)	Amenorrhea
Libido	Lost in 50 per cent	Declines

From A. V. Everitt, "The Pituitary Gland. Relation to Aging and Diseases of Old Age," *Postgraduate Medicine*, 40:644, 1966.

TABLE 9-II
COMPARISON OF LABORATORY DATA IN SIMMONDS' DISEASE
AND IN OLD AGE

<i>Determination</i>	<i>Simmonds' Disease</i>	<i>Old Age</i>
Red cell count	Decreased	Small decrease
Hemoglobin	Decreased	Small decrease
White cell count	Normal	Normal
Eosinophil count	Raised	Low
Blood cholesterol	Normal	High
Blood sugar	Decreased	Normal
Glucose tolerance	Increased	Diminished
Urinary FSH	Low	High
Urinary 17-ketosteroids	Low	Low
Urinary 17-hydroxysteroids	Low	Low to Normal
Basal metabolic rate	Very low	Low
Protein-bound iodine	Low	Low
Temperature	Subnormal (95.0 to 96.8°F.)	Low
Cold tolerance	Poor	Poor

From A. V. Everitt, "The Pituitary Gland. Relation to Aging and Diseases of Old Age," *Postgraduate Medicine*, 40:644, 1966.

status (Table 9-I) and laboratory tests (Table 9-II) resembles the normal elderly subject. Only in cardiovascular changes, glucose tolerance and in urinary gonadotropin excretion does Glinski-Simmonds' disease differ from normal old age (Everitt, 1966).

The patients are apathetic, frequently suffer from hallucinations or illusions and at times they manifest a catatonic stupor. Anthropometric measurements show that the upper part of the body may be larger than the lower.

The disease appears chiefly in women ($\frac{2}{3}$ of the cases) most frequently post partum.

In Sheehan's syndrome (Sheehan, 1937) there is a partial atrophy of the pituitary anterior lobe in women who suffered from severe hemorrhage after delivery.

Glinski-Simmonds' syndrome results from destruction of the anterior lobe of the hypophysis and cessation of its hormonal function, caused by necrosis (thrombi, emboli, hemorrhage), inflammatory states due to lues or tuberculosis (hypophysitis) or by crano-cerebral trauma.

To illustrate premature aging in this disease, here is one of my own cases (Herman, 1967).

A 39-year-old female, D. Z. (No. 121/35) was first admitted to hospital on December 9, 1935 (Fig. 9-2). A clinical diagnosis was made of Glinski-Simmonds' syndrome of luetic origin. The body-weight on admission was 29.3 kg, the height 148 cm. Extreme emaciation: subcutaneous fatty tissue impalpable bringing into prominence the contours of the whole skeleton. The individual muscles were distinct, and even the trunks of peripheral arteries were conspicuous, like strings.

The face was strikingly senile and sunken. The facial skin was of a peculiar colour, giving a parchment-like and pinkish-blue appearance, being wrinkled, especially on the cheeks. The upper jaw was retracted and atrophic, while the lower jaw protruded. The head was inclined forward, the whole trunk seemingly flexed upon itself.

In the anterior iliac region and over the front of the thighs, numerous translucent veins were visible due to atony of the skin. Over the lower half of the thorax and abdomen some scores of skin-lesions were seen, varying in size from a pinhead to a cherry-stone. These eruptions were partly elevated and mostly adherent to the cutaneous surface. They were compact, their colour varying from yellowish-brown to blackish (*verrucae seborrhoicae seniles naeviformes*). In the interscapular and lumbar regions a dozen or so similar but less distinct lesions were found.

The axillary and pubic hair was scanty and of feminine type, with numerous gray hairs. The hair of the scalp was thin and lusterless.

The dentition was very poor, and only a few teeth remained. No alteration in the nails was apparent. The breasts were completely absent, the mammary glands being scarcely palpable.



Figure 9-2. Glinsky-Simmonds' disease in a woman aged 39 years (from Herman (1967), by courtesy of the Elsevier Publishing Company).

Gynecological examination gave evidence of a senile vulva and narrow vagina. The vaginal portion of the uterus could not be felt, nor the uterine body and the tubes. There had been an amenorrhoea for many years.

Pulse rate 68; blood pressure 90/70 which fell on effort and could not be determined. Blood sugar 71 mg/100 ml. Hypersensitivity to insulin: drop of blood sugar level to 38 mg/100 ml persisting for 4 to 5 hours after administration of 10 U insulin.

On neurological examination there were found an anisocoria and irregularity of the pupils with almost complete loss of reaction to light, the reaction to convergence being intact. Mental deterioration, pleocytosis and increased globulin content in the cerebrospinal fluid. Lange curve of luetic type. Wassermann reaction in blood and cerebrospinal fluid markedly positive.

In short, we are concerned with a person with syphilis of the central nervous system and with marked Glinsky-Simmonds' syndrome.

The picture of senility consists here in the typical aged appearance of

the skin, with senile warts, typical hair-changes, sunken face, atrophic upper jaw and protruding mandible, lack of dentition, general cachexia, and loss of primary and secondary female sexual characteristics. In other words, there was a general asthenic involution of the organism, with a typical senile debility. The picture corresponded to an asthenic constitutional prototype of Kretschmer (1924) considering that asthenic women are not only thin but also short, "asthenic-hypoplastic," according to Kretschmer.

In the pluriglandular syndrome there is an early senility of individuals between 20 and 30 years old. Like Simmonds' disease there is atrophy of many endocrine glands, which in this syndrome is due to infection (tuberculosis, lues) or intoxication. The patient is affected by loss of hair, a gray and wrinkled skin, loss of weight and appetite, asthenia, arterial hypotension and sexual deficiency. Men look like old women (Breitman, 1949).

Cushing's Disease or Syndrome (Pituitary Basophilism, Hyperadrenocorticism)

Cushing (1932), when describing his syndrome, stated that the patients not only manifest external senile features, but also show some clinical and biochemical aspects of senescence, such as arterial hypertension, atherosclerosis, increase of blood cholesterol, hyperglycemia resistant to the action of insulin. The external habitus of the patients is that of an old man: the face is fat (moonface), there is also general trunk adiposity, the abdomen is large, the muscles atrophic, the skin is thin with purplish striae, there is adynamia, osteoporosis and virilismus in women.

In Chapter 17 in this monograph Wexler describes the metabolic and pathological derangements in Cushing's syndrome, which are associated with accelerated aging.

Here is a pertinent case of this syndrome.

A 23-year-old patient, W. Sz. (Fig. 9-3), already reported (Herman and Merenlender, 1936), revealed a diminution in height (from 176 cm before the onset of the disease to 165 cm on examination), a rounded full moon face, thick and short neck, grossly fat, rounded, prominent and protuberant abdomen, adiposity of the pelvic girdle and of the whole trunk with thin extremities; thin and atrophic skin with a fine branny desquamation, bluish-red in colour, and with arborescent red striae; delicate, lustreless, scanty, gray hair; impotence, muscular weakness; defective resistance against suppurative infections; osteoporosis of vertebrae with flattening; marked hypertension; hypercholesterolemia; hypercalcemia; and hyperglycemia.

Post mortem histological examination revealed, in addition to an anterior pituitary basophil adenoma and penetration of basophil cells into the posterior lobe of the hypophysis, hyaline degeneration and adrenal cortical hyperplasia with abundant deposit of lipoids in the adrenal cortex, atrophic testes and,



Figure 9-3. Cushing's syndrome in a young man aged 23 years (from Herman and Merenlender (1936), by courtesy of *Acta Dermato-Venereologica*).

finally, early conspicuous arteriosclerosis of the aorta and renal arteries. Cushing himself examined the pituitary gland of this patient and confirmed the diagnosis.

The patient revealed the characteristics of a pyknic constitution.

Raab (1936), in his paper on the analogy existing between the features of senility and Cushing's syndrome, drew our attention to the fact that there is a resemblance between the appearance, day by day, of various signs of normal aging, and of the most frequent pathological conditions of an advanced age, on one hand, and the signs and symptoms of Cushing's syndrome, on the other. In Cushing's syndrome according to Raab, we have a key to the understanding of all the unpleasant phenomena of our senility.

In the younger individual, the syndrome is nothing less than a caricature of old age, and if we agree that "senectus ipse morbus" let us transpose

this statement, in connection with the Cushing's syndrome, to that of "morbus ipse senectus."

Here belong such features of senility as osteoporosis, diabetes mellitus, hyperglycemia and glycosuria, arterial hypertension, etc.

Discussion

In the diseases of progeria, Werner's syndrome, ateliosis and Glinski-Simmonds' disease there is a premature senescence associated with a deficiency of one or more pituitary hormones. Whereas in Cushing's syndrome accelerated aging is associated with an excessive production of adrenocortical steroids, which in many cases is due to hypersecretion of ACTH by the pituitary.

It must be pointed out that the major criterion of increased aging in these clinical syndromes is the external appearance of the patient. Whether there is a real increase in the rate of aging or not can only be resolved with objective tests of aging. Tests of skin collagen (Villee, *et al.*, 1969) and lipofuscin pigment in the heart (Reichel and Garcia-Bunuel, 1970) in the few cases of progeria so far examined indicate increased aging of the skin and the heart.

Thus increased aging may result from either the lack or the excess of pituitary hormones. It is possible that any deviation from normal pituitary function may cause a disturbance of the normal pattern of aging. There could be more specific effects. For example the lack of growth hormone or the failure of tissues to respond to growth hormone could result in premature senility as in progeria. Similarly the excess of adrenocortical steroids as in Cushing's syndrome could accelerate the normal aging process. There appear to be two types of increased aging, a premature senility due to hypopituitarism and an accelerated aging due to hypersecretion of ACTH or corticosteroids.

TWO CONSTITUTIONAL TYPES OF SENILITY

The habitus of patients with Glinski-Simmonds' disease (Fig. 9-2) corresponds with the leptosomic (or asthenic) type of body physique, while that of Cushing's syndrome (Fig. 9-3) corresponds with the pyknic type.

It is proposed that there are two physiological types of senility associated with the leptosomic and pyknic types of constitution. This theory is based on the typology of Kretschmer (1924) who distinguishes three types of physical constitution, namely the asthenic or leptosomic, the athletic and the pyknic types.

The leptosomic or asthenic type of senility is—par excellence—an involutional type, with structural changes in all tissues such as skin, subcutaneous fat, muscles, bones and blood vessels. It differs from the juvenile

asthenic type by the lack of a disproportion between the so-called longitudinal and transverse dimensions. The leptosomic type of an aged person, thin and bent, is that of late senility. Kretschmer (1924) himself remarked that premature senility represents a frequent biological feature of asthenic persons.

The second type of aged individual, the pyknic one, is characterized, just as in the physiological conditions described by Kretschmer (1924), by an overgrowth of the body in the transverse and antero-posterior dimensions, and by a tendency towards adiposity of the trunk with delicate extremities, a "bloated" figure with a short neck, moon-like face, the head and shoulders craning forward, a barrel-shaped chest, and arched vertebral column. This type may sometimes resemble that variation of the athletic type which manifests itself by a general clumsiness ("allgemeine Plumpheit").

Each type of constitution is known to show a tendency for definite disease entities (Bauer, 1944; Schubert, 1962; Eitner, 1968). Individuals with the pyknic constitution usually have hypertension (Robinson and Brucer, 1940), raised serum lipid levels (Hellström, 1965) and coronary heart disease (Damon, *et al.*, 1969), while the leptosomics are usually affected by gastritis and intestinal ulcers (Schubert and Basel, 1965).

If there is a relationship between physique, disease and aging, to what extent is this linked to pituitary function? In order to answer this question studies of pituitary function will have to be made in old leptosomic and pyknic subjects.

CONCLUSION

In human subjects, normal pituitary function is associated with a normal rate of aging. Increased aging may result from either a lack or an excess of certain pituitary hormones.

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CHAPTER 10

PITUITARY AND COLLAGEN

ZDENEK DEYL, JAN ROSMUS and MILAN ADAM

SUMMARY

HYPOPHYSEAL HORMONES influence collagen metabolism either directly or indirectly through other endocrine glands. Growth hormone stimulates protein synthesis in general and thus collagen formation is increased. Growth hormone increases collagen breakdown and this action is specific for this fibrous protein. In hypophysectomized animals, according to Shoshan, there is a considerable slowing down of the first step in collagen cross-linkage, namely the conversion of lysine into α amino adipic acid- δ semialdehyde. It is concluded that lysyloxidase, the enzyme catalyzing this reaction, is pituitary dependent. According to our own data hypophysectomy reduces the solubility of the collagen structure. This finding could be interpreted as either blocked collagen formation or retarded maturation with subsequent rapid breakdown of the non-cross-linked forms of collagen.

Thyroid hormones exhibit both anabolic and catabolic effects on collagen. Thyroxine seems to be necessary for collagen formation. The catabolism of all types of collagen is increased in hyperthyroidism and decreased in hypothyroidism.

It is generally agreed that corticosteroids have an inhibitory effect upon collagen formation. According to our results adrenalectomized animals exhibit a high proportion of soluble collagen fractions. It seems that corticosteroids are capable of increasing collagenolytic activity and the breaking down of polymerized (mature) collagen structures.

Collagen formation is more pronounced in males than in females. The effect of estrogen upon collagen metabolism in nonreproductive organs is not yet clear. Progesterone is found to slow down the conversion of soluble collagen fractions into insoluble collagen in skin. Androgens in general exhibit an intensive anabolic effect upon the synthesis of proteins, including collagen.

INTRODUCTION

Collagen belongs to the family of fibrous proteins and appears to be one of the essential components of connective tissue. It is found almost everywhere in the vertebrate body. In the form of a loose fiber network this

protein penetrates different tissues, and, as the integral part of the extra-cellular phase, it plays an important role in establishing the microenvironmental conditions for many cells. It has been generally accepted for years (Chvapil, 1967) that the most important physiological functions of the connective tissue may be classified as the depository function, the transport function, and the supporting or mechanical function. Chemically these functions can be related to the formation of new cross-links between the polypeptide chains of collagen. Cross-linkage is regulated in part by pituitary hormones.

COLLAGEN CROSS-LINKAGE

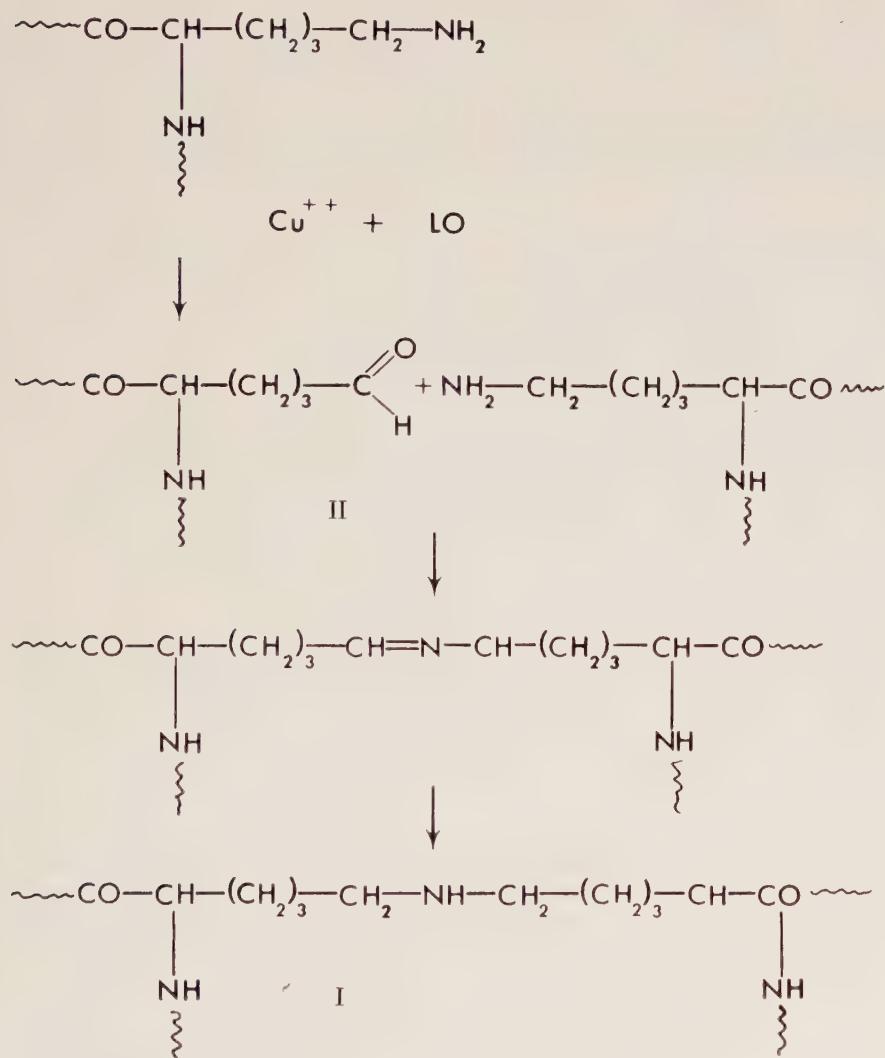
In order to form a reasonable basis for discussion it must be said that the collagen fiber is made up of molecules which adlineate in a quarter-stagger array. Each of these molecules is composed of three polypeptide chains which wind together and form the stiff rod-like helical molecule, monomeric collagen, which according to Schmitt, *et al.* (1942) is called tropocollagen.

The intra- and mainly inter-molecular forces, principally covalent interactions, are of first order importance in physiological regulation. It has been recognized for some time (Verzár, 1964) that covalent cross-links accumulate in collagen with age and this fact is reflected in the physiology of the organism in two different ways. First of all the very existence of new cross-links alters the properties of the connective tissue which becomes less swellable, less soluble and more resistant towards all types of degradation; this also means an increase in the metabolic half life. From the physiological point of view one would expect a change in transport processes, that is a decline in the circulation of materials between tissue cells and blood vessels. The second effect of cross-link accumulation is an increase in the overall proportion of the connective tissue within a particular organ. This is an immediate result of the increased catabolic resistance of polymerized collagen structures. The recognition of the slow accumulation of cross-links in collagen led to the formulation of the collagen-based theory of aging (Verzár, 1964).

This aging theory states that covalent cross-links accumulate with age, thereby increasing the overall proportion of collagen in organs and that this unfavourable increase in connective tissue (which occurs mainly at the expense of cells) is one of the causes of aging. The concept of covalent cross-links developed rapidly during the past two decades, and recently sufficient evidence has been acquired to describe these bonds in chemical terms.

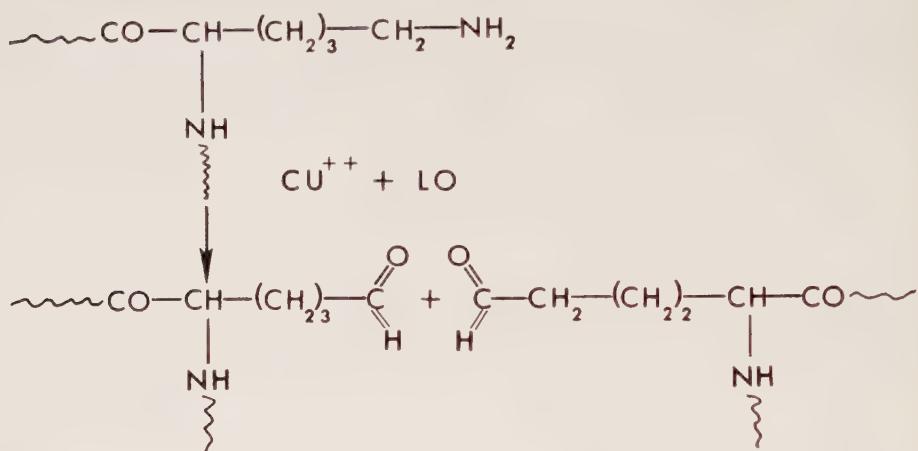
It should be stated that the most reactive region of the collagen molecule appears to be its N-terminal region in which two types of bonds oc-

cur. Both of these bonds are lysine derived. The first one Franzblau, *et al.* (1965) called lysinonorleucine (N-5-amino-5-carboxypentanyl lysine). It originates from lysine, in which the free α -amino group is oxidized to the α -amino adipic acid δ -semialdehyde by the enzyme lysyl oxidase (LO). In the next step this compound (sometimes referred to as allysine) undergoes a condensation reaction with an unmodified lysine residue and the resulting aldimine is stabilized by hydrogenation. The reaction is as follows.

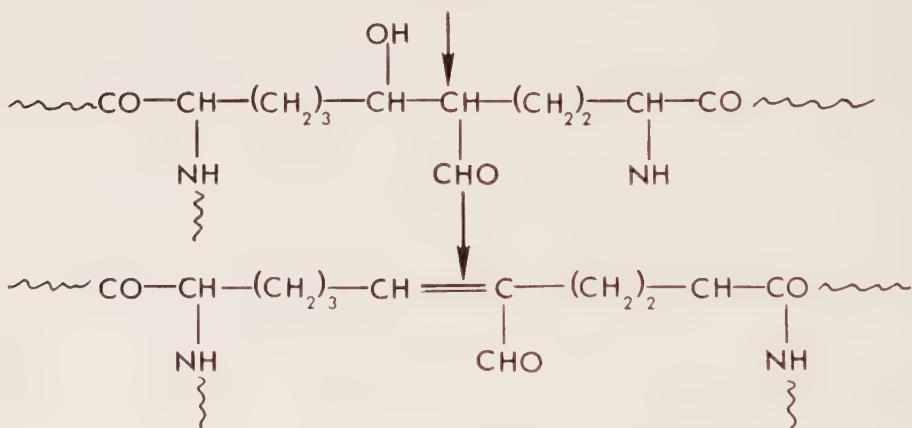


Lysinonorleucine
 N-(5-amino-5-carboxypentanyl)-lysine
 LO = lysyl oxidase

The second type of cross-link is essentially an alternative reaction of the first one: two residues of the α -amino adipic acid δ -semialdehyde are subjected to the aldol condensation. The reaction is as follows.



α amino adipic acid δ semialdehyde



Besides these terminal cross-links, there is considerable evidence to indicate the existence of covalent cross-links beyond the terminal region of the tropocollagen molecule. As far as the chemical nature of these cross-links is concerned they are presumably of the aldimine (Schiff base) type.

Cross-links discussed up to now may be also considered "physiological cross-links" since they occur in a healthy body. There is a great deal of speculation about pathological cross-links; tyrosine derived cross-linking interactions seem to be the most favored candidates.

It has been suggested that mainly two processes occur when the collagen fiber ages. Firstly it seems that intramolecular aldol-type cross-links (which are rather unstable) are converted into more stable ones like lysinonorleucine. This may occur both intra- and inter-molecularly. However, if this is to play a role in aging, the conversion has to end with the formation of an intermolecular cross-link. Secondly it is very likely that with increasing age, cross-links of the Schiff base type (aldimine bonds) are formed beyond the terminal region of the tropocollagen molecule.

The increase in the number of covalent cross-links is paralleled by a decrease in collagen solubility. A first approach to solubility changes is the estimation of the proportion of collagen fractions which are solubilized in 0.15 M NaCl phosphate buffer, in citrate buffer and in 6 M urea. During the phase of intensive collagen formation aging changes are readily detectable in the youngest (highly soluble) fractions, while in the phase of low-level collagen synthesis the most important fraction is the urea soluble collagen. Recently four types of collagen with different primary structures have been described. Of great importance is the existence of type III which is present in the insoluble fraction of various tissues together with type I and is stabilized with a disulfide bridge. The results discussed in this chapter have to be seen from this point of view.

For the normal mechanical and transport functions of the extracellular tissue it is necessary to maintain an appropriate degree of collagen cross-linking. This is ensured by different regulatory mechanisms of which hormonal control is of decisive importance.

PITUITARY HORMONES

Pituitary hormones may influence collagen turnover either directly or indirectly through other endocrine glands. Therefore, it appears necessary to include the effect of pituitary-dependent glands in the present review. In order to achieve a deeper understanding of the problem, one should not forget that hormonal imbalance may cause substantial changes in the animal's physiology. For example in pituitary dysfunction, anorexia with the consequent restriction in food consumption appears to be of considerable importance in the aging of collagen (Steinetz, *et al.*, 1966; Deyl, *et al.*, 1968).

GROWTH HORMONE (SOMATOTROPIN)

Growth hormone stimulates the proliferation of fibroblasts and consequently increases connective tissue formation. High doses of growth hormone result in generalized hypertrophy and hyperplasia (Taubenhaus and

Amromin, 1950). The local administration of growth hormone causes an increase in the mechanical strength of healing wounds (Chvapil, 1967). Besides collagen, the synthesis of other proteins *in vitro* is stimulated by growth hormone (Bartlett, 1955; Kostyo and Schmidt, 1962). A similar effect occurs in a cell free system isolated from somatotropin treated animals (Korner, 1962). Banfield (1958) has found that the administration of growth hormone to experimental animals increases the content of acid soluble collagen in the skin.

Growth hormone increases the level of hydroxyproline in serum (Kivirikko, *et al.*, 1958; Leroy and Sjoerdsma, 1965) as well as increasing its excretion in urine (Smiley and Ziff, 1964). Hydroxyproline excretion in urine is also increased in childhood if the growth is too fast (Jasin, *et al.*, 1962). On the contrary in the dwarfism of childhood hydroxyproline excretion in urine is considerably decreased (Jasin, *et al.*, 1962). Somatotropin administration increases the urinary hydroxyproline output in these individuals. It has been shown several times in the past that the incorporation of labeled proline into collagen and the formation of hydroxyproline are significantly decreased in hypophysectomized animals and on the contrary are strongly stimulated by growth hormone administration (Vaes and Nichols, 1962; Daughaday and Mariz, 1962; Chulkova and Orekhovich, 1965; Mikkonen, *et al.*, 1966; Valavaara, *et al.*, 1968).

In hypophysectomized rats, growth hormone stimulates fibroblast proliferation (Branwood, 1963), increases the thickness of collagen fibers in skin (Simpson, *et al.*, 1949) and increases the collagen content in skin and in thigh muscles (Scow and Hagan, 1965). A similar increase in the collagen content of skin and of thigh muscles was reported in thyroidectomized and thyroidectomized-hypophysectomized rats (Scow, 1951 and 1959). Kowalewski and Yong (1968a and b) found an increase in neutral salt soluble and acid soluble collagen in mice after growth hormone administration.

There is general agreement on the stimulatory effect of growth hormone upon collagen synthesis. Aer, *et al.* (1968) reported that growth hormone stimulates the formation of skin collagen to a much greater extent compared to its stimulatory effect upon the synthesis of noncollagenous proteins. The increased collagen formation is reflected in the positive calcium and strontium balance which occurs in hypophysectomized animals treated with growth hormone and in acromegalic patients. Scow (1951) has established that growth hormone stimulates collagen synthesis much more efficiently than it stimulates the synthesis of all other proteins. It has been assumed by Laitinen (1967) that growth hormone accelerates the conversion of soluble collagen into insoluble polymers.

As mentioned previously growth hormone increases the rate of hydroxyproline excretion in urine. This means that somatotropin simultaneously stimulates both the synthesis and catabolism of collagen. The effect of growth hormone is very distinct in acromegaly. In these patients the skin becomes very thick, the content of skin collagen is increased, collagen fibers are very coarse and more densely packed compared with normal adults (Gabrilove, *et al.*, 1962). In addition the bones start to grow in adult human subjects with this disease and this is accompanied by a raised serum hydroxyproline level and by increased urinary hydroxyproline and calcium output (Leroy and Sjoerdsma, 1965; Benoit, *et al.*, 1963; Lee and Lloyd, 1964; Kocher, *et al.*, 1965). All these facts indicate an increased collagen turnover. An increased urinary hydroxyproline output was observed also in human subjects treated with growth hormone (Ikkos, *et al.*, 1958). These findings are further supported by other workers (Bell and Bartter, 1967; Molinatti, *et al.*, 1961; Eisenberg and Gordan, 1961), who described increased bone resorption both in acromegaly and during somatotropin treatment. Jasin, *et al.* (1962) suggested that growth hormone exhibits a specific effect upon collagen breakdown, while other proteins are catabolized at the normal rate. The evidence for this statement comes from the fact that during active acromegaly and during growth hormone administration only the level of urinary hydroxyproline was increased, the urinary output of other amino acids remained normal.

According to Prockop (1962) urinary hydroxyproline originates partly from catabolized insoluble collagen and partly from newly formed soluble collagen. Aer, *et al.* (1968) were unable to prove an accelerated conversion of soluble collagen into the insoluble fraction. Also the degradation of soluble collagen was not influenced by growth hormone administration, while mature insoluble collagen was degraded more intensively in growth hormone treated animals. From this it is concluded that growth hormone stimulates predominantly the breakdown of mature collagen. It has to be stressed, however, that the doses of growth hormone used by Aer, *et al.* (1968) were extremely high and that the physiological action of growth hormone may differ from that described.

Elden (1969) assumes that growth hormone is a factor which accelerates collagen maturation. The above experiments of Aer, *et al.* (1968) are in apparent contradiction to this assumption.

HYPOPHYSECTOMY

Our results on hypophysectomized rats (Deyl, *et al.*, 1972) are in good agreement with the above mentioned findings. The retarded collagen synthesis and modified collagen catabolism result in distinct changes in the proportion of collagen in various organs (Table 10-I) and further, the

TABLE 10-I
COLLAGEN PROPORTION IN DIFFERENT ORGANS IN MALE HYPOPHYSECTOMIZED RATS OF AGE 113 DAYS

Organ	Organ Weight mg	Hypophysectomized		Hypro*		Controls		t-test Hypro*
		Hydro* μg/g Wet Weight	Dry Weight %	Organ Weight mg	Hydro* μg/g Wet Weight	Dry Weight %	Organ	
Kidney	\bar{x} 755.0	197.4	23.0	149.037	2,410.0	104.6	22.1	252.086
	s 44.8	3.93			273.0	6.08		5.98
Heart	\bar{x} 319.0	225.3	25.1	71.870	948.0	85.9	24.0	81.433
	s 14.4	7.26			58.2	5.32		10.5
Liver	\bar{x} 4,500.0	126.7	32.1	570.150	11,900.0	76.5	29.2	910.350
	s 400.0	3.9			1,025.0	3.02		6.23
Spleen	\bar{x} 310.0	136.0	24.4	42.160	1,040.0	103.6	22.8	107.744
	s 9.42	7.48			124.2	2.4		5.8
Aorta	\bar{x} 51.0	506.9	48.0	25.852	106.5	617.7	23.3	65.785
	s 46.7	94.1			13.35	17.3		0.87
Epididymal fat	\bar{x} 687.0	82.4	70.0	56.609	3,440.0	55.4	58.8	190.576
	s 137.0	36.2			1,005.0	30.9		1.04
Lung	\bar{x} 1,490.0	345.0	24.4	514.050	1,520.0	221.0	21.5	335.920
	s 164.0	129.1			244.0	28.9		0.1
Testes	\bar{x} 211.0	495.0	22.9	104.445	2,620.0	130.9	11.1	342.958
	s 35.9	81.7			174.0	23.1		13.5

* Hypro = hydroxyproline; \bar{x} = mean from 21 estimations; s = standard deviation.
(Reproduced from Deyl, et al. (1972) with permission of Pergamon Press Ltd.)

TABLE 10-II
SOLUBILITY OF SKIN COLLAGEN IN HYPOPHYSECTOMIZED, ADRENALECTOMIZED, GONADECTOMIZED AND CONTROL RATS

Group	Age in Days	Body Weight g	Dry Skin	Neutral				Insoluble Collagen			
				Total Hydroxypro- line Cont. mg/100 mg	Soluble in 0.15 M NaCl	Soluble in 0.45 M NaCl	Acid Soluble Collagen	6 M Urea Extractable Collagen	Residue		
Hypophysectomized (63 days ago)	113	136.6	6.98	0.15	0.86	0.36	0.53	34.80	54.67		
Controls		237.4	8.00		4.20		2.34	35.30	51.50		
Adrenalectomized (18 days ago)	68	142	5.3	1.11	6.76	9.55	29.34	48.56			
Controls		155	5.8	0.83	5.53	8.60	28.2	49.83			
Gonadectomized (74 days ago)	124	258	4.76	0.39	2.09	3.4	22.91	67.29			
Controls		239	7.58	0.80	4.50	6.04	34.59	51.41			

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qualitative features of collagen, i.e. its solubility, are changed, as well (Table 10-II).

Hypophysectomized rats in our investigation were substantially retarded in their growth (hypophysectomized animals weighed on the average 136.6 g and controls 337.4 g) at the end of the experiment. The ratio of collagen (hydroxyproline per g of wet organ) in almost all organs investigated at age 113 days (kidney, heart, liver, epididymal fat, testes and spleen) was increased in hypophysectomized animals. The differences compared to controls were highly significant ($p < 0.001$). The greatest difference observed was that in testes where the hydroxyproline concentration after hypophysectomy increased more than three times. While in most organs the hydroxyproline concentration was increased in experimental animals, in aortae the final hydroxyproline concentration was lower by about 20 percent but this difference was insignificant. These data, however, represent a strictly one-sided view of the collagenous stroma of various organs. The total amount of collagen per organ was significantly decreased in hypophysectomized animals in comparison to controls (Table 10-II). This holds for all organs investigated except for lungs, in which the discrepancy is presumably due to infectious lung disease which was quite frequent in our rats.

Data summarized in Table 10-II confirm that hypophysectomy reduces the solubility of the collagen structure. This finding has to be understood in terms of blocked collagen synthesis, i.e. that the decreased solubility of the collagen structure is a result of decreased collagen synthesis.

It is, however, necessary to stress that our data relate to male rats surviving 63 days after hypophysectomy. It appears to be well established that in late hypophysectomy the absence of growth hormone is of prime importance while shortly after hypophysectomy other factors may prevail. It has also to be stressed that firstly this situation occurs in rats, i.e. in continuously growing animals, and secondly that some strain differences were noticed.

The above conclusions are further supported by findings of Steinertz, *et al.* (1966), who reported changes in the shrinkage properties of tendon collagen in hypophysectomized rats which resembled increased aging of the tendon. In discussing these results the above authors took into consideration all the important factors like age at hypophysectomy and strain differences. These authors further ascribed the differences observed to impaired collagen synthesis after hypophysectomy rather than to decreased collagen cross-linking. Steinertz, *et al.* (1966) also reported a similar extractability of collagen in urea when controls and hypophysectomized animals of age 60 days are compared. This statement can be confirmed from our own data (see Table 10-II), as the changes in collagen solubility ap-

parently do not occur at the expense of this particular fraction, but at the expense of the more easily soluble collagen fractions.

In judging the overall effects of hypophysectomy on the collagen metabolism, cross-link formation must also be considered. As shown recently by Shoshan, *et al.* (1972) it appears that the conversion of lysine into the α -amino adipic acid δ -semialdehyde is considerably slowed down and also the polymerization of implanted collagen is retarded. Therefore it has been concluded that either the production and/or the activity of lysyl oxidase has been decreased after hypophysectomy and it is suggested that lysyl oxidase may be pituitary dependent.

TSH AND THYROID HORMONE

In many respects the effect of growth hormone resembles the effects of thyroid hormones. Under the influence of pituitary thyrotropic hormone (TSH) the thyroid gland produces thyroxine and triiodo-thyronine, which in general stimulate metabolic processes. With respect to collagen, thyroid hormones exhibit both anabolic and catabolic effects. Thyroxine seems to be necessary for collagen formation. Kivirikko, *et al.* (1963 and 1965b) and Gries and Lindner (1966) followed the hydroxyproline excretion in urine and accumulated a considerable volume of evidence showing that in hyperthyroidism the excretion is decreased. This is in agreement with the report of Giles and Everitt (1967) on the inhibition of collagen maturation in thyroidectomized animals. Hypothyroidism induced by propylthiouracil administration during a period of 62 weeks resulted in a significant decrease of both soluble and insoluble collagen fractions in rat skin (Freihoffer and Wellband, 1963). In animals, from which the thyroid gland has been removed, decreased bone formation has also been observed by Detenbeck and Jowsey (1969), which may be related to changes in the metabolism of the collagenous matrix. Tracer studies with proline-¹⁴C of Kivirikko and coworkers (Kivirikko and Laitinen, 1965a and b; Laitinen, *et al.*, 1966; Kivirikko, *et al.*, 1967) have shown that catabolism of all types of collagen is increased in hyperthyroidism and decreased in hypothyroidism.

Collagen synthesis in hypothyroidism may be normalized by thyroxine administration (Kivirikko, *et al.*, 1967; Valavaara, *et al.*, 1968). Increased thyroxine administration, however, decreases collagen formation (Mikkonen, *et al.*, 1966; Vaes and Nichols, 1962; Fink, *et al.*, 1967; Kivirikko, *et al.*, 1963 and 1967). It seems, therefore, that in a normally functioning thyroid the conditions for collagen synthesis are optimal.

Thyroxine simultaneously stimulates the breakdown of both the soluble and insoluble fractions of collagen (Kivirikko, *et al.*, 1967). In hyper-

thyroidism an increased urinary hydroxyproline output was observed (Benoit, *et al.*, 1963; Dull and Henneman, 1963; Kivirikko, *et al.*, 1965b). Accordingly in hyperthyroidism urinary hydroxyproline excretion was decreased (Keiser and Sjoerdsma, 1962; Kivirikko, *et al.*, 1963 and 1964). The relation between thyroid activity and collagen metabolism can be very nicely demonstrated in the metamorphosis of the tadpole, *Rana catesbeiana*, in which collagenous structures are degraded by a specific tissue collagenase (Gross and Lapierre, 1962). It is thus possible to conclude, that collagen catabolism is considerably increased in hyperthyroidism and decreased in hypothyroidism (Kivirikko and Laitinen, 1965b; Laitinen, *et al.*, 1966; Kivirikko, *et al.*, 1967). Giles and Everitt (1967) observed in young rats five months after thyroidectomy a slow down of maturation in rat tail tendon collagen. Thyroxine administration normalized the "age" of tail tendons. These authors explained the above results by the thyroxine induced increase in food consumption. Conversely our own results indicate that prolonged food deprivation has the opposite effect (Deyl, *et al.*, 1968 and 1971).

ACTH AND CORTICOSTEROIDS

Both ACTH and cortisone inhibit the formation of the granulation tissue in rats (Taubenhaus and Amromin, 1950). It is generally agreed that corticosteroids have an inhibitory effect upon collagen formation. This effect is not specific for collagen but it is observed with all proteins studied (Kivirikko, 1963; Wegelius and Koch, 1966; Laitinen, 1967; Lorenzen, 1969).

The effects of cortisone and its derivatives have been intensively studied. Cortisone, when administered to adult animals, decreases the amount of soluble forms of collagen in the skin (Siuko, *et al.*, 1959; Houck, 1962; Sakata, 1960; Günther and Carsten, 1961; Kühn, *et al.* (1964). The decrease in both bound and free hydroxyproline was observed in newborn rats, if their mothers were given cortisone during pregnancy (Chvapil, 1958). Daughaday and Mariz (1962) reported a decrease in hydroxyproline formation after cortisone administration by following the proline-¹⁴C incorporation in their samples. Kowalewski (1969) found that in pups whose mothers were treated with cortisone during the lactation period, there was less young collagen and more mature insoluble fibers, than in pups of control dams.

Quite similar results were published by Kühn, *et al.* (1964) who in addition were able to prove that by the action of prednisone the rate of insoluble collagen formation is increased. On the other hand, an increase in free hydroxyproline within 6-10 days has been observed after a single 1 mg

dose of cortisone (Roberts, *et al.*, 1951; Chvapil, 1959). It was, however, not definitely proved that this increase is not a relative one caused by the decrease of noncollagenous structures. The decrease of both free hydroxyproline and the soluble forms of collagen by cortisone in chick embryo was exploited very extensively by Kivirikko (1963). This author describes an increase in the turnover of newly synthesized forms of collagen by the action of cortisone. Additional evidence for the same finding was presented by Trnavsky (1967). A very strong inhibitory effect on collagen synthesis is also described for the 6-methyl-prednisolone "Medrol" (Baretta, *et al.*, 1962). Günther and Carsten (1961) observed in adrenalectomized animals an increase of the soluble collagen in skin. There are some speculations that the solubility changes are due to changes in cross-links between individual tropo-collagen molecules. Our own data (Table 10-II) indicate that adrenalectomized animals exhibit a higher proportion of soluble collagen fractions which is in agreement with the observations of Günther and Carsten (1961).

Corticosteroids inhibit the growth and migration of fibroblasts *in vitro* (Cornman, 1951; Kaufman, *et al.*, 1953; Ruhmann and Berliner, 1965).

The effect of corticosteroids upon collagen is more distinct in growing individuals than in adults. Lorenzen (1969) has observed an inhibitory effect upon collagen formation caused by corticosteroids during the proliferation of connective tissue in healing wounds in young animals. High doses of corticosteroids reduced the number of fibroblasts which are also morphologically altered (Wegelius and Koch, 1966; Lorenzen, 1969). According to Gould and Manner (1967) corticosteroids are bound to polyribosomes and result in their disaggregation. These authors reported a decrease of polyribosomes to 50 percent; the incorporation of proline-C-14, however, was decreased to a greater extent (by 90%) which is evidence for more than one mechanism of corticosteroid action. Hirayama, *et al.* (1971) have followed cortisone formation and collagenolytic activity in liver during cortisone administration. They established a decrease in collagen formation and an increase in collagenolytic activity.

The effect after hypophysectomy should be a combination of the effects of the absence of somatotropin, corticotropin and other pituitary hormones. While adrenal hypofunction should result in facilitated collagen synthesis and therefore an increase in the soluble collagen pool, the absence of somatotropic hormone causes a strong inhibition of collagen synthesis and finally it decreases the amount of soluble collagen forms. These effects oppose each other and from the data obtained from young hypophysectomized animals it appears that the effect of growth hormone enhances that of corticosteroids. It should also be noted that the presence of

corticosteroids might affect even the rate of collagen maturation as indicated by the work of Árvay and Takács (1965), described in Chapter 18 of this monograph.

Based on their findings of substantial losses of dermal collagen within two hours of corticosteroid administration, Houck and Patel (1965) formulated a hypothesis that corticosteroids increase collagen catabolism. The results of Sakata (1960) and Kowalewski (1966) were interpreted in the same way. This hypothesis is obviously due to the well known antianabolic effect of corticosteroids. However, such pronounced changes are unlikely to be due to changes in collagen metabolism because of the small turnover of insoluble collagen. In spite of some contradictory results it seems to be well established that at least under specified circumstances corticosteroid administration increases the content of acid soluble collagen fractions. On the other hand, it has also been proved that cortisone administration reduces the urinary content of hydroxyproline in young rats as reported by Kivirikko and Laitinen (1965a).

The apparent discrepancy between this finding of Houck and Patel (1965) and that of Kivirikko and Laitinen (1965a) should be discussed in more detail. Firstly, as reported by Kivirikko and Laitinen, the effect of cortisone upon the hydroxyproline level in urine occurs in young animals only; in older animals it is without any effect. Secondly, it has been shown that the changes in urinary hydroxyproline excretion are due to the change in collagen synthesis, while collagen catabolism was shown to be unaffected (Kivirikko, *et al.*, 1965a). Laitinen (1967a) admits also that the urinary hydroxyproline may not be an adequate assay for reflecting a small change in collagen catabolism, and that the acid soluble collagen pool after corticosteroid administration may not be due to collagen breakdown processes only. It seems thus that cortisone and its derivatives inhibit collagen formation. For collagen metabolism the decreased rate of enzyme release from lysosomes is no doubt important. Cathepsin D belongs to the category of enzymes which are of particular interest because of their collagenolytic properties (Bensusan and Klein, 1965). This fact could explain why, after small doses of cortisone, the total amount of collagen in tissues increases.

Cortisone and its derivatives also influence the formation of mucopolysaccharides and glycoproteins. These substances play an important role in the aggregation of tropocollagen into fibrils. It is possible that collagen turnover may be modified in this manner.

In conclusion it could be stated that cortisone decreases collagen formation, but its possible role in the breakdown of collagen has to be further elucidated. One has to bear in mind that any corticosteroid if given in sufficient amount has a cytotoxic effect. Consequently unless one is dealing

with physiological amounts of those hormones in model experiments one cannot draw conclusions concerning the functional significance of the hormone in question (Munck, 1965).

In addition it should be noted that the effect of glucocorticoids upon the connective tissue generally opposes the action of mineralocorticoids.

SEX HORMONES

As far as sex hormones are concerned there are distinct discrepancies in their relation to collagen metabolism. It is generally agreed that collagen formation is more pronounced in males than in females (Kao, *et al.*, 1957; Boucek, *et al.*, 1959; Boucek and Noble, 1961). It is also very likely that the different results reported by different authors reflect the fact that the action of sex hormones varies according to species, sex and the tissue studied.

The collagen content of the uterus increases after estradiol administration (Smith and Allison, 1966a and b) and decreases after ovariectomy (Harkness, *et al.*, 1954). The effect of ovariectomy upon the collagen content of the uterus may be reversed by estrogen administration (Morgan, 1963a). The collagen content of the uterus is subjected to changes during the estrous cycle (Smith and Kaltreider, 1963). The lowest collagen content has been found in the uterus shortly before ovulation, while the highest collagen level appeared during metestrus. Morgan (1963b) reported significant decrease in the collagen content of the uterus between diestrus and estrus and thus confirmed the finding of Smith and Kaltreider (1963). During pregnancy the collagen content of the uterus increases and after delivery is rapidly reduced (Harkness, 1964; Schaub, 1964 1965). The post-partum degradation of collagen is inhibited by estrogen administration (Woessner, 1969).

The effect of estrogen upon the collagen metabolism in nonreproductive organs is not yet clear. For example, the incorporation of lysine-C-14 into sponge-granuloma, is less intense in females than in males (Boucek and Noble, 1961). According to Taubenhaus, *et al.* (1952), estrogens inhibit granulation tissue formation and decrease collagen synthesis. On the contrary, Bavetta, *et al.* (1962) and Robertson and Sanborn (1958) described a stimulatory effect of estrogens upon collagen formation in carageenin granuloma. It has been established by Kao, *et al.* (1965) that estradiol increases lysine-C-14 incorporation into carageenin granuloma collagen. However, the total amount of this protein is decreased. Based on her own experiments with carageenin granuloma in guinea pigs, Henneman (1968) suggested that estradiol has a stimulatory effect upon both collagen formation and degradation. There are also data showing a decreased collagen content in rat skin after estradiol administration (Sobel, *et al.*, 1953).

Henneman (1971) found in experiments with proline-C-14 a reduced collagen biosynthesis and increased collagen degradation in skin caused by estradiol administration. Estradiol had the opposite effect on collagen in uterus and bone. Henneman (1968 and 1970), Smith and Allison (1966a and b) and Riggs, *et al.* (1969) studied the effect of estrogen upon the bone tissue in osteoporotic patients, in which bone resorption was increased before medical treatment. According to these studies it seems that the main effect of estrogen in osteoporosis is to block bone resorption. Estradiol administration in males decreases hydroxyproline output in urine and decreases the high excretion of hydroxyproline due to excess growth hormone, parathyroid hormone or thyroxine (Katz and Kappas, 1968).

Kühn, *et al.* (1965) using glycine-C-14 have found in progesterone treated rats a slower conversion of soluble fractions into insoluble collagen in rat skin, because of the retarded formation of the interchain bonds and also because of a more intensive breakdown of the neutral salt soluble fraction. Holzmann, *et al.* (1965) reported an increased catheptic activity in progesterone treated rats. The effect of progesterone upon collagen formation has not been elucidated. Smith and Kaltreider (1963) reported that progesterone increases collagen content of the uterus but Harkness and Harkness (1959) came to contradictory conclusions.

Androgens in general exhibit an intensive anabolic effect upon protein synthesis. Collagen formation with respect to testosterone has been studied in capon skin (Herrick and Brown, 1952). It has been proved that testosterone increases collagen synthesis under these conditions. A similar effect has been found by Kühn, *et al.* (1962) in male rats.

Our own results (Table 10-II) indicate that in castrated male rats, collagen formation is decreased in skin and that the proportion of insoluble collagen is thus indirectly increased (Deyl, *et al.*, 1972). Smith and Allison (1965) reported an increased collagen content of skin and femur in young female rats after testosterone administration. An increased mechanical strength of wounds after testosterone treatment has been described by Jørgenson and Schmidt (1962) and Pearce, *et al.* (1960).

CONCLUSION

The influence of the pituitary on the final degree of collagen maturation is very complex. Generally speaking the action of the pituitary upon collagen structure and aging may be related to the following processes:

1. via somatotropin, which regulates the proportion of collagen synthesis to collagen breakdown during the growth period. Obviously this hormone regulates collagen maturation only indirectly;
2. via lysyl oxidase. After hypophysectomy the general decrease in pro-

tein synthesis is reflected in a decrease of lysyl oxidase activity, which means that there is reduced formation of new cross-links of the lysinonorleucine type.

These effects, together with the effects of other hormones are briefly summarized in Table 10-III.

TABLE 10-III

THE EFFECT OF PITUITARY HORMONES AND SOME RELATED HORMONES UPON COLLAGEN SYNTHESIS, BREAKDOWN AND AGING

Hormone	Mode of Action	Effect on Collagen		Aging
		Formation	Breakdown	
Growth (Somatotropin)	Direct	Stimulated	Stimulated	?
Thyrotropic	Thyroxine mediated	Reduced in both hyper- and hypothyroidism	Stimulated	Increased
Adreno-corticotropic	Cortisol mediated	Reduced	?	Increased ?
Gonadotropic	Progesterone mediated Estrogen mediated Testosterone mediated	Reduced ? Stimulated	Stimulated ? None	? ? ?

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CHAPTER 11

THE ROLE OF THE PITUITARY AND THE THYROID IN THE AGING OF COLLAGEN IN RAT TAIL TENDON

ARTHUR V. EVERITT and LEIGH DELBRIDGE

SUMMARY

AGE CHANGES IN THE COLLAGEN fibers of rat tail tendon were studied physically, by measuring the time to break in 7 M urea at 40°C under a load of 2 g; and chemically, by chromatography of the subunits of soluble collagen. With increasing age collagen fibers become progressively more insoluble and physically stronger. These changes are due to the accumulation of cross-links between collagen molecules as demonstrated by chromatography.

The long-term effect of hypophysectomy is to retard certain aging changes in tail tendon collagen. The age-related decline in solubility is abolished by hypophysectomy. The breaking time of a collagen fiber from the tail of a two-year-old hypophysectomized rat is approximately the same as that from a one-year-old control rat. Thyroidectomy has a similar anti-aging effect on collagen in tail tendon, and thyroxine accelerates these age changes. The effects of thyroidectomy and thyroxine are strongly correlated with food intake.

INTRODUCTION

Collagen, as an extracellular protein, is not renewed during cell division, and consequently accumulates aging changes. Since collagen is a major constituent of the connective tissue in all organs, aging changes in collagen could affect all body functions. It is therefore of great importance to understand the factors which regulate the aging of collagen. Probably the most significant hormonal factors are the hormones secreted by the pituitary and thyroid glands.

THE AGING OF COLLAGEN

Collagen is one of the few proteins (together with elastin) which show clear cut changes with age. During maturation and aging the collagen content of most tissues increases and the fibers become increasingly insoluble. These age changes are shown in Figure 11-1 for the tail tendon of the male Wistar rat.

Probably the most conspicuous age changes in collagen are to be seen in its physical properties. Verzár (1955) was the first to develop a test of biological aging utilizing these age changes. He found that the tension developed during the thermal contraction of collagen at 65°C increased markedly with age. Variations of this test of collagen age consist in measuring contraction, relaxation and rupture (or breaking) of the collagen fiber in potassium iodide, sodium perchlorate or urea solutions (Boros-Farkas and Everitt, 1967). The increase with age in the time to break an isolated collagen fiber under a load of 2 g in 7 M urea at 40°C is shown in Figure 11-2.

Age changes in the physical properties of collagen are usually attributed to the formation and accumulation of covalent cross-links. Gel filtration

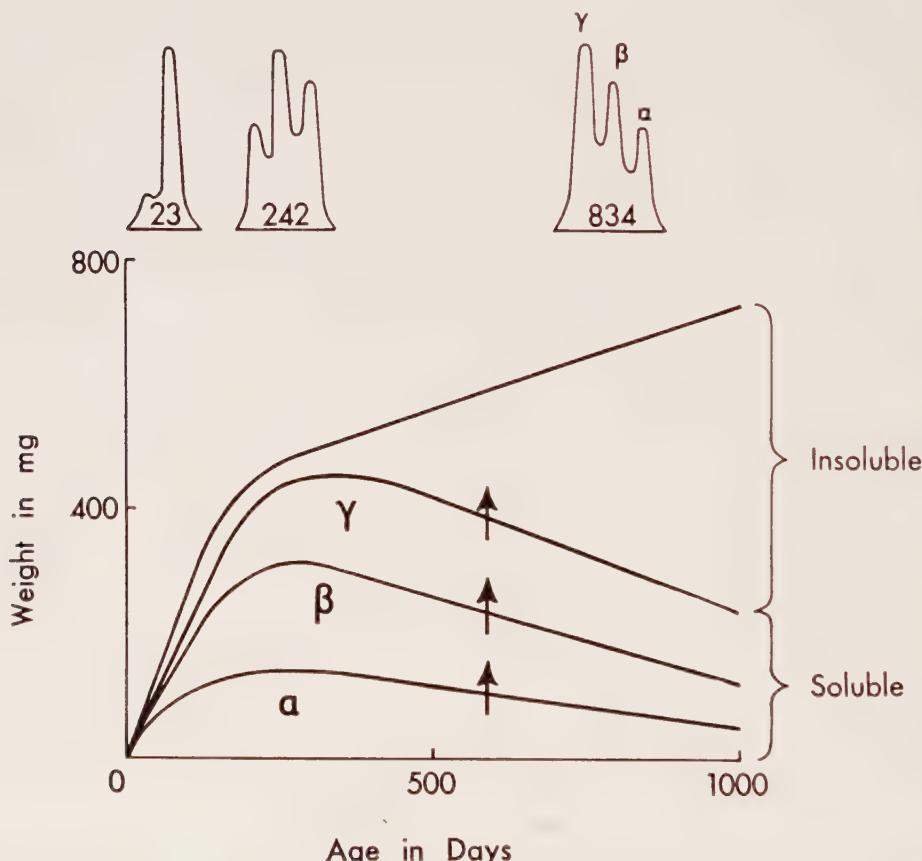


Figure 11-1. The increase with age in the total weight of tail tendon collagen and the amount of insoluble collagen. Gel filtration patterns on Biogel A-15 m show an increase with age in γ collagen subunits and a corresponding decrease in α subunits (From Delbridge and Everitt (1972a), reproduced with permission of S. Karger, Basel).

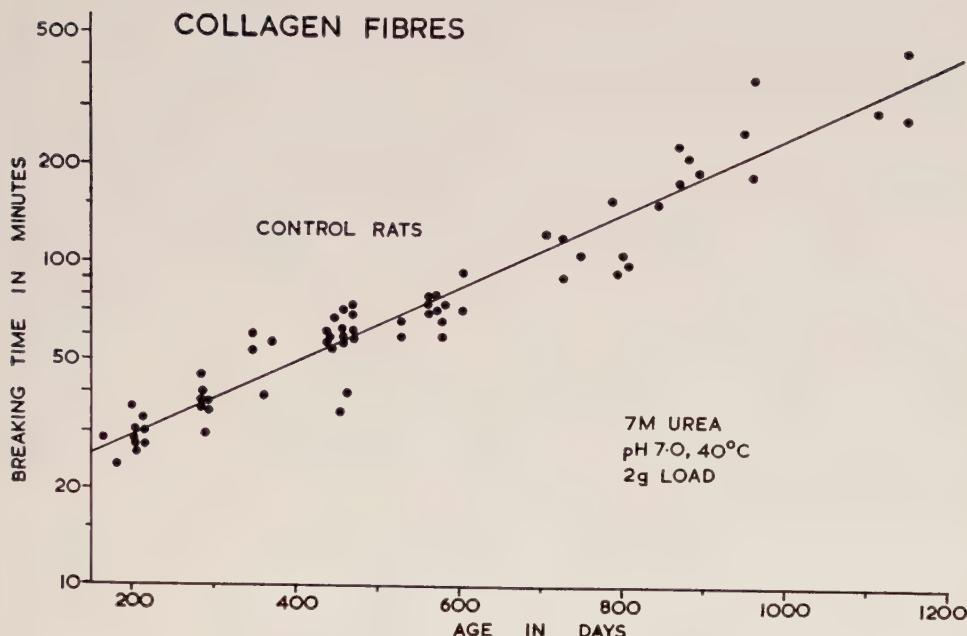


Figure 11-2. The increase with age in the time to break an isolated collagen fiber from rat tail tendon in 7 M urea at 40°C (From Everitt, *et al.* (1968), reproduced with permission of the *Journal of Gerontology*).

chromatography of denatured collagen (Delbridge and Everitt, 1972a) shown in Figure 11-1 clearly demonstrates the increasing cross-linkage of collagen with age. There is a rise in the proportion of γ (cross-linked 3 chain) and a fall in the proportion of α (uncross-linked single chain) components of denatured soluble collagen with increasing age.

EFFECTS OF HYPOPHYSECTOMY

Surgical removal of pituitary brings about striking changes in the physiology of the organism, including the cessation of growth and the inhibition of protein synthesis. The synthesis of collagen, as with other proteins, is inhibited in the hypophysectomized rat (Smith and Armstrong, 1961; Valavaara, *et al.*, 1968; Franchimont and Denis, 1969).

The failure of collagen synthesis is associated with the demonstration of accelerated "aging" or cohesion of collagen fibers 25 days after hypophysectomy (Steinetz, *et al.*, 1966; Everitt, *et al.*, 1968). In collagen extracted from the tail tendon of the hypophysectomized male rat, 3 weeks after operation, there is a reduction in the proportion of α subunits (uncross-linked chains) as shown by gel-filtration chromatography (Everitt and Delbridge, 1972) in Figure 11-3. Thus the collagen fibers from the tail

tendon of the recently hypophysectomized rat appear to be older than those from a normal rat of the same age, because they contain less newly-synthesized or young collagen.

This temporary acceleration of collagen aging in tail tendon soon after hypophysectomy is subsequently followed by a permanent retardation of aging (Fig. 11-4) as shown by measurements of the time to break in 7 M urea (Everitt, *et al.*, 1968) and by studies of thermal contraction (Verzár and Spichtin, 1966). The retardation of aging is so great that an 800-day old hypophysectomized rat has collagen fibers in its tail tendon with physical properties corresponding to those of a 400-day-old sham operated rat (Fig. 11-4). Hypophysectomy also decreases the aging of collagen in the dorsal skin of the rat (Verzár and Spichtin, 1966). Chromatographic studies by Deyl, *et al.* (1967) on rat skin suggested that hypophysectomy may be an effective block in the cross-linking of collagen.

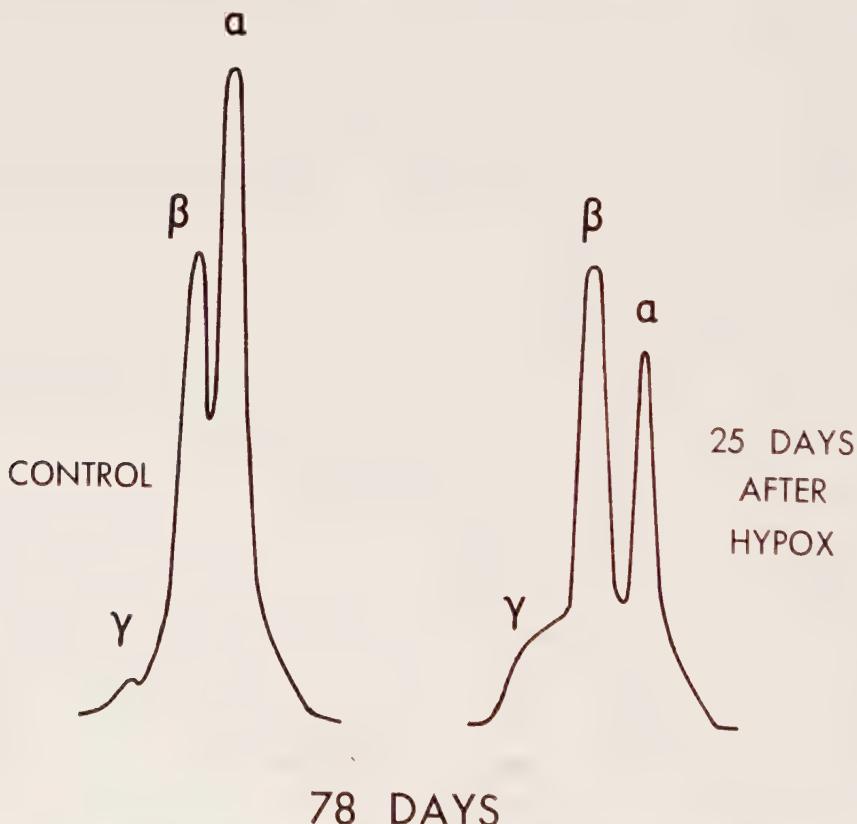


Figure 11-3. The decrease in α -subunits (newly synthesized collagen) 25 days after hypophysectomy, indicating a fall in collagen synthesis and hence an apparent rise in collagen age (From Everitt and Delbridge (1972), reproduced with permission of Pergamon Press Ltd.).

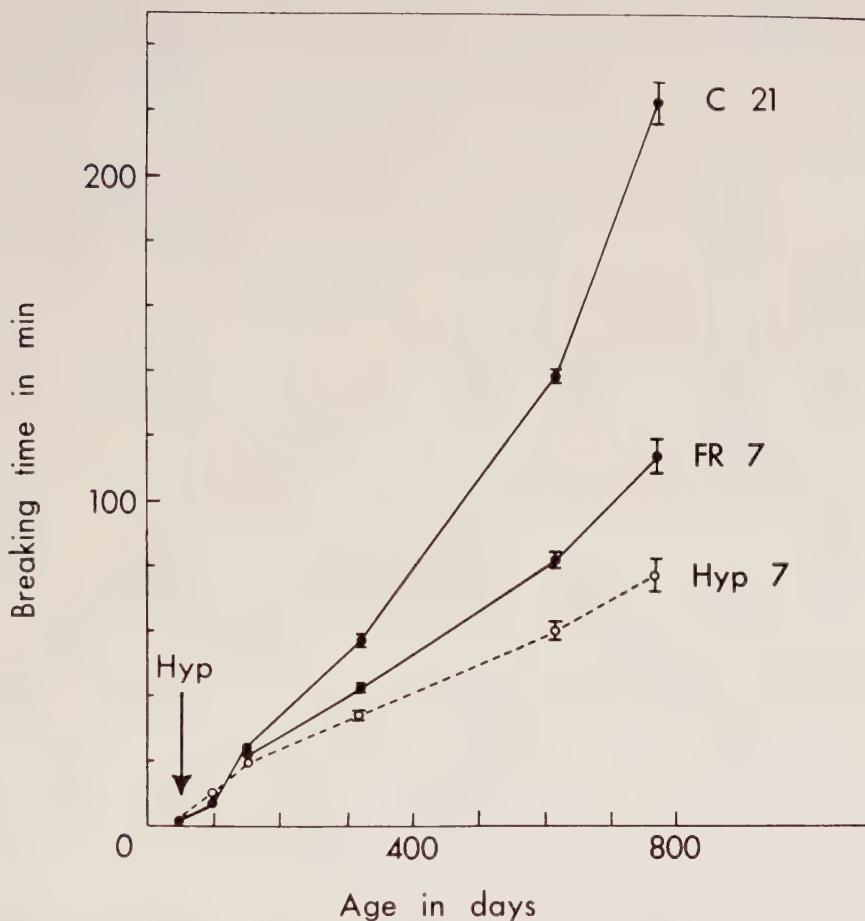


Figure 11-4. The retarded aging of collagen fibers from tail tendon in food restricted (FR7) and hypophysectomized rats (Hyp 7). The 800-day-old hypophysectomized rat has a collagen age (measured by time to break collagen fiber in 7 M urea at 40°C) equal to that of a 400-day-old intact control (From Everitt (1971a), reproduced with permission of the Australian Association of Gerontology).

Collagen fibers washed in acid phosphate have much shorter breaking times in 7 M urea (Fig. 11-5). Delbridge, *et al.* (1972) showed that acid phosphate washing breaks the intermediate labile form of the lysine-derived aldehyde type of cross-link described by Bailey (1969). The chemistry of cross-links is discussed in the preceding chapter by Deyl, Rosmus and Adam. As the control rat ages, phosphate washing has less effect on breaking time indicating that the labile bonds are becoming stabilized with age. However in the hypophysectomized rat this does not occur, indicating that hypophysectomy inhibits the conversion of the labile into the stabilized form of the cross-link (Delbridge and Everitt, 1972b).

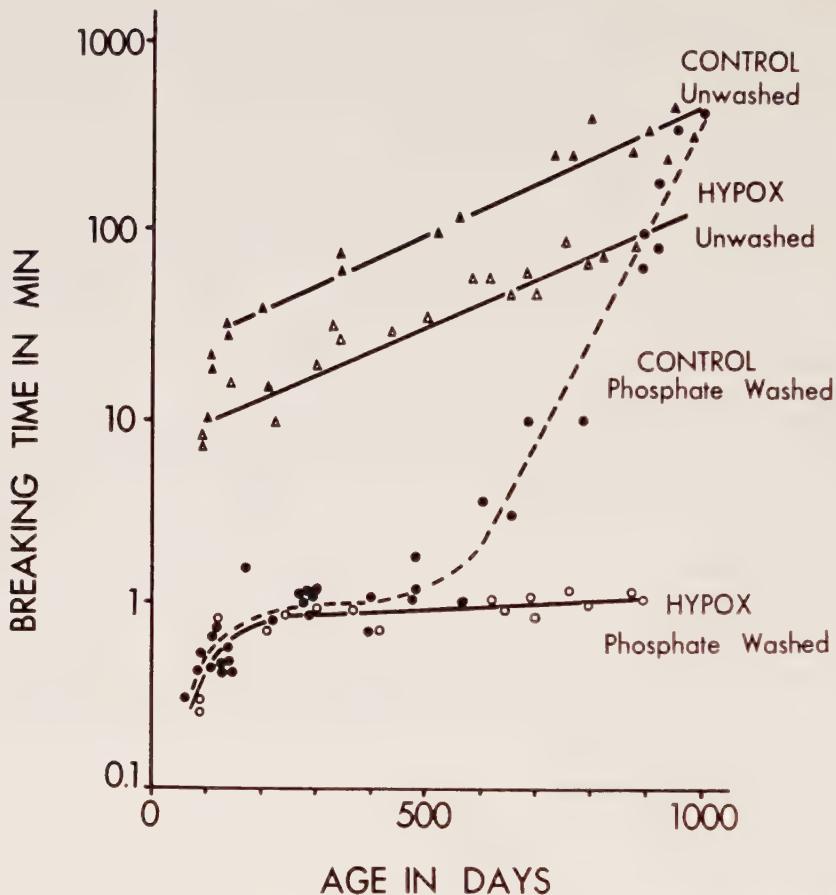


Figure 11-5. The breaking times in 7 M urea of acid-phosphate washed and unwashed collagen fibers from intact control rats and hypophysectomized rats. Acid phosphate washing breaks the labile form of the lysine-derived aldehyde cross-link. This cross-link becomes stabilized late in life, and the stabilization requires the presence of the pituitary gland (From Delbridge and Everitt (1972b), reproduced with permission of Pergamon Press Ltd.).

Shoshan, *et al.* (1972) showed that the cross-linking of collagen implants was inhibited in hypophysectomized rats. These results were interpreted by Shoshan as indicating that hypophysectomy inhibits the first step in intermolecular cross-linking of collagen by affecting the production or activity of lysyl oxidase. Howarth and Everitt (1974 and Fig. 11-6) failed to demonstrate any activity of an amine oxidase (believed to be lysyl oxidase) in the aortas of 10 hypophysectomized rats aged 100 days (50 days after operation). At this age the amine oxidase is readily demonstrated in intact rats. Enzyme activity was partly restored by injecting hypophy-

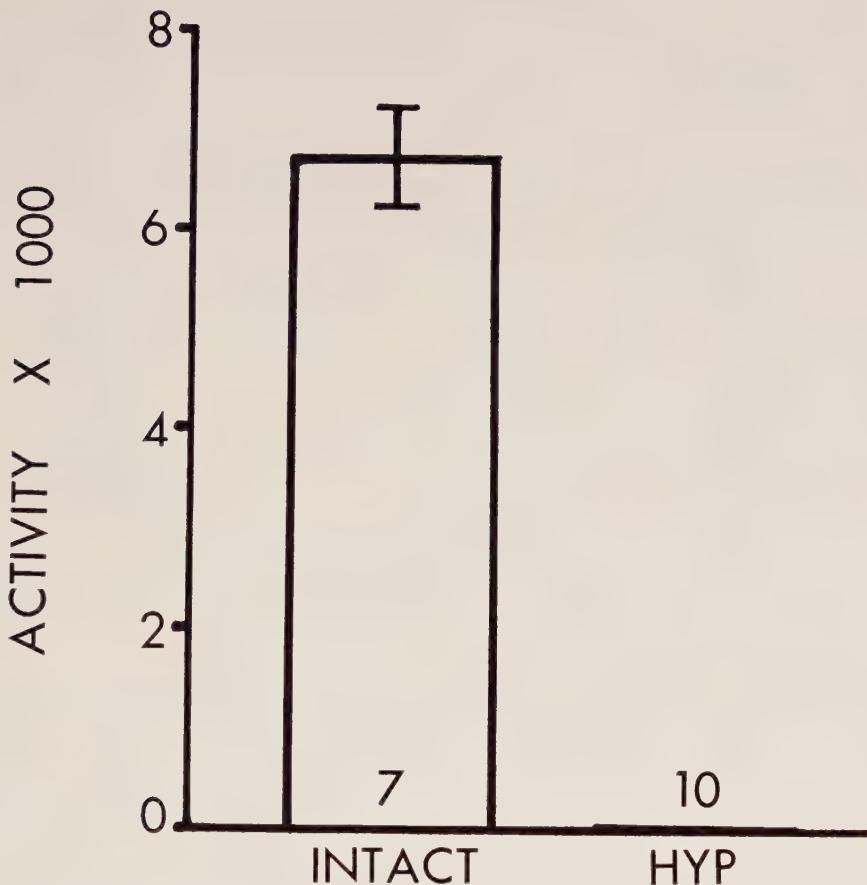


Figure 11-6. Amine (lysyl) oxidase activity in the aorta of male Wistar rats aged 100 days. Intact controls (INTACT) are compared with hypophysectomized (HYP). Amine oxidase was assayed using benzylamine as substrate by a variation of the technique of Tabor, *et al.* (1953) (From Howarth and Everitt (1974), reproduced with permission of S. Karger, Basel).

sectomized rats with growth hormone and cortisone (Fig. 14-4, Chap. 14). This study lends support to the work of Shoshan, *et al.* (1972) suggesting that lysyl oxidase is a pituitary-dependent enzyme.

Age changes in collagen solubility are also abolished by hypophysectomy (Fig. 11-7). The biphasic effect of hypophysectomy on collagen aging is also seen in the solubility of tail tendon (previously washed in 0.5 M NaH₂PO₄) in water at 65°C (Everitt and Delbridge, 1972). One month after hypophysectomy the collagen fibers in tail tendon contain twice as much insoluble or old collagen (20%) as in the intact control (10%) and consequently are "older" than normal for that age. However, in the 800-day

old hypophysectomized rat the collagen is "younger" than normal because it contains 20 percent insoluble or old collagen, compared with 40 percent in the intact control.

Hypophysectomy prevents the fall in collagen solubility with age, and so the youthful property of high collagen solubility is preserved even into old age. Normal age changes do however occur in the soluble collagen of the hypophysectomized rat (Everitt and Delbridge, 1972) as seen in the normal pattern of aging in the chromatogram on denatured soluble collagen (Fig. 11-8). Therefore the site of the anti-aging action of hypophysectomy is not on soluble collagen, but on the insoluble fraction. Presumably hypophysectomy blocks the cross-linkage of collagen leading to the formation of more insoluble collagen.

The retarded aging of collagen in the hypophysectomized rat is not asso-

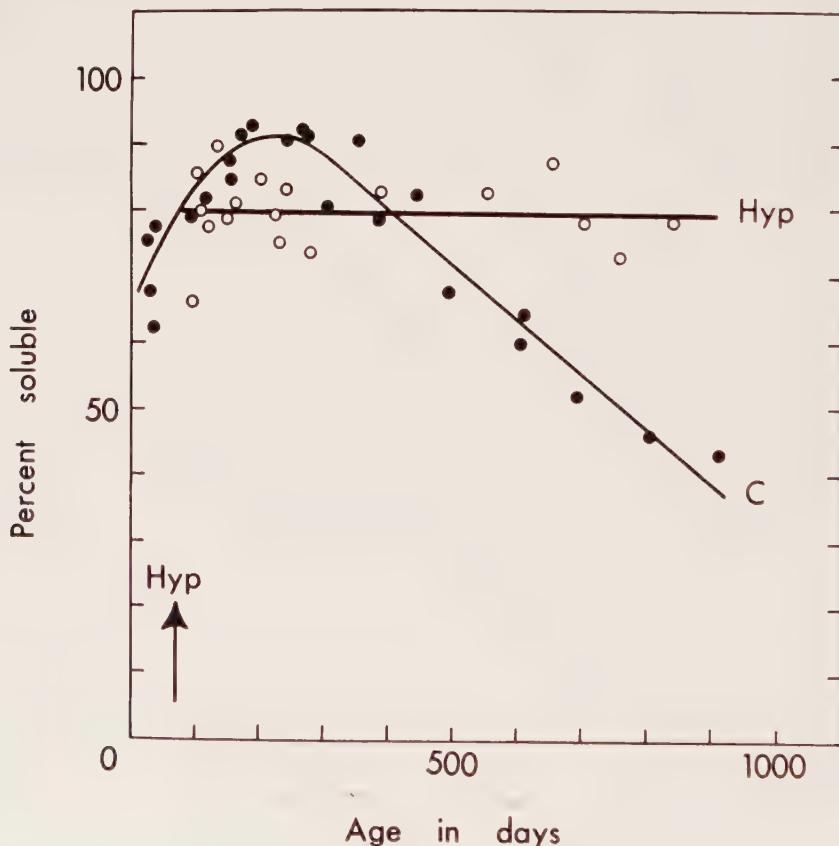


Figure 11-7. Effect of age on the solubility of tail tendon collagen (acid phosphate washed) in water at 65°C for 10 minutes in intact control (C) and hypophysectomized (Hyp) rats. Hypophysectomy abolishes the age change in solubility (From Everitt and Delbridge (1972), reproduced with permission of Pergamon Press Ltd.).

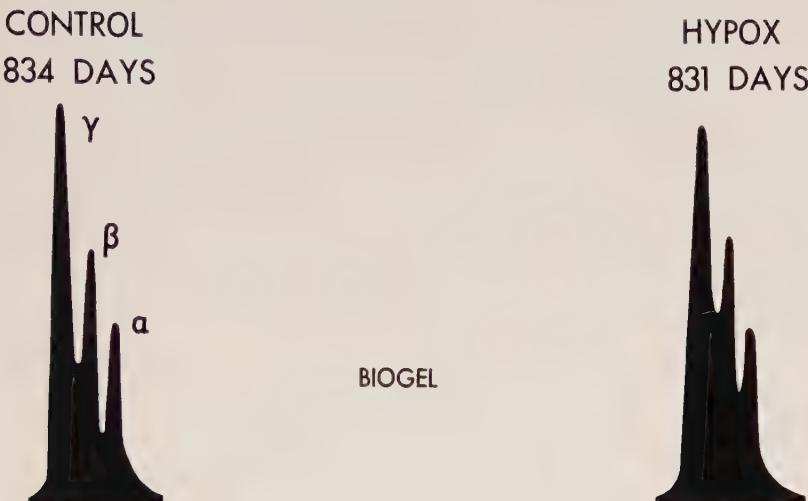


Figure 11-8. Gel filtration (Biogel A-15 m) patterns of α , β , γ collagen subunits in soluble collagen from the tail tendons of intact controls and hypophysectomized rats of age 834 days (From Everitt and Delbridge (1972), reproduced with permission of Pergamon Press Ltd.).

ciated with an increased lifespan. The hypophysectomized rat has, in fact, a shortened lifespan (Everitt and Cavanagh, 1965). Life duration is determined by pathology and is only indirectly related to the physiological aging of collagen.

Food intake is an important factor in collagen aging (Chvapil and Hruza, 1959). The lowered food intake of the hypophysectomized rat (Andik, 1957; Everitt and Cavanagh, 1965) has been suggested as the main reason for its retarded aging (Olsen and Everitt, 1965). Food is required for the synthesis of collagen and for the aging or cohesion of collagen fibers (Elden, 1969; Everitt, 1971b). When a comparison is made of the collagen age, as measured by the breaking time in urea (Fig. 11-4) of fibers from hypophysectomized rats and food restricted rats eating the same amount of food, the hypophysectomized rats are found to be significantly "younger." Therefore the pituitary gland secretes a factor which accelerates the aging of collagen independently of changes in food intake. This factor is probably ACTH, which is secreted during the stress of food restriction and is known to have an aging action on collagen.

EFFECTS OF PITUITARY HORMONES

Adrenocorticotropic hormone (ACTH) was shown by Árvay and Takács (1965) to increase the aging of collagen fibers in rat tail tendon. An aging effect of ACTH on collagen in rat aorta has been reported by Borkowski,

et al. (1966). It is well known that under conditions of stress, ACTH is released and stimulates the secretion of corticosteroid hormones from the adrenal cortex. Stresses reported to increase collagen age are repeated breeding of female rats (Árvay and Takács, 1965), prolonged psychological stress (Paré, 1965), severe undernutrition (Rassaert and Steinertz, 1968) and exposure to light, sound and electric shocks (Árvay and Takács, 1965). The long term treatment (600 days) of hypophysectomized rats with the corticosteroid, cortisone, accelerates the aging of collagen in tail tendon, without any significant change in food intake (Fig. 11-9).

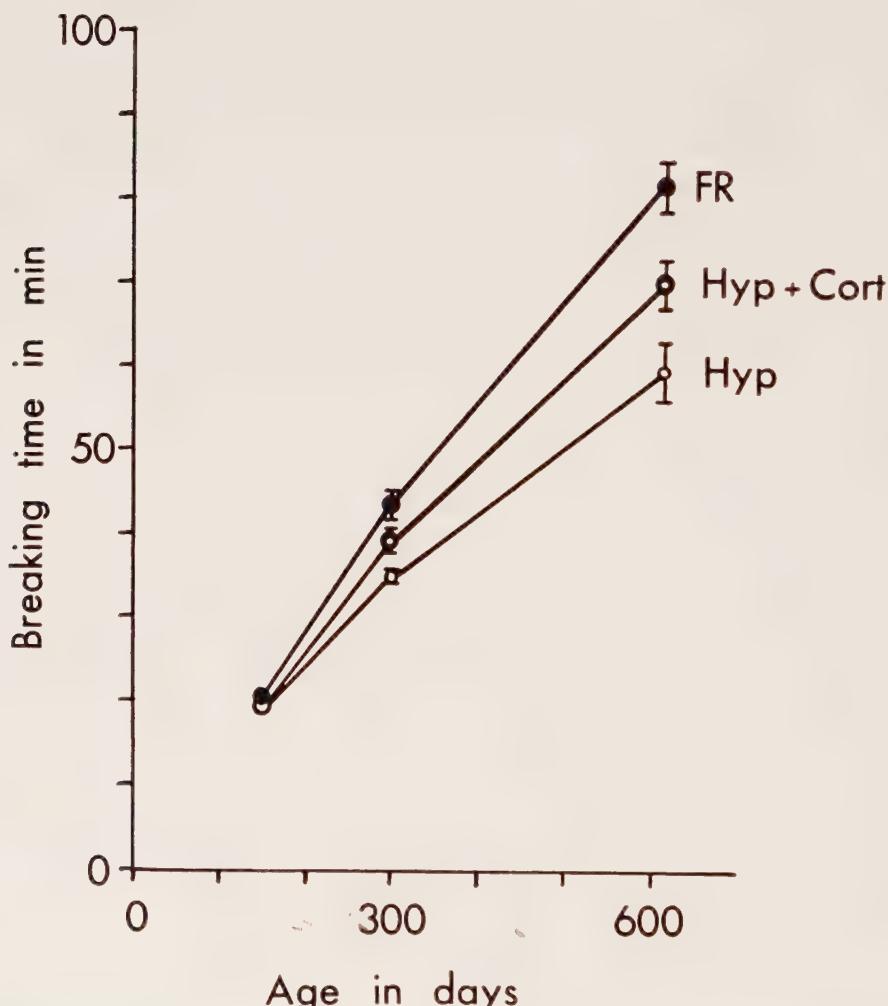


Figure 11-9. The aging effect of physiological doses of cortisone (Hyp + Cort) on collagen fibers in the tail tendon of the hypophysectomized rat (Hyp) (From Everitt (1973), reproduced with permission of Pergamon Press Ltd.).

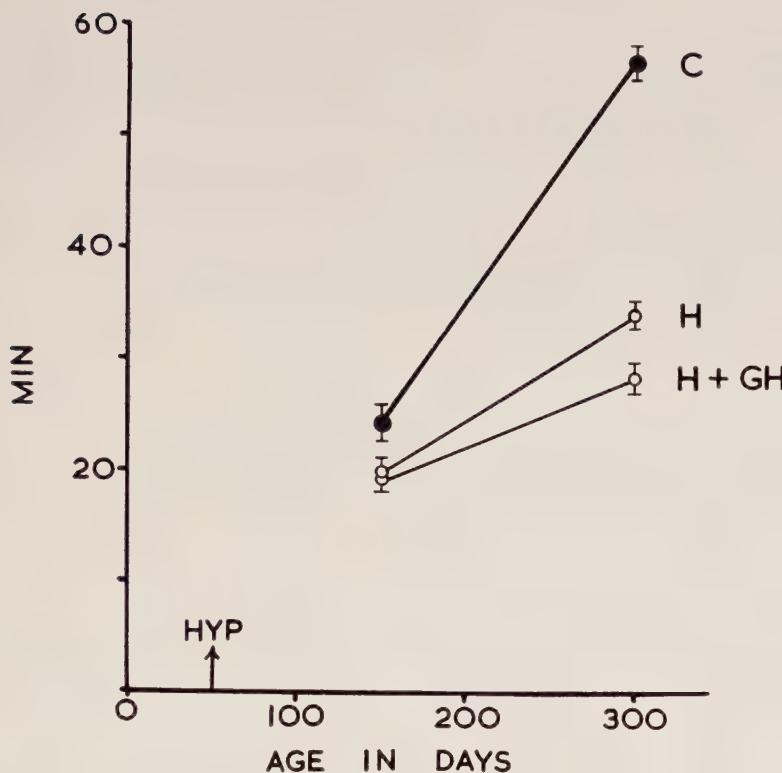


Figure 11-10. The anti-aging effect of physiological doses of growth hormone ($H + GH$) on collagen fibers in tail tendon of the hypophysectomized rat (H) (From Everitt (1973), reproduced with permission of Pergamon Press Ltd.).

Elden (1969) postulated that growth hormone is the pituitary factor which accelerates the aging of collagen. The chronic administration of growth hormone to hypophysectomized rats actually retards the aging of collagen (Fig. 11-10) as measured by the breaking time test. This apparent antiaging effect of growth hormone is probably due to the well known effect of growth hormone in stimulating the synthesis of collagen (Aer, *et al.*, 1968; Valavaara, *et al.*, 1968). As the result of the increased synthesis of collagen, the collagen fiber of the growth-hormone-treated hypophysectomized rat contains more "young" or newly synthesized collagen.

There is no direct evidence of an effect of thyroid stimulating hormone (TSH) on the aging of collagen, although TSH undoubtedly accelerates the aging of tail tendon through the mediation of the thyroid gland.

EFFECTS OF THYROID HORMONES

Thyroid hormones stimulate metabolic processes generally, including those metabolic changes leading to increased aging of collagen. Thyroxine increases collagen aging in tail tendon (Fig. 11-11).

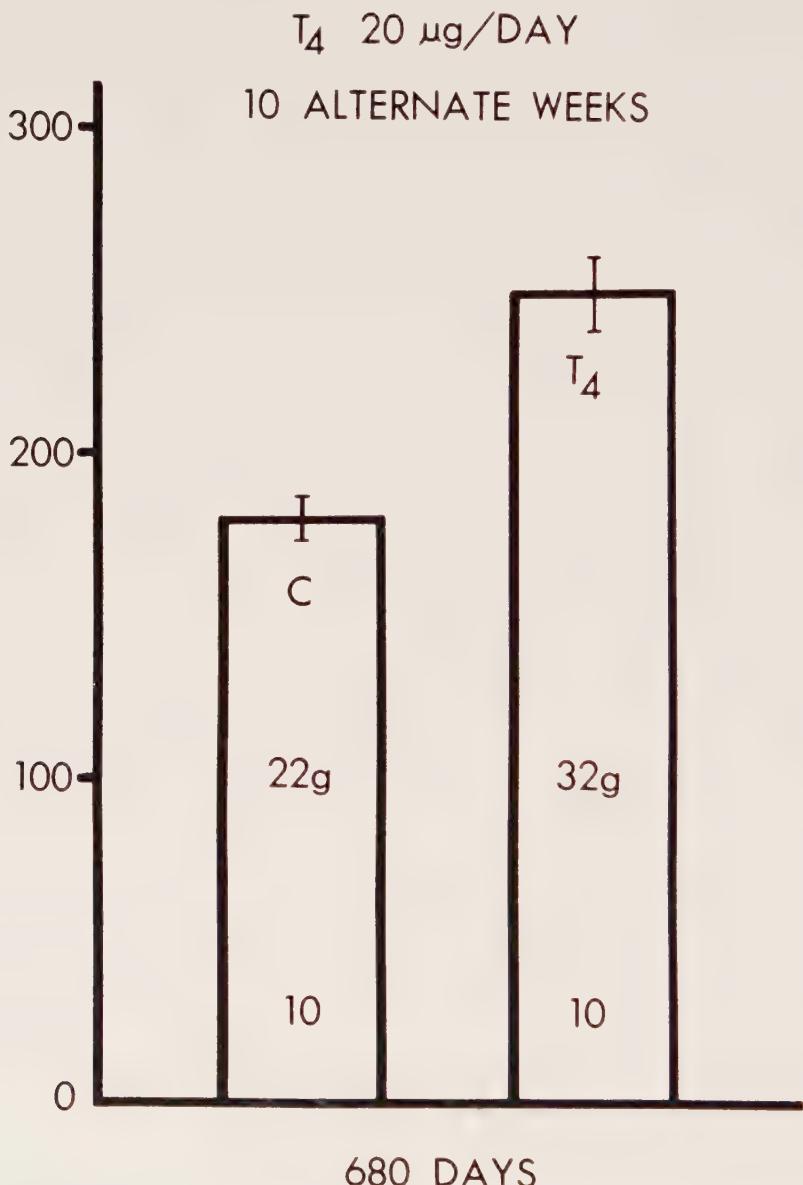


Figure 11-11. The effect of thyroxine (T_4) injections over a period of 5 months on the breaking time of tail tendon collagen fibers in 7 M urea, compared with a control group. The food intake was 32 g per day in thyroxine injected rats and 22 g in controls (From Everitt, *et al.* (1969), reproduced with permission of S. Karger, Basel).

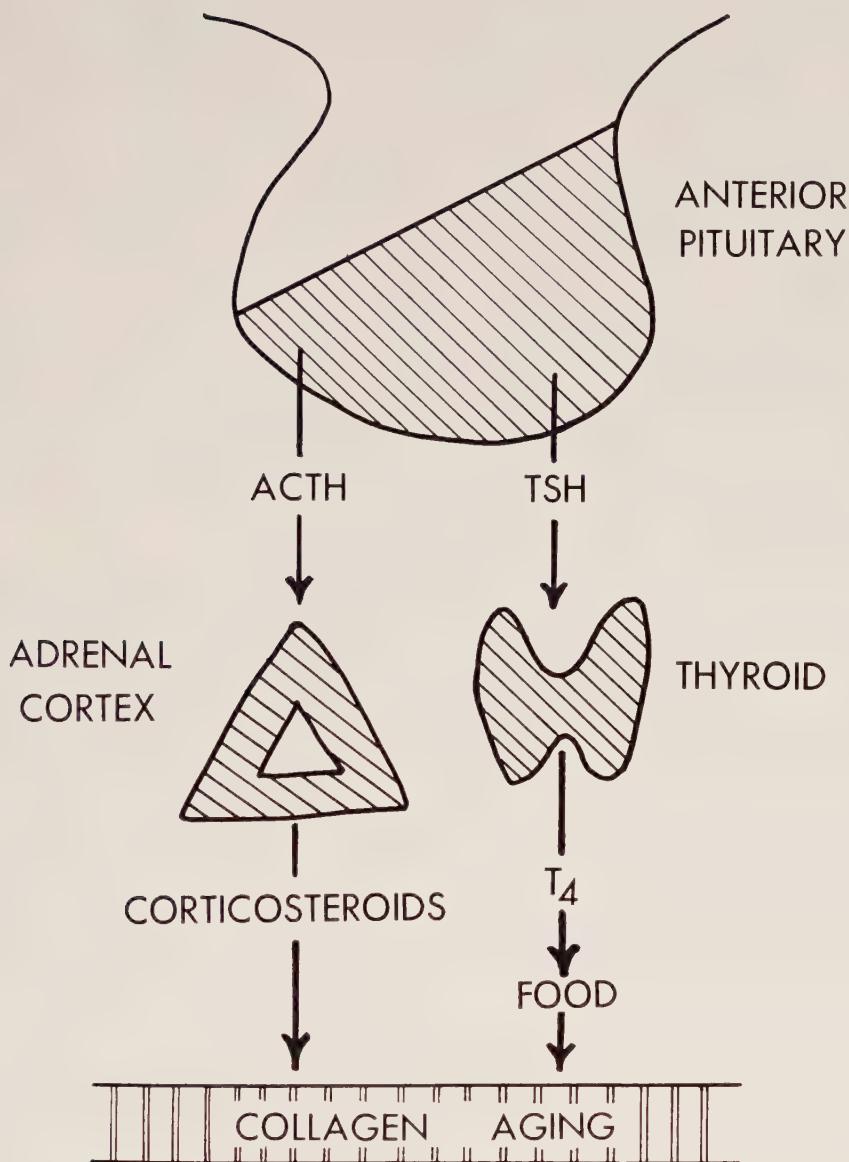


Figure 11-12. The role of the pituitary and the thyroid in controlling aging through the mediation of corticosteroids and the food consumed.

Surgical thyroidectomy like hypophysectomy, causes an initial acceleration of aging in collagen (Steinetz, *et al.*, 1966). This aging effect is probably due to the reduction in collagen synthesis arising from the loss of thyroid hormones or from the increased production of corticosteroids (Elden, 1969).

The long term effect of thyroidectomy (TX) is to significantly retard

the aging of rat tail tendon collagen over a period of 5 months (see Fig. 26-1 in Ch. 26). The aging process can be restored to normal over this period by replacement therapy with the thyroxine (TX + T₄). Thyroxine is able to increase the aging of tail tendon in young intact rats (Steinetz, *et al.*, 1966), in young thyroidectomized rats (Steinetz, *et al.*, 1966; Giles and Everitt, 1967) and in old rats (Everitt, *et al.*, 1969; Fig. 11-11). Old rats treated with thyroxine over a 5 months period also show an increased percentage of insoluble (or old) collagen in their tail tendons (Everitt, *et al.*, 1969).

The aging effect of thyroxine is associated with the increased food intake. There is a strong correlation between total food intake and collagen age as measured by breaking time. However, the factorial study of Giles and Everitt (1967) showed that thyroxine increases collagen aging in tail tendon, but only when food intake rises (see Fig. 26-1). Thus collagen aging is more directly related to food intake and metabolism than to thyroxine (Fig. 11-11).

Collagen aging is relatively independent of food intake up to 14 g per day (Everitt, 1971b), but above this threshold the aging of collagen increases as the food intake rises. The threshold of 14 g per day corresponds to the food intake of the thyroidectomized rat. As this is the lowest level of collagen aging it is taken as the intrinsic level of aging. Thyroxine is then regarded as the major factor accelerating the intrinsic aging of collagen.

CONCLUSION

There is an intrinsic aging process in the collagen of rat tail tendon, which continues even in the absence of the pituitary and thyroid glands. This aging process is accelerated by thyroxine (Fig. 11-12). The acceleration of collagen aging is closely related to the amount of food consumed, which is regulated by pituitary and thyroid hormones. Corticosteroids also increase the aging of collagen, but act independently of food intake. The effects of pituitary and thyroid hormones on collagen aging in other organs have received little or no attention, and could be different from those found in rat tail tendon.

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CHAPTER 12

THE PITUITARY IN RELATION TO SKELETAL AGING AND DISEASE

RUTH SILBERBERG

SUMMARY

SKELETAL AGING IS REGARDED as a continuous process starting during the period of active growth and continuing through life. The relationship of hypophyseal hormones and of pituitary-dependent hormones to skeletal aging and disease has been reviewed.

Somatotropin accelerates all phases of skeletal turnover; in addition to its effect on growth, the hormone promotes skeletal aging at the molecular, ultrastructural, cellular, tissue and clinical levels. Besides its well-known role in the pathogenesis of acromegaly, somatotropin may be involved in the skeletal lesions of diabetes mellitus. Evidence in favor of an endocrine origin of osteitis deformans Paget is inadequate.

Thyrotropin, gonadotropins, and adrenocorticotropin act on the skeleton through intermediation of the respective dependent hormones. Thyroxine accelerates skeletal turnover rates and thus promotes both skeletal growth and aging; the latter effect predominates and results in increased tendency to osteoporosis. Sex hormones when acting during the growth period accelerate early phases of aging; then effectiveness decreases with increasing age. The pathogenesis of hyperostosis frontalis interna and its relation to aging changes of the skull await further clarification. Gonadal deficiency before and during adolescence retards skeletal aging by preventing adequate bone development; past maturity, absence of gonadal secretions hastens aging changes. Glucocorticoids, by virtue of their anti-anabolic action, retard bone formation, thus promoting the development of a bone deficit.

Senile osteoporosis is considered an intrinsic skeletal disorder, related to aging changes and subject to modification by hormones. The latter may exert their effects either while the disease is in progress or prior to its onset. Senile osteoarthritis, likewise an intrinsic skeletal disease, appears related to pituitary activity because of the profound effects that somatotropin and

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other hormones controlled by the pituitary gland exert on growth and aging of cartilage. The disease may be modified by the cumulative effects of minor hormonal imbalances occurring prior to the onset of the lesions.

INTRODUCTION: THE CONCEPT OF AGING IN RELATION TO THE SKELETON

For the purpose of the present discussion, aging is defined as change progressive with time and associated with loss of adaptability to change in the environment. In accordance with the views of investigators who consider the distinction between maturation and aging as untenable (Child, 1915; McGavack, 1963; Chvapil and Deyl, 1964), such a distinction seems especially arbitrary in regard to skeletal tissues. At all times during the lifespan of an individual, structural material is deposited and removed, resulting in continuous remodeling (Silberberg and Silberberg, 1960; Frost, 1963; Johnson, 1966). Only the rates or the order of magnitude of these processes change with age, and neither change abruptly as the presumed state of maturity is reached. Further difficulties in differentiating between maturation and aging arise if the levels of observation are considered. Events, appearing as phases of maturation on the tissue level, may at the cellular level, comprise regression and even death of cells. Early investigators contended that dying chondrocytes contribute to the production of matrix (Schaffer, 1930). Electron microscopic studies gave qualified support to this view: about the periphery of dying chondrocytes whorls of dense collagen fibrils are deposited (Silberberg, 1968). Furthermore, epiphyseal-diaphyseal union constitutes the typical state of maturity of a tubular bone at the macroscopic level—yet the microscopic events leading up to it include not only proliferation but also degeneration and death of chondrocytes, calcification of matrix and resorption of cartilage and bone. The latter processes, on the other hand, do not cease as “maturity” is reached; they continue long past this stage, and contribute ultimately to the senile transformation of the skeleton. Accordingly, “maturation” of bone is considered as a phase of the aging process.

PROBLEMS ASSOCIATED WITH THE EVALUATION OF SKELETAL AGING

Owing to technical difficulties, investigation of skeletal aging has lagged behind, and there has been some tendency to extrapolate from basic data obtained by studying less unwieldy types of connective tissue. However, skeletal collagen and mucopolysaccharides may not be identical with those of soft connective tissues (Klein and Curtiss, 1962; Glimcher and Katz, 1965; Meyer, 1966). Results obtained with one type of connective tissue do therefore not necessarily apply to another type. Moreover, the rate as well

as certain morphological manifestations of aging vary not only from tissue to tissue within the same individual (Merkel, 1891; Loeb, 1941), but also from location to location within cartilages and bones (Hammett, 1925; Trotter, *et al.*, 1960; Harkness, 1961; Amprino and Marotti, 1964; Kao, *et al.*, 1962, 1965; Israel, 1967; Miller, *et al.*, 1969). These local differences in spontaneous aging may modify the local response to hormonal imbalances, and they may also be responsible for seemingly divergent experimental observations: whereas the growth promoting action of somatotropin (STH) predominated in the tibia, the effect of the hormone in the metacarpal manifested itself chiefly in stimulation of resorptive processes (Asling and Evans, 1956).

The skeletal effect of hormones is influenced by the physiologic age of the tissue at the time of hormone action. With the loss of growth potential occurring in aging epiphyseal cartilage, growth stimulation becomes more difficult (Silberberg and Silberberg, 1943; Asling and Evans, 1956; Everitt, 1959a). Conversely, processes that are active at the time the hormonal imbalance sets in, may still be stimulated. This applies to degeneration of cartilage and resorption of both cartilage and bone at the growth zones and to formation and resorption of membranous bone (Silberberg and Silberberg, 1939; Harris, *et al.*, 1972). Results of experiments of short duration may seem opposed to those of long range observation; this discrepancy need not be due to secondary involvement of other hormones; after brief treatment with STH the number of chondrocytes in a growing area may be increased, or the tissue may contain more water, or the matrix may be more basophilic than in untreated controls—signs that would make the cartilage appear younger than that of untreated animals. However, under continued treatment stimulation is followed by accelerated degeneration, calcification, ossification and resorption. The age-promoting influence of the hormone may thus be missed, unless observations are conducted over an adequate period of time. Conversely, following hypophysectomy, cartilage is relatively poor in cells as it is during certain phases of aging (Silberberg and Silberberg, 1960; Stockwell, 1967): this effect of hypopituitarism has therefore been interpreted as a sign of premature aging. However, epiphyseal cartilages of aged and hypopituitary animals respectively differ from one another (Fig. 12-1a, b), and, following insulin challenge, STH secretion of aged subjects equalled that of young adults (Cartlidge, *et al.*, 1970).

Additional problems of interpretation are created by the fact that a static histological picture present at a fixed point in time may not reflect just one, but one of several processes. Thus, osteosclerosis may be the result of overproduction or of decreased resorption of bone.

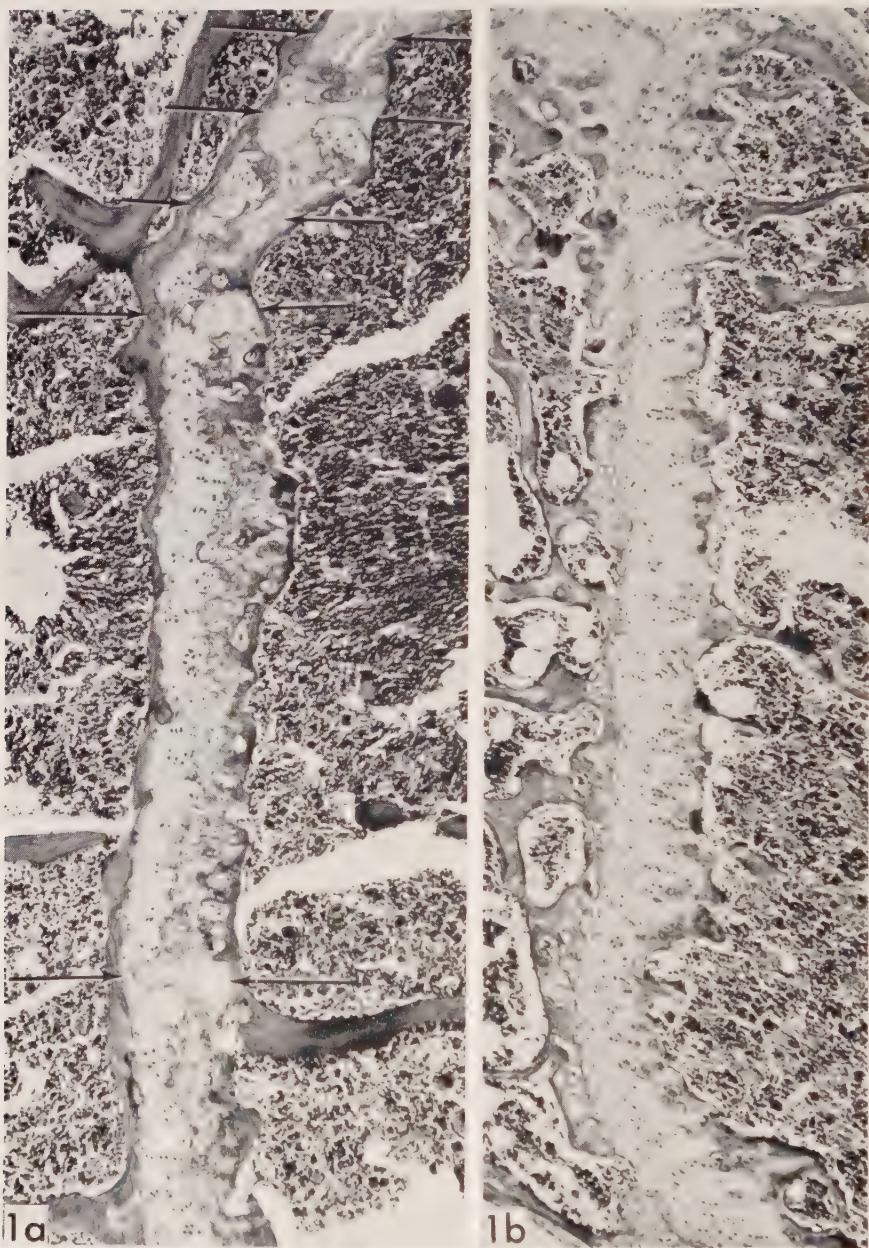


Figure 12-1. Growth zones at the upper end of the tibia of aged mice. Hematoxylin and eosin, 80 \times . Untreated 2-year-old animal with intact hypophysis. "Plugs" of necrotic, ossifying cartilage traverse the inactive growth zone (arrows). b. Untreated hypopituitary dwarf mouse, 41 months of age. The cartilage of the epiphyseal plate is poor in cells, shows diffuse hyalinization but no "plugs."

Decreased turnover rates have often been considered as a sign of aging (Child, 1915) and conditions associated with altered turnover rates have accordingly been thought to indicate accelerated or retarded aging respectively. However, skeletal metabolism as well as growth of cartilage and resorption of bone may remain at a high level or rise again in old age after having dropped during midlife (Silberberg and Silberberg, 1952; Sedlin, *et al.*, 1963; Stockwell, 1967). High turnover rates as such thus do not indicate youthfulness and may even cause premature aging (Pearl, 1928; Loeb, 1941; Silberberg and Silberberg, 1955; Everitt, 1959b; Silberberg and Lesker, 1971).

TABLE 12-I
PRINCIPAL AGING CHANGES IN MAMMALIAN HYALINE CARTILAGES
AND IN BONES

I. Before Maturity					
	Gross	Micro. and Submicro.	Biophys. and Biochem.	Kinetics	
Cartilage	----	No. cells No. mitoses Nucl./cyt. vol.	permeability Viscosity O ₂ consump./cell H ₂ O Mineral Ca, P, CO ₂ , SO ₄ Non-prot.N Collagen DNA	MPS CS-A/CS-C PPL/PPH CH/PPL Uronic acid Galactosamine Glucosamine KS Enzyme act./wt Enzyme act./cell	Time curve chondrocytes retarded Uptake of H ³ - thymidine C ¹⁴ -glycine S ³⁵
Bone		Cell size Intracell. glycogen PAS matrix Diam. collagen-fibrils, fibers			
Bone	Mass ↑	Woven bone No. bone form. foci No. bone resorp. foci Lamellar bone Crystal size PAS	Spec. gravity Density Mineral Ca, P, CO ₂		Exchange of Ca Uptake of P ³² , glycine-C ¹⁴
II. Past Maturity*					
Cartilage	Atrophy or hypertrophy Fibrillation Fraying Erosion	No. cells abs. or rel. Cell size Cell death Calcification Ossification Lipid KS in art. surfaces Metachromasia ↓	Elasticity Viscosity H ₂ O, SO ₄ Uronic acid Galactosamine Glucosamine PPL/PPH CH in PPL	Non-collagen prot. Glycoprotein Some glycolyt. enz. Some citr. acid cycle enz. UDPGDH Sulfatase Cathepsins Myokinase B-galactosidase B-glucuronidase	Art. cart.: Turnover of cells Uptake S ³⁵
Bone					
Bone	Mass (exc. skull & focal osteosclerosis) Width of cortex No. trabec. Diam. ind. bones	Dead bone No. low density osteons No. incomplete osteons No. resorp. foci Calcific occlusion Haversian canals	Mineral ↑ Density Glycoprotein Collagen solubility	Bone form. rate Collagen turnover Form. of resorp. foci retarded	

*Variable age at onset.

Explanation of symbols and abbreviations: ↑ increase, ↓ decrease, () slight change, MPS mucopolysaccharide, CH carbohydrate, CS chondroitin sulfate (A and C), KS Keratan sulfate, PPL light fraction of protein polysaccharide, PPH heavy fraction of protein polysaccharide, UDPGHDH Uridindiphospho-glucose-dehydrogenase, PAS periodic acid Schiff stain

Within the limited scope of this chapter skeletal aging cannot be discussed in detail; the principal aging changes observed in nasal, costal, epiphyseal or articular cartilage and in bone have therefore been summarized in Table 12-I. Only those aspects of the subject will presently be dealt with in which hormone action has been demonstrated or suspected. Hormonal effects will be reviewed as they modify skeletal aging at the molecular, ultramicroscopic, histologic, macroscopic or clinical levels.

EFFECT OF HYPOPHYSEAL HORMONES ON SKELETAL AGING AND DISEASE

The Action of Somatotropin (STH)

On the molecular and electron microscopic levels STH acts on the aggregation of skeletal collagen: in rats with intact hypophysis given STH and tracer doses of C^{14} -proline, total and specific activities of C^{14} -hydroxyproline of femur collagen were increased. If the isotope was administered some time prior to the injection of STH, urinary excretion of C^{14} -hydroxyproline was likewise increased (Aer, *et al.*, 1968). Assuming that some of the excess hydroxyproline was derived from femoral collagen, both synthesis and breakdown of bone were promoted. Injections of STH caused an increase in the diameter of collagen fibrils and accelerated formation of microscars in joints of growing mice with intact pituitaries (Silberberg, 1968). In dwarf mice, which owing to a genetic defect do not produce STH, collagen fibrils in the hip joint remain more delicate than in normal mice (Fig. 12-2). Dwarf animals also develop secondary thyroid and gonadal insufficiencies and these might contribute to the retardation of collagen aggregation. The presence of many thin collagen fibrils in bone of patients suffering from acromegaly, was interpreted as evidence of accelerated turnover of collagen (Stolpmann and Remagen, 1963); however, these findings are also reminiscent of physiologic age changes in the distribution of collagen fibrils of varying thickness (Schwarz and Pahlke, 1953).

Production of mucopolysaccharides as indicated by uptake of S^{35} by costal or epiphyseal cartilage was increased after administration of STH (Dziewiatkowski, 1964), while incorporation of the isotope was diminished following hypophysectomy (Murphy, *et al.*, 1956; Collins and Anilane, 1959). The half-lives of chondroitin-4-sulfate of cartilage and of keratan sulfate of nucleus pulposus were shortened in rabbits given STH; thus, both breakdown and synthesis of MPS were stimulated (De Sario, 1958; Davidson, *et al.*, 1962). Fibrils and ground substance are thus affected concordantly by the hormone.

In the femora of hypophysectomized rats, the ratio of amorphous bone

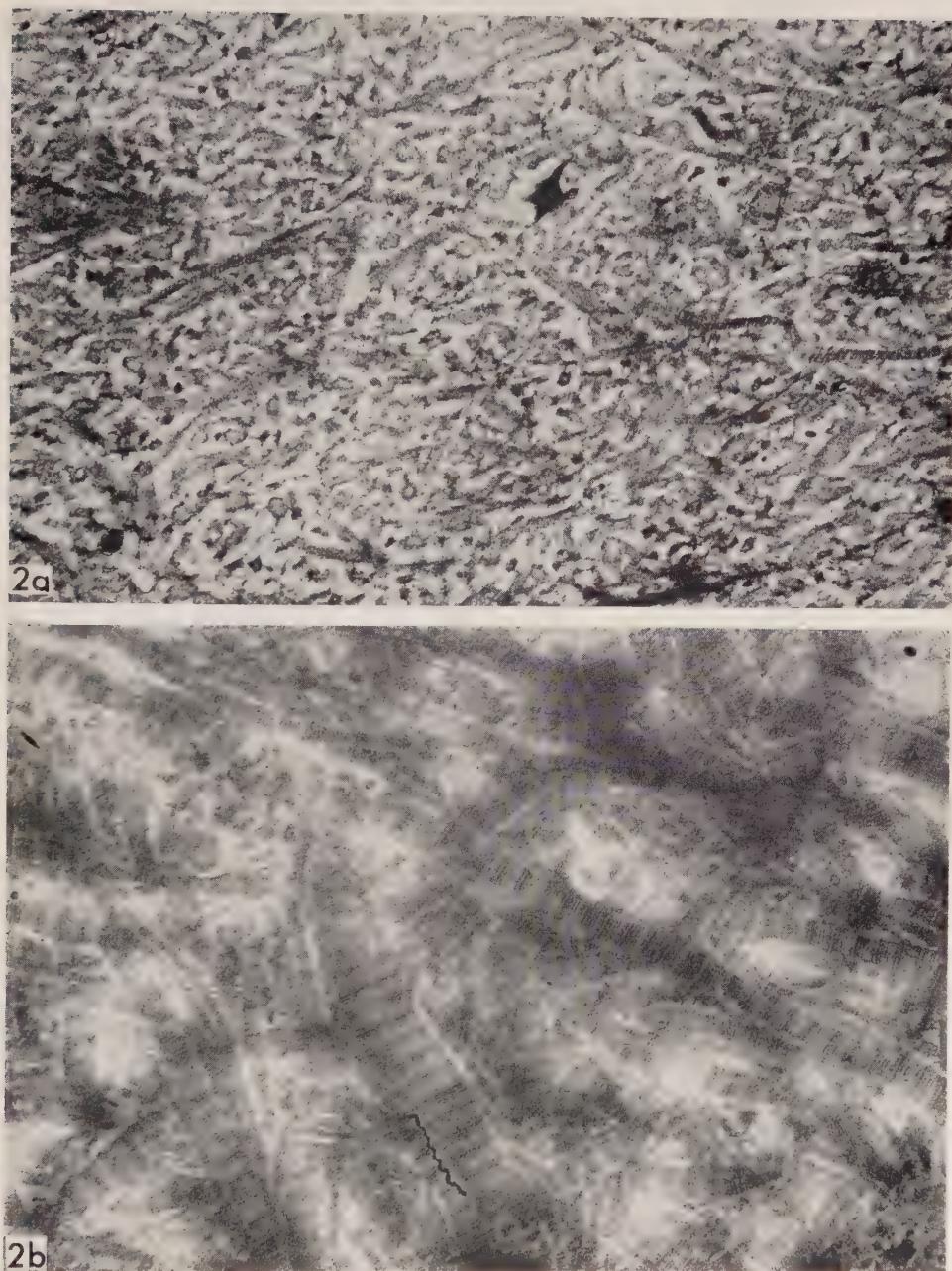


Figure 12-2. Electron micrographs of the deep zone of the cartilage of the femoral head of mice. Uranyl acetate and lead citrate stains, magnification, 67,000 \times . a. Hypopituitary dwarf mouse, 41 months old. Delicate, haphazardly arranged collagen fibrils. b. Twenty-five-month-old mouse with intact pituitary. Collagen fibrils, in haphazard arrangement, are several times thicker and are more densely packed than those of the dwarf. Black line on fibril in center is due to an artefact on the photographic plate.

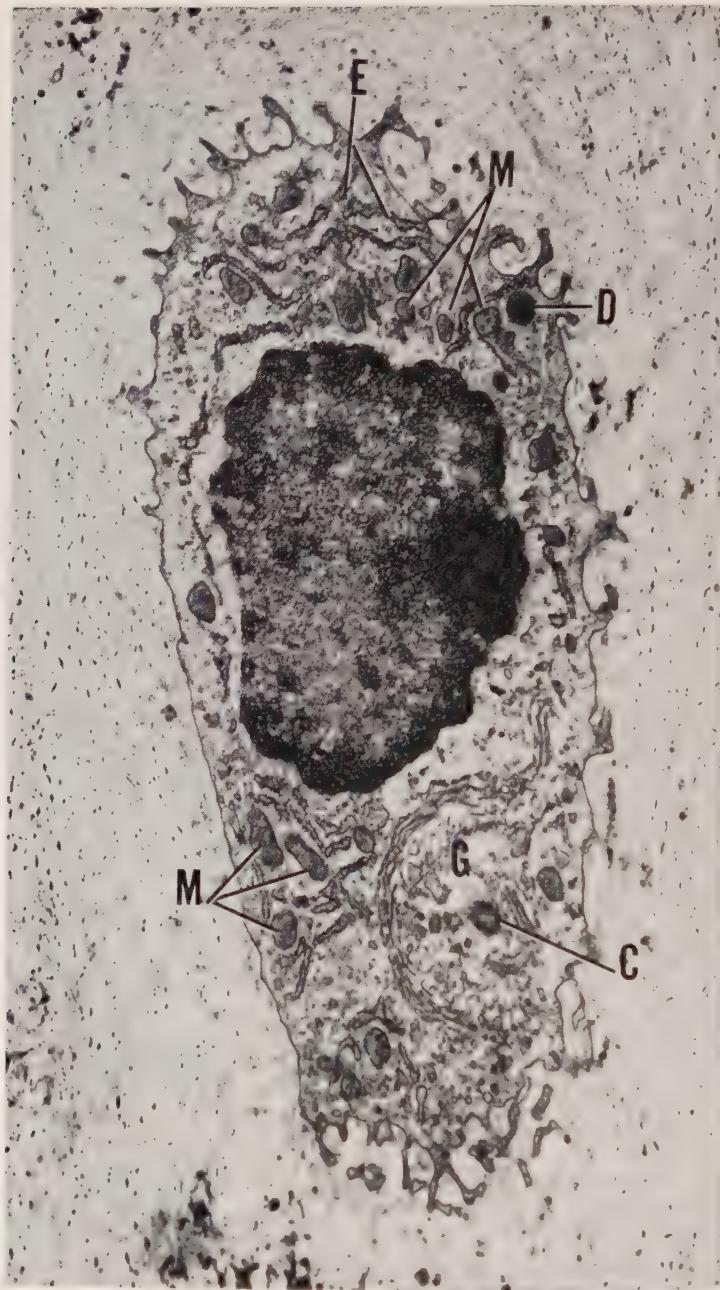


Figure 12-3. Forty-one-month-old dwarf mouse. Retarded cell: small amounts of endoplasmic reticulum, small mitochondria, a fairly large but delicate Golgi apparatus.

Figures 12-3-5. Midzonal chondrocytes of the articular cartilage of the hip joint of mice. Stained with uranyl acetate and lead citrate, magnification, 18,000 \times . Key to lettering: E—endoplasmic reticulum, G—Golgi apparatus, M—mitochondria, B—multivesicular body, Y—lysosome, D—dense body, C—centrosome, S—glycogen, L—lipid vacuole.



Figure 12-4. Forty-one-month-old hypopituitary dwarf mouse. This is one of the largest cells found. Endoplasmic reticulum extremely scanty and narrow, mitochondria small, Golgi apparatus delicate but quite large. The bizarre shaped nucleus and the lipid vacuoles in the cytoplasm are signs of aging.

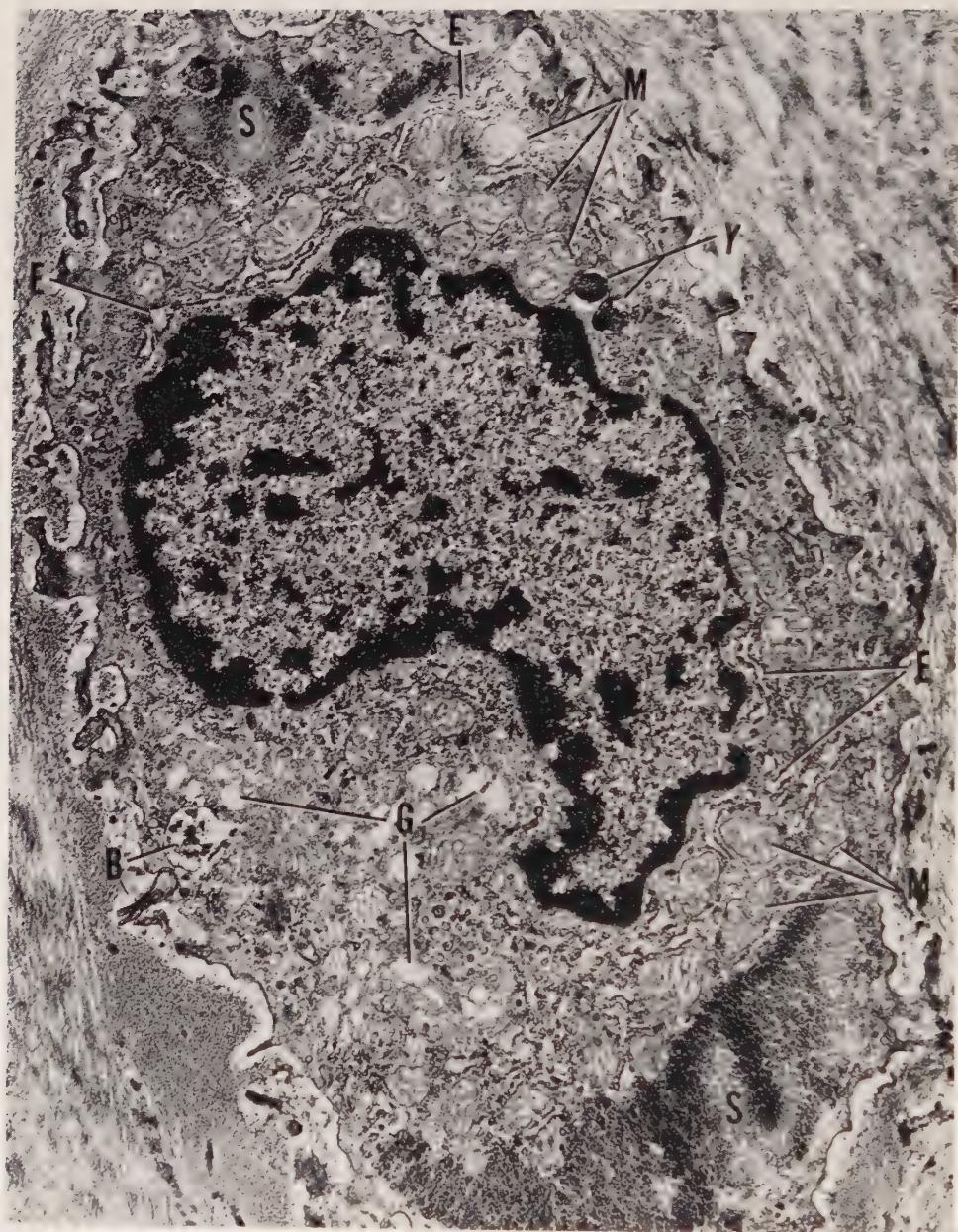


Figure 12-5. Normal mouse, 25 months of age. Compare with Figures 12-3 and 12-4. The irregular, drawn-out nucleus indicates that this is a comparatively old cell. The cytoplasm is crowded with organelles, and at both cell poles considerable amounts of glycogen are present.

mineral to crystalline apatite was increased (Posner, 1967). This finding is of interest in view of the age-linked physiologic change from small to larger crystal size (Hodge, 1949; Best, 1959; Dallemande, 1964). In hypophysectomized rats, chondrocytes of the intervertebral discs contained less glycogen than chondrocytes from normal rats (Racite and Bindoni, 1971), a sign of retarded aging of the cartilage cells.

The age promoting effect of STH manifests itself also in the ultrastructure of articular chondrocytes: after administration of the hormone: nuclear and organellar development, deposition of glycogen, degeneration and disintegration of cells are accelerated. These processes are retarded in hypopituitary dwarf mice; there are fewer dead cells and fewer microscars than in normal controls. Yet, in old dwarfs chondrocytes age to some extent, as indicated by the presence of nuclear and organellar regression or by accumulation of cytoplasmic lipid (Figs. 12-3, 12-4, 12-5).

The action of STH on the *tissue level* was demonstrated by early conventional histologic investigations (Silberberg and Silberberg, 1943). Not only proliferation of chondrocytes, but the entire sequelae of endochondral ossification, including hypertrophy and death of cells, production and calcification of matrix, resorption of cartilage, formation and resorption of primary spongiosa are accelerated following administration of crude or purified preparations of STH to guinea pigs, rats or mice (Silberberg, 1936, 1943; Asling and Evans, 1956). STH promoted both formation and resorption of callus in guinea pigs (Silberberg and Silberberg, 1943) and in combination with thyrotropin, aided in the healing of non-uniting fractures in human individuals (Koskinen, 1963). Contrariwise, low STH levels were found in the serum of a patient with delayed fracture union, both before and after insulin challenge (Misal, *et al.*, 1971). Differences in methods of investigation, species differences and differences in interpretation of histologic findings have caused the evolution of two opposing concepts of the action of STH on the skeleton. Since in guinea pigs and mice with intact hypophyses the growth promoting action of STH was associated with a conspicuous acceleration of aging, STH was considered to be not merely a "growth" hormone. Furthermore, some stimulation of growth was also seen after administration of thyroid hormone. It was therefore concluded that the skeletal response to stimulation by STH was less specific than originally assumed (Silberberg and Silberberg, 1943). On the basis of results obtained primarily in hypophysectomized, but also in nonhypophysectomized rats, only growth promoting action was originally attributed to STH, while stimulation of maturation was considered to be due to the action of thyroid hormone. Over the years, this extreme view was modified to allow for some maturation promoting action of STH and for a limited growth stimulating potential of TH (Asling and Evans,

1956). While aging is advanced more markedly in the presence of the thyroid gland, STH is effective in the absence of the latter (Smith, *et al.*, 1927; Laqueur, *et al.*, 1941; Silberberg and Silberberg, 1943; Asling and Evans, 1956).

Retardation of skeletal growth and aging was observed in human hypopituitarism before the analogous change in experimental animals was known (Erdheim, 1916; Smith and MacDowell, 1930; Mortimer, 1937). Thyroid function in such human dwarfs may be unimpaired (Roth, *et al.*, 1967); thyroid deficiency may thus not be implicated in the skeletal disorder accompanying hypopituitarism.

The response of the articular cartilage to STH illustrates the close relationship between skeletal growth and aging. Early stimulation of cell multiplication (Silberberg and Silberberg, 1949) and of synthesis of mucopolysaccharides (Dziewiatkowski, 1964) is quickly followed by degeneration of cells and matrix with erosion of the articular surface. The ensuing disorderly cycle of overgrowth and degeneration of cartilage, overgrowth of neighboring bone and of the soft articular tissues results in severe disfiguration of the joints and typical arthrosis in guinea pigs and mice (Silberberg, 1936, 1952) and in rats (Reinhardt and Li, 1953). By contrast, hypopituitary dwarf mice did not develop senile osteoarthritis of the knee joints, although they lived to a remarkably old age (Silberberg, 1972).

At the *clinical level* excess of STH manifests itself as gigantism or as acromegaly. The skeletal manifestations of acromegaly first analyzed by Arnold (1891), Dietrich (1909), and Erdheim (1931), have since been extensively studied and reviewed (Layani and Durupt, 1952). The disease is age-linked, inasmuch as it involves the skeleton only after the growth zones have closed. Skeletal alterations of acromegaly have two aspects, one of growth and another of aging; growth is elicited wherever a growth potential is left: at the bony shafts or at the epiphyses, including the articular surfaces; all such growth is associated with regression, and stimulated bone formation with increased resorption.

The aging effect of STH, in acromegaly, is reflected in the early appearance of osteoporosis (Layani and Durupt, 1952; Remagen, 1965; Villanueva, *et al.*, 1966) or of arthrosis (Arnold, 1891; Dietrich, 1909; Erdheim, 1931). Calciuria considered as causing osteoporosis (Fraser, 1962), may be secondary to the latter. The osteoporosis may also result from the intervention of thyroxine (Layani and Durupt, 1952) or of adrenocorticotropin (Casuccio, 1962). Histogenetically, the bone deficit may be due to decrease in the number or to functional exhaustion of osteoblasts or to accelerated resorption in the presence of a continued high level of bone formation. The former concept agrees with recent findings in senile osteoporosis (Kelin and Frost, 1964) and would explain the osteoporosis of

acromegaly in terms of an acceleration of the spontaneous disease of old age.

The role of hormones, other than STH, in osteoporosis of acromegaly is poorly understood. Notwithstanding the presence of skeletal disease, not all acromegalics suffer from hyperthyroidism or hypercorticism (Davis, 1940; Roth, *et al.*, 1967). However, if the levels of thyroxine, adrenocorticotropic or glucocorticoids are increased, their participation in the production of osteoporosis cannot be ruled out.

Acromegalic arthropathy is often considered as specific (Erdheim, 1931; Waine, *et al.*, 1947); however, more recent observations in experimental animals and in human acromegaly (Di Giulio and Masdena, 1960; Stolpmann and Remagen, 1963) do not support this view. Differences between acromegalic and osteoarthrotic joint lesions are quantitative, as far as bone and cartilage are concerned. The "diagnostic" features of acromegalic joint disease are due to associated changes in soft tissues and in extra-articular skeletal locations. The distinction between the two types of arthrosis is made still more uncertain by the finding of increased serum levels of STH in menopausal women suffering from arthrosis as compared to STH levels in menopausal women with intact joints (Franchimont, 1970).

Involvement of the vertebral column, acromegalic spondylosis, has likewise been described as specific, different from senile spondylosis (Layani and Durupt, 1952), and, in particular, unrelated to changes in the intervertebral discs. Yet, in experimental hyperpituitarism of rats, the annulus fibrosus of the intervertebral discs was conspicuously altered (Asling, *et al.*, 1955). Furthermore, accelerated turnover of keratan sulfate in the nucleus pulposus of rabbits given STH (Davidson, *et al.*, 1962), suggests involvement of the intervertebral discs in acromegalic spondylosis. The latter and spondylosis of old age might thus also have a histogenetic mechanism in common.

Little is known as to the effect of age on the course of acromegalic arthropathy. STH is less capable of promoting arthrosis in old than in young mice, and in aged acromegalic patients, proliferation of articular chondrocytes was less conspicuous than in a young adult acromegalic (Silberberg and Silberberg, 1952, 1964).

Osteitis Deformans Paget

The disorder, occurring most commonly in late adulthood, is characterized by increased resorption and necrosis of bone and by excessive but inadequate bone formation as well as fibrosis of the bone marrow. One or several bones may be affected.

Since the disease was found associated with hypercalcemia (Cassano and Tronchetti, 1939), abnormal glucose tolerance and a family history of

diabetes, obesity and tallness (Moehlig and Adler, 1937), a metabolic and probably hypophyseal origin was suggested. However, the absence of severe changes in serum calcium and phosphorus (Kay, *et al.*, 1934) and the patchy distribution of the bone lesions (Reifenstein and Albright, 1944) were taken as proof that the disease is an intrinsic skeletal disorder. Endocrine abnormalities may be secondary to bone lesions at the base of the skull impinging on the pituitary or on the hypothalamus. Localized Paget's disease is quite common in old individuals, and coexistence with other pathological conditions prevailing in such populations may strictly be on the basis of statistical probability. There is thus no adequate evidence favoring an endocrine origin of osteitis deformans.

Skeletal Involvement in Diabetes

Although diabetes is basically not a disorder of old age, a discussion of the associated skeletal lesions falls within the scope of this chapter, because of their similarity to and their overlap with skeletal diseases of old age proper. In childhood diabetes, bone and dental age are accelerated during the immediate prediabetic period; advanced bone age was also found in 6- to 16-year-old children of diabetic mothers (White, 1960). The co-existence of diabetes and skeletal disease has been known for some time (Boyd and Nelson, 1926), yet the problem was not investigated systematically until the recent past.

Two basic manifestations characterize the adult diabetic skeleton; both commonly occur together. The joint lesions are most often in the form of hypertrophic ossifying spondylosis, involving vertebral bodies and the anterior ligaments of the spine; however, there is also a high incidence of hypertrophic osteoarthritis of knees, hips, feet, hands, and sternoclavicular joints (Boulet and Mirouze, 1954; Silberberg, *et al.*, 1959; Waine, *et al.*, 1961; Ott, *et al.*, 1963; Hajkova, *et al.*, 1965; Julkunen, *et al.*, 1966; Forgaes, *et al.*, 1972). Grossly, the lesions resemble those of acromegaly, or of senile osteoarthritis, being, however, more severe and developing more rapidly than the latter. The hyperostotic overgrowth, especially at the spine, has been attributed to the action of STH, which fluctuates excessively in diabetes (Williams and Wood, 1965); accordingly, diabetic arthropathy may be pathogenetically related to that of acromegaly. Insulin failure may, however, be directly involved in the causation of the lesions. During insulin deficiency produced by alloxan, production of mucopolysaccharides is decreased (Schiller and Dorfman, 1957) and articular chondrocytes are less numerous than in untreated controls (Cicala, *et al.*, 1962). In mice made diabetic with anti-insulin serum, articular chondrocytes fail to develop or degenerate (Silberberg, 1968).

The second type of skeletal disease associated with diabetes manifests

itself as osteoporosis of vertebrae, pelvis, ribs, skull and long bones (Hernberg, 1952a; Klein, *et al.*, 1964; Recordier, *et al.*, 1966). The lesions occur spontaneously in human patients as well as in rats treated with alloxan (Hernberg, 1952b) and appear to result from a decrease in the number of osteoblasts or from osteoblastic failure to function properly. Synthesis of proteins and of mucopolysaccharides by osteoblasts is inadequate; calcium metabolism is unaltered, but phosphaturia is present in all patients (Recordier, *et al.*, 1966). Decreased urinary excretion of hydroxyproline indicates decreased turnover of collagen. The interaction of aging changes with the hormonal imbalance is highly complex; post-menopausal osteoporosis may be attenuated in diabetic females (Meema and Meema, 1967); to compound the problem, generalized osteoporosis is occasionally accompanied by focal osteosclerosis (Boulet and Mirouze, 1954), a finding observed occasionally also under normal conditions (Silberberg, 1971).

Thyrotropin

Skeletal changes occurring in the presence of increased levels of TSH are apparently due to stimulated thyroid activity. TSH decreased the half-life of chondroitin sulfate A in young cartilage, but did not change turnover rates of mucopolysaccharides in the nucleus pulposus of aging rabbits (Davidson, *et al.*, 1962). The hormone increased S³⁵ uptake in the growth zone (Collins, *et al.*, 1961), promoted fracture healing in rabbits (Eitel and Lexer, 1936) and supported the accelerating effect of STH on fracture repair in human individuals (Koskinen, 1963).

Earlier data dealing with the acceleration of all phases of skeletal growth and aging by thyroid hormone (Lit. cited: Silberberg and Silberberg, 1943) were supplemented by roentgenologic and histologic studies in rats (Asling and Evans, 1956) and more recently, by the use of modern methods: thyroxine increased uptake and accelerated disappearance of S³⁵, and thiouracil decreased these processes in the articular cartilage of rats (Dziewiatkowski, 1957), results reflecting the increase of both anabolism and catabolism caused by thyroid hormone. Electron microscopically aging and death of articular chondrocytes and calcification of the matrix were accelerated following treatment with thyroxine (Silberberg, 1968).

In the femora of rats, thyroxine lowered the ratio hexosamine/collagen (Garay, *et al.*, 1958) but also increased the breakdown of bone collagen; the more marked decrease of soluble collagen as compared to that of non-soluble collagen (Fink, 1967) and the resulting decrease in the ratio soluble/nonsoluble collagen are consistent with an age-promoting effect.

Osteoporosis may be associated with hyperthyroidism in man (Askanazy and Rutishauser, 1933; Williams and Morgan, 1940; Steyer, 1952), and in several species of birds during molt (Meister, 1951; Urist and Deutsch,

1960a). While both formation and resorption of bone are accelerated, predominating resorption ultimately causes the bone deficit (Askanazy and Rutishauser, 1933), and thus hastens skeletal aging. This acceleration of tissue aging which is associated with high turnover rates of mineral and organic components (Krane, *et al.*, 1956; Dziewiatkowski, 1964) illustrates the fallacy of using turnover rates as an index of aging.

Lactogenic Hormone

Prolactin slightly stimulated growth of cartilage in hypophysectomized male rats (Asling, *et al.*, 1955) and increased the uptake of S³⁵ in costal cartilage (Collins, *et al.*, 1961). Long range administration of prolactin failed to modify the course of osteoarthritis of mice (Silberberg and Silberberg, 1962b).

Gonadotropins (FSH, LH)

Neither follicle stimulating nor luteinizing hormone are known to exert direct skeletal effects. Aging changes produced in tracheal and epiphyseal cartilage of rats given FSH or LH were attributed to the intermediation of gonadal hormones (Tinacci, *et al.*, 1962).

The age-promoting effects of androgenic and estrogenic steroids have been reviewed recently (Silberberg and Silberberg, 1971). The problem of increased osteogenesis following estrogen treatment has been further studied with the help of radioautography. Estradiol stimulated transformation of fibroblasts into osteoblasts rather than production of osseous matrix (Holzer, 1965; Simmons, 1966). Following treatment with estradiol, uptake of S³⁵ was decreased in epiphyseal and costal cartilage and in metaphyseal bone of young rabbits, while the hydroxyproline content of these tissues was unchanged (Priest, *et al.*, 1960; Berntsen, 1968). The increased fibrillarity of articular cartilage of mice treated with estradiol (Silberberg, 1968) may therefore be due to increased concentration of collagen in an absolutely decreased amount of ground substance. In contradistinction to the effect of large doses, small doses of estradiol valerate stimulated both linear growth and resorption of bone of mice (Suzuki, 1958). In hens exogenous estrogen caused resorption of cortical bone, while producing hyperostosis in the medullary cavity (Urist and Deutsch, 1960a). Estradiol decreased the incidence of senile osteoarthritis of mice, the effect of the hormone changing with age at the beginning of the treatment (Silberberg and Silberberg, 1964, 1970).

Absence of sex hormones during early adolescence causes the skeleton to retain its youthful appearance (Silberberg and Silberberg, 1971). Gonadal insufficiency, starting in adulthood but prior to menopause, hastens the onset of a hormonal dysequilibrium characteristic of post-menopausal years; only then does gonadal deficiency promote aging. Accordingly, osteoporosis

of hypogonadism may be brought about in several ways: the skeleton may remain delicate owing to the absence of either the protein-anabolic effects of androgens or of the resorption-inhibiting action of estrogens. As the tendency to osteoporosis increases with advancing age, these eunuchoid skeletons reach a critical bone deficit more quickly than normal skeletons (Labhart and Courvoisier, 1950; Nowakowski and Gaderman, 1952). The second type of hypogonadal osteoporosis is related to the menopause and thus involves a previously normal skeleton. Menopausal osteoporosis is attributed to the absence of the resorption-inhibiting effect of estrogens (Albright, 1941). The role of the menopause in osteoporosis has been denied (Donaldson and Nassim, 1954). Changes caused by menopause and age, respectively, may overlap; but while an age effect may be mistaken for one caused by the menopause, a truly menopausal effect may also be masked by aging: in oophorectomized women, bone mass was decreased for 20 years, but equalled that of controls thereafter (Smith, 1967). After a lapse of 20 years, the aging effect had thus caught up with that of the menopause.

Adrenocorticotropin (ACTH)

ACTH acts on the skeleton through intermediation of adrenal glucocorticoids (Becks, *et al.*, 1944; Baker and Ingle, 1948). Differences between the effects of ACTH and glucocorticoids, respectively, are quantitative or in time required to become manifest; the response to ACTH may be related to the degree of cortical stimulation effected, and the latter varies in different species (Kass, *et al.*, 1954).

ACTH inhibited uptake of S^{35} (Collins, *et al.*, 1961); the hormone retarded growth and endochondral ossification in growth zones (Becks, *et al.*, 1944) and in the temporo-mandibular joint of growing rats (Ratcliff, 1965); in rabbits, growth of cartilage and osteogenesis were slowed down, while resorption of bone was unaffected during a brief period of observation (Storey, 1957). In kneejoints of aging mice, the incidence of osteoarthritis was decreased following administration of ACTH (Silberberg and Silberberg, 1964); this effect declined with increasing age of the animals at the beginning of treatment.

The response of skeletal tissues to adrenal glucocorticoids, particularly to cortisone, has been thoroughly investigated. At the molecular level, cortisone inhibited sulfation of polysaccharide in developing cartilage of chicken embryos (Whitehouse and Lash, 1961), and of costal cartilage of rats (Boström and Odeblad, 1953; Dziewiatkowski, 1964); the hormone decreased the uptake of C^{14} -glycine in both, articular chondrocytes of rabbits (Mankin and Conger, 1966), and femoral collagen of weanling, adult and old rats (Smith and Allison, 1965). The inadequate production of matrix retards skeletal aging in young and facilitates bone resorption in

old animals. Methylprednisolone raised both total glucosamine and the ratio glucosamine/galactosamine, in costal cartilage of rabbits (Kaplan and Fischer, 1964), suggesting an increase of keratan sulfate. While such a change would usually indicate aging (Kaplan and Meyer, 1959), it was, in view of the anti-anabolic effects of glucocorticoids, interpreted as due to retarded breakdown. However, a decreased ratio hexosamine/collagen (Sobel and Marmorston, 1954) and the deposition of lipid in the bone matrix of cortisone-treated rats (Sakai and Cruess, 1967) are likewise compatible with an aging effect.

At the histological level, glucocorticoids inhibit growth of cartilage in the growth zones of rats, rabbits and mice (Follis, 1951a; Sissons and Hadfield, 1955; Storey, 1957; Rosa and Vallario, 1962) and retard formation of lamellar bone (Frost, *et al.*, 1962). Resorption of cartilage and bone were decreased in rats (Follis, 1951a; Bernick and Ershoff, 1962) but not in rabbits (Sissons and Hadfield, 1955; Storey, 1957). Similarly, fracture healing was not delayed in rats (Key, *et al.*, 1952), but all phases of callus formation were retarded in rabbits (Sissons and Hadfield, 1951). Massive doses of cortisone accentuated the physiologic osteoporosis of laying hens, promoted osteoporosis in the capon and initiated it in the rooster (Urist and Deutsch, 1960b).

Clinically, hypercorticism of Cushing's disease is associated with osteoporosis (Mooser, 1921; Follis, 1951b). The bone deficit stems from both, increased resorption and impaired osteoblastic activity related to the anti-anabolic action of the excessive glucocorticoids present. In ribs of human individuals treated with cortisone the number of osteogenic foci was decreased by 50 percent, while the area of bone resorption was more than twice the normal; both bone formation and resorption proceeded about ten times faster than in untreated controls (Duncan, 1967).

Hyperostosis Frontalis Interna (Morgagni's Syndrome)

The diffuse or patchy thickening of the internal table of the frontal bones of the skull is considered an endocrine stigma, because of its association with hirsutism and obesity (Moore, 1936; Henschen, 1937; Pedersen, 1947). Clinically, the syndrome has features of adrenal hypercorticism; yet, hyperostosis frontalis interna occurs in association with acromegaly, toxic goiter, and diabetes (Junet, 1955). The incidence of the disorder increases with age, and is higher in women than in men. This and the co-existence with testicular atrophy has been considered as suggestive of an inhibitory role exerted by the male sex hormone on the bony overgrowth (Burkhardt, 1968). Hyperostosis frontalis interna occurred in 3 percent of a clinically normal population and in 7 to 12 percent of adipose patients, free from endocrine disorders; 24 percent of diabetic individuals 40 years

of age and older were affected as compared to 8 percent of nondiabetic subjects of comparable age. An etiologic role of pituitary basophilism was originally suspected (Henschen, 1937; Mellgren, 1945). Objections to classifying hyperostosis frontalis interna with endocrine disorders include its local character and the apparent paradoxon of hyperostosis of the skull, while hypercorticism causes osteoporosis elsewhere in the skeleton. The key to the situation may be provided by the similarly paradoxical behavior of the skull during physiologic aging: unlike the rest of the skeleton, which undergoes rarefaction, the skull often becomes osteosclerotic or thickened (Erdheim, 1935; Israel, 1967; Burkhardt, 1968). If the combination of hyperostosis and osteomas produced in the skull of mice by administration of estrogen or of gold thioglucose can be proved to be an analogue of the human disease, hyperostosis frontalis interna may have to be classified as a hypothalamic disorder (Rudali, 1968).

Diseases Subject to Modification by Various Hormones

Senile Osteoporosis

The discussion of etiology and pathogenesis of osteoporosis has produced a voluminous literature (Pommer, 1925; Schmorl and Junghanns, 1932; Schmidt, 1937; Albright, 1947; Follis, 1954; Fourman, 1955; Bartelheimer and Schmitt-Rhode, 1956; Whedon, 1957; Reifenstein, 1957; Jowsey, 1960; Nordin, 1960; Casuccio, 1962; Urist, *et al.*, 1962; Sans Sola, 1963; Heaney, 1965; Birkenhaeger-Frenkel, 1966; Garn, *et al.*, 1967; Rose, 1967; Newton-John and Morgan, 1970).

Osteoporosis manifests itself grossly in increased porosity of the bones and histologically, in rarefaction of the spongiosa, widening of the Haversian canals, minute foci of necrosis and microfractures of interstitial bone and paucity of osteoblasts. Osteoclasts and fibrosis of the bone marrow or osteoid tissue are not features of senile osteoporosis, which is thus basically different from osteitis fibrosis or osteomalacia. Senile osteoporosis is more common and more severe in females than in males, regardless of species (Silberberg and Silberberg, 1962a; Newton-John and Morgan, 1970). Histogenetically, the bone deficit has been attributed to absolutely decreased deposition, absolutely increased resorption, absolutely and relatively increased resorption in the presence of normal or decreased deposition of bone, to primary or secondary demineralization, or to a deficiency in the organic matrix of bone. Perhaps, several of these mechanisms are involved in the process.

As to its relation to skeletal aging, osteoporosis is variously considered as an exaggeration of physiological aging (Fourman, 1955; Casuccio, 1962; Newton-John and Morgan, 1970) or as unrelated to the latter (Kelly, *et al.*, 1959). Endocrine involvement has been postulated or denied, respectively.

The concept of aging as a continuous and overlapping shift in balance between anabolic and catabolic processes makes it conceptually difficult to consider senile osteoporosis as resulting from disease independent of the aging process. In the absence of either major biochemical or morphologic differences between osteoporotic and nonosteoporotic aging bone, the differences between the two types of bone appear quantitative (Casuccio, 1962; Frost, 1963; Urist, 1962, 1964). Minor biochemical and ultrastructural differences (Kelly, *et al.*, 1959; Dollerup, 1964; Birkenhäger, 1966) may be due to the presence of foci of necrosis in osteoporotic bone (Urist, 1964) and of unorganized, calcified connective tissue in aging bone (Jowsey, 1960). The basic disease may thus constitute a primary intrinsic skeletal disorder, most likely related to and continuous with the aging process.

Conclusive evidence speaking for or against the role of hormones in senile osteoporosis is difficult to establish. In accordance with the basic concept that endocrine secretions do not initiate physiologic processes, but merely alter the rates at which the latter operate, hormones should have only a modifying influence in the pathogenesis of osteoporosis. But since changes in rates are characteristic of the disease, hormonal influences may well play a role in its evolution. Furthermore, inasmuch as severe hormonal dysequilibria give rise to advanced osteoporosis in specific circumstances, the possibility that similar, though attenuated effects are produced by minor hormonal imbalances, cannot be ruled out.

More specifically, gonadal insufficiency or relative adrenal hypercorticism have been incriminated as the main promoting influences in senile osteoporosis (Albright, 1947; Reifenstein, 1957); yet, STH should not be overlooked as a potential contributing factor. The cumulative effects of minor, repeated hormonal disturbances may then, over a period of time, superimpose themselves either actively, as in the case of ACTH or STH, or permissively, as in gonadal deficiency—on the intrinsic skeletal aging process. Or, hormonal imbalances by exerting their effects prior to the appearance of the disease, may accentuate the physiologic aging tendency of the skeleton and thus accelerate the development of the critical bone deficit. The failure of hormone therapy in senile osteoporosis might be partly explained on this basis.

Senile Osteoarthritis (Senile osteoarthritis, senile degenerative joint disease)

This joint disease, one of the most widespread disorders in man and animals (Heine, 1926; Silberberg, 1943; Sokoloff, 1968), develops on the basis of aging changes in the articular cartilage (Weichselbaum, 1877; Bennett, *et al.*, 1942; Silberberg and Silberberg, 1952). The early lesions are char-

acterized by disturbances in the equilibrium between chondrocytes and matrix and between fibrils and ground substance, respectively. The role of hormones in the pathogenesis of the disease has been disregarded by earlier investigators (Lang, 1934). Recent research has shown, however, that senile osteoarthritis is a metabolic disorder of articular cartilage and subject to modification by systemic factors, such as STH, adrenal glucocorticoids, or sex hormones (Silberberg and Silberberg, 1952, 1964). Inasmuch as aging of connective tissues is controlled by hormones (Schiller and Dorfman, 1957), the susceptibility of articular cartilage to such influences is to be expected. As in the case of senile osteoporosis the role of hormones in human osteoarthritis is difficult to demonstrate. However, in mice, growth stimulating agents, of which STH is but one, promote the de-

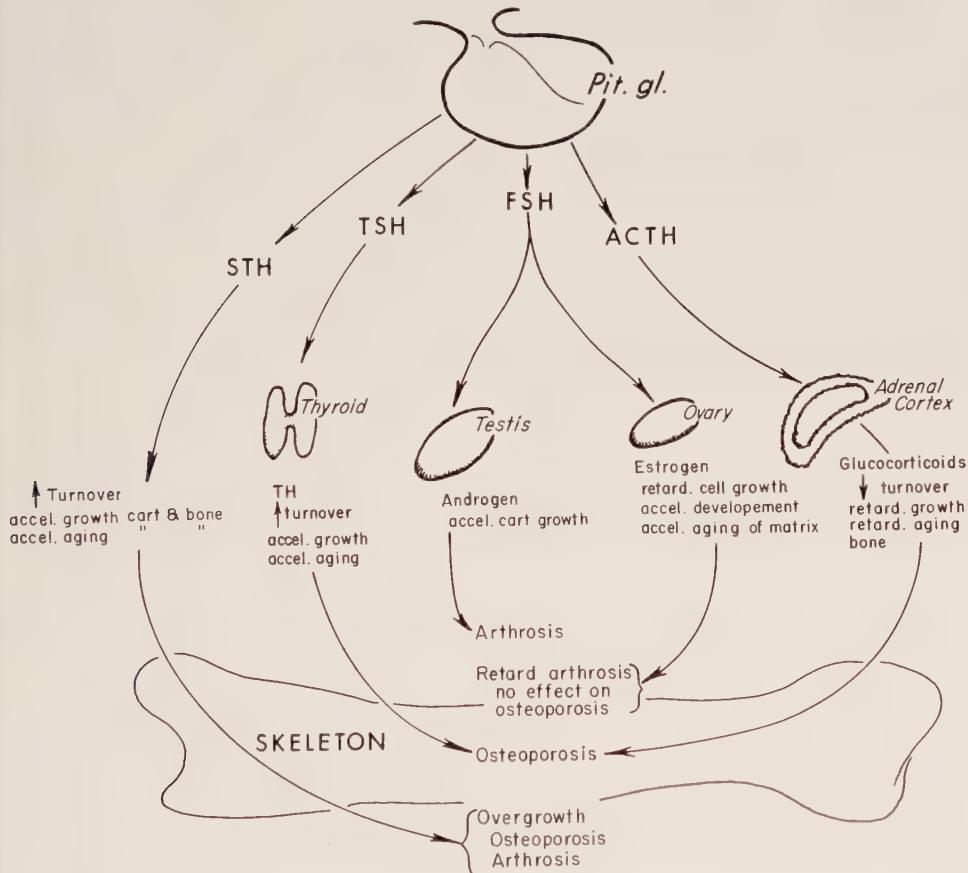


Figure 12-6. Diagram illustrating the endocrine pathways by which the pituitary gland may act on the skeleton. Only somatotropin (STH) acts directly on the skeleton without the intermediation of other endocrine glands. Other tropic hormones—TSH, FSH and ACTH—act through intermediation of their primary targets: Thyroid, gonads and adrenal cortex, respectively.

velopment of the lesions whereas the latter are attenuated by agents that retard growth. The modifying action of hormones may take place during early phases of articular aging, long before the actual onset of disease (Silberberg and Silberberg, 1964).

CONCLUSIONS

In the skeleton processes of growth and maintenance are inseparably linked to processes of aging. Growth may not be stimulated without simultaneous or subsequent acceleration of aging. The various phases of the skeletal time curve overlap and differ from one another only by the relative intensities with which the respective processes of growth or aging go on.

Hormones of the pituitary and of pituitary-dependent endocrine glands may intensify and accelerate or decrease and retard the biologic processes that determine skeletal aging (Fig. 12-6). The changes brought about by such hormone action are thus quantitative and not associated with changes in the basic qualities of cartilage or bone.

The susceptibility of the skeleton to stimulation generally decreases with advancing age; hormonal effects are modified by the physiologic age of the tissues and by local factors.

The pathogenesis of some skeletal disorders known to be or suspected of being endocrine in origin may be understood in terms of an intensification or acceleration of either the aging process or the spontaneous diseases of old age, respectively.

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CHAPTER 13

PITUITARY CONTROL OF CARDIOVASCULAR AND RENAL FUNCTION

KALMAN KOVACS and EVA HORVATH

SUMMARY

FOLLOWING HYPOPHYSECTOMY, characteristic morphologic, histochemical and biochemical alterations develop in the cardiovascular system and the kidneys, accompanied by changes in the renal excretory function; thus cardiac weight, cardiac output, cardiac work, oxygen consumption, blood and plasma volume, blood pressure, renal weight, renal blood flow and glomerular filtration rate are all decreased. No diuretic reaction can be elicited by oral water loads. Stimuli which lead to cardiac or renal hypertrophy under normal conditions fail to cause an enlargement of the heart or kidneys. Since the administration of a single pituitary hormone does not completely restore cardiovascular and renal performance it is justified to conclude that the lack of more than one pituitary hormone is responsible for the changes found in hypopituitary states. The cardiac alterations occurring in hypopituitarism are primarily influenced by STH and by TSH via thyroxine release. However, ACTH exerts the most marked effect on the kidneys through an increased secretion of glucocorticoids. The hormonal correlations have not been sufficiently explored at present and further studies are needed particularly as hormonal synergism or antagonism considerably modifies various responses. The pituitary influence on cardiovascular and renal functions at the molecular level is not yet known. From the study of this field important new advances can be expected.

INTRODUCTION

The pituitary gland plays an important role in regulating cardiac, vascular and renal functions. The first convincing evidence of this hypophyseal influence dates back to 1895 when Oliver and Schäfer discovered that pituitary extracts elevate blood pressure. A few years later Howell (1898) demonstrated that the posterior lobe is responsible for this effect. In 1913, von den Velden and, independently, Farini found that neurohy-

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pophyseal extracts decrease urinary output in patients suffering from diabetes insipidus. In 1918, von Hann published the results of her studies (based on careful observations of extensive autopsy material) of the role of the pituitary gland in water metabolism. She was the first to postulate that not only neurohypophyseal damage but also the preservation of the anterior lobe is necessary for the development of diabetes insipidus; if the adenohypophysis is also destroyed, polyuria and polydipsia are considerably reduced. Then, in 1932, Smith described procedures for hypophysectomy in rats. The application of this surgical technique facilitated the study of the consequence of hypophyseal insufficiency in experimental animals. Crude hypophyseal extracts and, later, purified pituitary hormones also became available. The administration of biologically active substances made it possible to investigate the effects of pituitary hormone overdosage. In the thirties, extensive research was started along these lines thus opening up new avenues in endocrinology.

At present, research concerning the pituitary gland is still of main interest. Clinicians learned that a number of pathologic conditions, including some vascular diseases such as diabetic retinopathy, malignant nephrosclerosis, Kimmelstiel-Wilson syndrome, etc., are greatly ameliorated by those procedures which destroy the pituitary gland. These findings have given impetus to the use of radioactive and surgical hypophysectomy in clinical medicine. Hypopituitarism is now also more frequently recognized due to the improvement of diagnostic methods. In addition, hypophyseal hormones are readily available to physicians in their everyday practice for the treatment of various diseases. A large number of human case histories has been collected thus enabling an evaluation of the role of the pituitary gland not only in experimental animals but also in man. It has also been established that the hormonal secretion of the pituitary gland is markedly controlled by the hypothalamus (Harris, 1955; Szentagothai, *et al.*, 1962; Reichlin, 1963, 1967, 1968; Guillemin, 1964; Harris and Donovan, 1966; Martini and Ganong, 1967). Great progress has been made in studies of the regulatory hypothalamo-hypophyseal pathways and in determining how the diencephalic centers and the median eminence influence hypophyseal hormone production and release. At present, the action of pituitary hormones is under investigation at a molecular level.

Although a great deal of information has been accumulated in this field, there are still many contradictory data, and several problems are not sufficiently understood. It would be impossible in this review to discuss all the problems and to give a complete list of references pertaining to pituitary control of cardiovascular and renal functions. Therefore, only a few aspects are briefly summarized.

It should also be emphasized that this review deals only with the physi-

ology of the cardiovascular and renal systems, and provides a framework for the data on aging relationships described in other chapters.

HYPOPHYSEAL INFLUENCES ON CARDIOVASCULAR SYSTEM

Cardiac Weight

McQueen-Williams and Thomson (1940) were the first to describe that the heart gradually atrophies in hypophysectomized rats. This finding was confirmed by several authors (Hajdu and Beznák, 1945; Beznák, 1959; Kovács, *et al.*, 1965). Beznák very thoroughly investigated the relationship between cardiac weight and hypophyseal function and found a more pronounced decrease in heart than in body weight (Beznák, 1954a, b, 1956, 1967). This was shown by the fall in the heart weight/body weight ratio.

It is well established that increased demands made on the heart lead to hypertrophy. However, cardiac enlargement does not develop in hypophysectomized rats. This was tested in several experimental models such as aortic constriction, renal hypertension caused by unilateral nephrectomy + DOC + NaCl, etc. With the notable exception of the results of Hall, *et al.* (1960, 1962), it was unequivocally found that cardiac hypertrophy occurred in normal but failed to develop in hypophysectomized rats (Beznák, 1954a, b, 1964; Girerd and Rassaert, 1962).

According to Beznák, cardiac atrophy is an adaptation to the decreased demand made on the heart. It may be due to hypophysectomy per se or to secondary atrophy of one or more endocrine glands. The question was which hormonal deficiency was responsible for cardiac atrophy. It was observed that lyophilized anterior pituitary powder inhibited the development of cardiac atrophy when given immediately after hypophysectomy and restored cardiac weight when treatment began a few weeks after the extirpation of the pituitary gland. In testing various hormones it was found that STH and thyroxine administered conjointly produced the best results in normalizing cardiac weight; they were also effective after aortic constriction, which causes marked hypertrophy. The hearts of hypophysectomized rats gained weight after STH treatment but remained below those of normal rats of similar size. Eartly and Leblond (1954) and other authors (de Grandpre and Raab, 1954; Scow, 1954) found that thyroxine given to hypophysectomized rats prevented a fall in the heart weight/body weight ratio; not only did it protect against cardiac atrophy but reversed it once it had developed. Thyroxine also potentiated the cardiac weight increasing effect of a saline suspension of cattle anterior pituitary tissue in the nonhypophysectomized rat (Selye, *et al.*, 1945).

Aortic constriction does not produce cardiac enlargement in hypophysectomized rats treated with ACTH, cortisone or DOCA (Beznák, 1954a, b). However, ACTH is not without effect as the heart weight after aortic

constriction was shown to be greater in hypophysectomized rats treated with ACTH than in untreated controls.

Cardiac Output

Hypophysectomy is followed by a rapid and considerable (as much as 50%) decrease in cardiac output as demonstrated in the rat (Beznák, 1959, 1960, 1963a, b, 1964; Kovács, *et al.*, 1965) and in dog (White, *et al.*, 1947a, b). In man, pituitary insufficiency (Stamler, *et al.*, 1949) or surgical removal of the gland (Bojs, *et al.*, 1962) also leads to the reduction of minute volume. Beznák (1959) showed that the decrease of cardiac output is not caused by the inability of the heart to do more work. She demonstrated by applying load to the heart that the response by hypophysectomized animals was almost as great as that observed in the controls (Beznák, 1959). The reduced cardiac output found shortly after hypophysectomy cannot be explained by the decreased blood volume which, in fact, is a subsequent development. Beznák (1959) assumed that the drop in cardiac output was induced possibly by the slower circulation and reduced venous return as well as by the decreased tone of veins and capillaries resulting from the lack of pituitary, thyroid and adrenal hormones. In support of this view it was found that the concomitant administration of STH and thyroxine completely normalized (Beznák, 1963a), while large doses of cortisone partly restored (Kovács, *et al.*, 1965) the diminished cardiac output of hypophysectomized rats. Thyroxine also caused a considerable rise in the minute volume of intact animals (Beznák, 1959).

Under physiologic conditions the hormones secreted by the posterior pituitary do not markedly affect cardiovascular parameters. Large doses of vasopressin (octapressin), however, lead to a considerable reduction of cardiac output in man (Pippig and Schmitt, 1966). According to Beznák (1959) infusion of pitressin is capable of increasing the minute volume in both intact and hypophysectomized rats.

Stroke Volume

The stroke volume showed no significant reduction in rats hypophysectomized several weeks prior to examination (Beznák, 1963b, Korecky, *et al.*, 1966). These values could be raised above normal with STH in both *in vivo* (Beznák, 1963a) and *in vitro* (Korecky, *et al.*, 1966) experiments thus pointing to the direct effect, not involving extracardiac factors, which STH exerts on the heart muscle (Korecky, *et al.*, 1966). On the other hand the effects of thyroxine *in vivo* differ from those *in vitro*. Conjoint treatment with STH and thyroxine markedly raises the diminished stroke volume (calculated on the weight of the left ventricle) above the normal value in hypophysectomized rats. This result was not achieved with the heart-lung

preparation which elicited values even lower than those produced by STH alone. Korecky, *et al.* (1966) supposed that certain substances (catecholamines, cardiotropin, etc.), not adequately present under *in vitro* conditions, were necessary for the whole thyroxine effect to manifest itself.

Cardiac Work

Beznák (1959, 1963b) found cardiac work (done by the left ventricle) greatly reduced in rats two weeks after the removal of the pituitary. She (1963b) also demonstrated that, during input-load or resistance-load the working capacity of the heart of hypophysectomized animals rises almost to the same extent as that of intact controls. Thyroxine given for 5 weeks is capable of normalizing the significantly decreased cardiac work under basal conditions even in rats hypophysectomized 2 to 3 months previously (Beznák, 1963b). However, the maximal work produced by such hearts during load remained below the values expected in intact animals. STH in itself only somewhat raises cardiac work in hypophysectomized rats. Nevertheless, in the case of simultaneous treatment with STH and thyroxine both the basal and maximal values of the cardiac work overtake those of the controls (Beznák, 1963a).

Vascular Resistance

Cardiac vascular resistance does not markedly change after hypophysectomy (Kovács, *et al.*, 1965) and the values for total peripheral resistance generally also remain within the normal range (Beznák, 1959, Kovács, *et al.*, 1965) or may show a slight tendency to increase (Beznák, 1963b). These data point out that the vascular resistance does not play an important role in modifying other cardiovascular parameters after removal of the pituitary.

Oxygen Consumption

An early consequence of the removal of the pituitary is a decrease in oxygen consumption, as demonstrated in rats (Beznák, 1959, 1963a, b, 1964) as well as in man (Falkheden, *et al.*, 1963). The rapid reduction of oxygen consumption can be attributed to the lack of the pituitary trophic hormones and of thyroxine.

Thyroxine given to hypophysectomized rats normalizes oxygen consumption and restores, at least under basal conditions, several hemodynamic parameters (Beznák, 1959, 1963b, 1964). Despite this, it is certain that the hypothyroid state is not the only factor responsible for diminished oxygen consumption. Falkheden, *et al.* (1962) found that low oxygen consumption and basal metabolic rate constantly occur also in those cases of hypophysectomized humans in whom the clinical signs of hypothyroidism are not apparent. They emphasize the lack of STH and its possible role in in-

ducing decreased O₂-consumption. This assumption is supported by the hypermetabolism found in patients suffering from acromegaly (Henneman, *et al.*, 1960). On the basis of the data of Falkheden, *et al.* (1962) it also seems obvious that the hemodynamic alterations developing after hypophysectomy are not primarily of cardiac origin.

Blood Volume, Plasma Volume

The removal of the hypophysis is regularly followed by the diminution of blood volume and plasma volume. The data of various authors are somewhat different concerning the onset of these changes. Enerbäck and Belin (1958) reported that both the absolute plasma volume and the ratio of plasma volume to body weight were significantly decreased in rats hypophysectomized only 6 to 9 days previously. On the other hand, Berlin, *et al.* (1950) and Bond and Leonard (1957) did not observe a reduction in the blood volume 12 to 14 days after the removal of the pituitary gland. From the data of Bond and Leonard (1957) it was also apparent that on the 12th postoperative day the plasma volume was slightly decreased presumably because of the contemporary mild elevation of the erythrocyte volume. After that time the blood volume, plasma volume and erythrocyte volume were equally reduced.

According to Enerbäck and Belin (1958) treatment with STH gives complete protection against the diminution of blood volume as well as of plasma volume /body weight ratio occurring in hypophysectomized rats. Bond and Leonard (1957), however, found that the removal of the pituitary leads to the establishment at a lower level of a new plasma volume / body weight ratio which could not be influenced by growth hormone in spite of the increase of plasma volume and body weight values. The most probable cause of the discrepancy found between the data of various authors is that the preparations of STH used differed in purity. Bond and Leonard (1957) did not test their preparations in this respect but the substance used by Enerbäck and Belin (1958) certainly also possessed corticotrophic and gonadotrophic activity. It is apparent from Beznák's (1956) comparative studies that the effects of various STH preparations on the cardiovascular parameters differ and depend presumably upon their contamination with other tropic hormones.

The decrease of total blood volume was also observed in man after hypophysectomy (Falkheden, *et al.*, 1963) and in pituitary insufficiency (Stamler, *et al.*, 1949). Analyzing the causes of this change Falkheden, *et al.* (1963) found the reduction of blood volume developing after hypophysectomy very similar to that observed in hypothyroidism. However, as was demonstrated by Falkheden, *et al.* (1963), this reduction in the blood volume also occurred in those patients in whom hypothyroidism did not

develop or to whom thyroxine was given. The authors assumed that the lack of STH played an important role in the decrease of blood volume since it remained low also in those patients to whom thyroxine, sex-hormones and cortisone were administered concomitantly.

Blood Pressure

Lyophilized anterior pituitary powder (LAP), STH (Beznák, 1954, 1956) as well as TSH, LTH and ACTH (Aoki, 1963; Hunter and Haist, 1965; Skelton, *et al.*, 1969) raises the blood pressure of intact rats over the normal values. Among the hormones of the peripheral endocrine glands, mainly thyroxine (Beznák, 1962; Aoki, 1963) and cortisone (Aoki, 1963) proved effective. Chronic treatment with ACTH could lead to development of hypertension also in man (Treadwell, *et al.*, 1964).

Amongst the posterior pituitary hormones, vasopressin (pitressin) given in large doses elevates the blood pressure both in man (Lipton, *et al.*, 1960) and in experimental animals (Beznák, 1959; Friedman and Paul, 1952); oxytocin has the opposite effect (Lipton, *et al.*, 1960).

Hypophysectomy leads to a significant fall of blood pressure in various experimental animals (Braun-Ménendez, 1932: dog; Page and Sweet, 1937: dog; Odgen, *et al.*, 1944: rat; Leathem and Drill, 1944: rat; Beznák, 1954a, b, 1956, 1959: rat; Lico, 1962: toad). A similar effect was also observed in human patients (Bojs, *et al.*, 1962).

According to Beznák's assumption (1959) the low blood pressure following hypophysectomy is the consequence of reduced cardiac output; thus blood pressure values could be raised or normalized by the increase of minute volume. Beznák demonstrated in 1954 that LAP and STH can restore the blood pressure of hypophysectomized rats, though, as was observed later (Beznák, 1963a) STH neither increases the cardiac output nor the blood pressure to any considerable extent. Naturally, the possibility arises that the STH preparations used in the earlier experiments were contaminated with other tropic hormones whose effect might add to that of STH.

The literary data concerning the effect of ACTH are contradictory. Beznák (1954b) stated that ACTH neither changed the blood pressure nor potentiated the effect of STH in hypophysectomized rats. Hunter and Haist (1965) and Aoki (1964), on the other hand, found it effective in raising blood pressure. According to the results of Hunter and Haist (1965) TSH and LH can also cause an elevation in blood pressure.

Administration of thyroxine normalizes the low blood pressure of hypophysectomized rats (Beznák, 1963a, b, 1964) and raises it above normal when given together with STH (Beznák, 1963a). Cortisone also elevates the blood pressure (Kovács, *et al.*, 1965).

Beznák (1954a, 1956, 1963b, 1964), in her careful studies, also demon-

strated that mechanical load imposed on the heart (constriction of the aorta), while inducing hypertension in intact animals, fails to do so in hypophysectomized rats. The mild blood pressure response following aortic constriction observed in hypophysectomized rats could be intensified with LAP, STH (Beznák, 1954a, 1956) and thyroxine (Beznák, 1964); nevertheless only the simultaneous administration of thyroxine and STH produced the blood pressure elevation expected in intact animals. ACTH and cortisone were not effective under similar experimental conditions (Beznák, 1954b). This finding renders questionable the observations of Anderson, *et al.* (1944) who reported the restoration of earlier existing renal hypertension of hypophysectomized rats by the administration of ACTH.

Neither do the experimental results of Beznák (1967) confirm the data of Hall, *et al.* (1960, 1962) who described the induction of mineralocorticoid hypertension in hypophysectomized rats. Beznák (1967) demonstrated that the blood pressure of unilaterally nephrectomized, DOC + NaCl treated and hypophysectomized animals approached the blood pressure values of similarly treated, nonhypophysectomized rats only when thyroxine and STH were also given after removal of the pituitary. Green, *et al.* (1952) as well as Girerd and Rassaert (1962) also failed to observe the signs of mineralocorticoid hypertension in optimally conditioned hypophysectomized rats.

HYPOPHYSEAL INFLUENCES ON THE KIDNEY

Renal Weight

Contrary to Collip's (1938) finding that the pituitary gland has no effect on the growth of the kidneys because compensatory renal hypertrophy occurred to the same extent in hypophysectomized as in normal rats, it is now unequivocally established that the anterior lobe plays a major role in regulating the size of the kidneys (Selye, 1941; Astarabadi, 1963a, b; Astarabadi and Essex, 1952). Several experiments have furnished convincing evidence that renal weight decreases significantly in hypophysectomized rats and dogs (McQueen-Williams and Thomson, 1940; Winternitz and Waters, 1940). Renal enlargement which develops after unilateral nephrectomy is also prevented by prior or simultaneous hypophysectomy (Astarabadi and Essex, 1953). It was also shown in rats that the remaining kidney which had already hypertrophied in response to the removal of the contralateral kidney, undergoes a considerable regression in size when hypophysectomy is performed two weeks after unilateral nephrectomy (Astarabadi, 1962). It seems that the behavior of the kidneys is similar to that of the adrenals, gonads and thyroids. These endocrine glands also atrophy after hypophysectomy and are no longer capable of compensatory hypertrophy after uni-

lateral or partial resection when the pituitary gland is previously or simultaneously removed.

Two main questions arise from these findings: which hormonal deficiency is responsible for the regression of the kidney and for the failure of compensatory hypertrophy after unilateral nephrectomy in hypophysectomized rats, and how do pituitary influences act upon the renal mass?

Treatment with crude hypophyseal extracts or lyophilized anterior pituitary powder produced compensatory hypertrophy in hypophysectomized rats and dogs and enhanced its occurrence to an extent which was greater than normal (Astarabadi and Essex, 1953). This experiment unquestionably proved the presence of a renotrophic principle in the adenohypophysis and furnished convincing evidence that this unidentified substance is required for compensatory renal hypertrophy. However, administration of ACTH, TSH, gonadotropic hormone or prolactin, was unable to completely normalize kidney weight (Selye, 1949; Astarabadi, 1963a, b). STH also appeared to be ineffective in maintaining the size of the kidneys when injected into hypophysectomized rats, although body growth continued at a normal rate (Astarabadi, 1961). Unlike hypophysectomy, bilateral adrenalectomy (Astarabadi, 1963a), gonadectomy, and thyroidectomy (McQueen-Williams and Thomson, 1940; Zeckwer, 1945; Selye 1949) failed to prevent compensatory renal hypertrophy in unilaterally nephrectomized rats and the administration of adrenal (Selye, 1941; Earle, *et al.*, 1953), gonadal or thyroid (White, *et al.*, 1947a) hormones did not restore the size of the kidneys in hypophysectomized animals. It was supposed that a specific renotrophic principle is secreted by the pituitary gland (Astarabadi and Essex, 1953); however, this suggestion has not yet been convincingly proved. The other possibility may be that the simultaneous action of several pituitary hormones is needed for the maintenance of the normal renal size (Selye, 1941).

The mechanism whereby the pituitary gland affects the growth of the kidneys is not clearly understood. It is known that hypophysectomy is followed by many alterations, such as a decrease of cardiac output, glomerular filtration rate, renal blood flow and metabolism, etc., and it seems reasonable to suppose that some of these changes are responsible for the development of renal atrophy. It has been suggested that the reduction in renal circulation is the most important factor in inducing a decrease in the size of the kidneys (White, *et al.*, 1949a, b; Earle, *et al.*, 1953). This postulate is, however, not supported by the fact that corticoids given in sufficient amounts are capable of restoring renal blood flow to normal level but, at the same time, do not beneficially influence renal atrophy (Kovács, *et al.*, 1965). Nor can lowered oxygen consumption, which is a characteristic

change in hypophysectomized rats, be the causative factor, since treatment with thyroxine does not prevent the decrease of the renal mass following removal of the pituitary gland (Astarabadi and Essex, 1953). Obviously, further experiments are needed to clarify the mechanism whereby the pituitary influences the size of the kidneys.

Morphologic Changes in the Kidneys

The restricted renal function observed after hypophysectomy is accompanied by mild but well defined morphologic alterations in the kidneys (Kovács, 1963; Horváth, *et al.*, 1964). The glomeruli do not show pathologic changes in spite of the conspicuous atrophy of the cortex which develops several weeks after hypophysectomy. However, the tubular lumina are moderately dilated and the flattened epithelium stains less intensely than normally. According to Cafruny, *et al.* (1957) this atrophy of the renal cortex can be inhibited by STH treatment.

In spite of the fact that a considerable atrophy of the renal cortex is apparent only a few weeks after hypophysectomy, Schwarz and Wolff (1966) could demonstrate ultrastructural changes in the epithelial cells of the convoluted part of the proximal tubuli and in the connecting capillaries as early as 5 days after removal of the pituitary. Compared to the normal picture, the electron density of the cytoplasm was increased in numerous epithelial cells (transitory and dark cells). Ultrastructural changes such as the widening of basal infoldings and widening or disappearance of the intervillous spaces in the brush border which were observed in these cells, might play a role in the diminution of tubular reabsorption. Besides the changes of tubular epithelium the capillaries also showed characteristic alterations, such as the broadening of the subendothelial space, the separation of the basement membranes of the cell and capillary, as well as the vacuolation of the capillary endothelium.

Schwarz and Wolff (1966) assumed that the lack of adrenocortical hormones is responsible for these observed alterations since similar changes could be demonstrated in the kidneys of adrenalectomized rats. Selye, *et al.* (1945) found that injections of pituitary extracts result in pronounced hypertrophy and hyperplasia of the tubular cells in nonhypophysectomized rats. The renotrophic effect was greatly enhanced by the simultaneous administration of thyroxine although the latter hormone possessed only moderate kidney-stimulating properties when given alone.

Biochemical and Histochemical Changes in the Kidneys

There are only a few references in the literature to the biochemical and histochemical alterations observed after hypophysectomy. Reid (1956)

found a diminution in the yield of the microsomal-fraction; however, the concentration of RNA-P increased in the kidneys of hypophysectomized rats. The majority of other biochemical data deals mainly with the behavior of alkaline phosphatase. Various authors (Dempsey, *et al.*, 1949; Matthies, *et al.*, 1949; Kochakian and Robertson, 1950) demonstrated decreased concentration and activity of this enzyme after hypophysectomy. According to Kochakian and Robertson (1950) the maximum decrease in activity was observable between 19 and 26 postoperative days after which an increasing tendency became manifest. The reduced activity could be restored with STH treatment (Matthies, *et al.*, 1949). Dempsey, *et al.* (1949) and Deimling, *et al.* (1966, 1967) described the decrease of the histochemically detectable alkaline phosphatase activity in the kidneys of hypophysectomized rats. Horváth, *et al.* (1964) found no significant changes in the intensity of the reaction when studying the kidneys of rats hypophysectomized several weeks previously, although here too the amount of enzyme should have diminished in view of the reduced size of the brush border.

One of the most characteristic histochemical alterations found in the kidneys of hypophysectomized rats is the strong decrease of the PAS-positivity of the brush border (Horváth, *et al.*, 1964). This change, as the ultrastructural alterations of this structure (Schwarz and Wolff, 1966), might be due to the lack of adrenocortical hormones. The fact that cortisone treatment normalizes both the diuretic reaction and the PAS-positivity of the brush border in hypophysectomized rats, supports this view (Kovács, 1963, Horváth, *et al.*, 1964).

Cafruny, *et al.* (1957) studied the concentration of protein-bound sulfhydryl groups in the kidneys of hypophysectomized rats and found that it decreased in the terminal portion of the proximal tubuli and in the collecting ducts. The amount of SH-groups could be elevated by thyroxine and STH in the proximal part of the nephron and was reduced by thyroxine in the distal tubuli. The hormones administered failed to modify the SH-content in the collecting ducts. Cafruny, *et al.* (1957) tended to conclude that different hormones have their own target cells in the nephron and the histochemical alterations observed reflect functional changes. It is difficult to decide whether this hypothesis is justified or not. By all means the depletion of the SH-content can hardly be responsible for the restricted tubular function since the diuretic reaction can be restored with cortisone in hypophysectomized rats without any change occurring in the diminished SH-content of the tubuli (Kovács, 1963; Horváth, *et al.*, 1964).

It was also demonstrated (Horváth, *et al.*, 1964) that hypophysectomy decreases the activity of succinic dehydrogenase and cytochrome oxidase in the collecting tubules. However, this change also does not seem to be close-

ly connected with impaired renal function because these enzymatic activities remain diminished even after normalization of the renal hemodynamic parameters.

Renal Circulation

Numerous investigations have proved convincingly that renal blood flow and glomerular filtration rate are significantly diminished in patients suffering from severe hypopituitarism caused by various organic diseases as well as in hypophysectomized man and experimental animals (Pickford and Ritchie, 1945; White, *et al.*, 1947a; Luft and Sjögren, 1950; Boss, *et al.*, 1952; Bojs, *et al.*, 1961, 1962; Falkheden, 1963; Kovács, 1963; Kovács, *et al.*, 1965; Isaacs, *et al.*, 1969). It is well known that changes in renal hemodynamics may lead to alterations in the excretory capacity of the kidney. Reduction of renal circulation results in a decrease of kidney function which is easily demonstrable by the failure of a diuretic reaction after an oral water load (Boss, *et al.*, 1952; Herrmann, 1955; Mertens, 1955; Sheehan, 1955; Robson and Lambie, 1959; Kovács, *et al.*, 1959; Kleeman, *et al.*, 1960).

The pituitary influences on renal hemodynamics has been extensively studied in the last thirty years. Numerous publications have dealt with the problem of identifying the pituitary hormones responsible for the maintenance of a normal renal circulation. Earlier reports emphasized the significance of STH and TSH and, indeed, many experiments supported this view (de Bodo, *et al.*, 1951; Davis, *et al.*, 1954; White, *et al.*, 1949b). At present, however, it seems to be fairly well-established that the ACTH-adrenocortical axis plays the most important role (Brunner, *et al.*, 1956b; Gale, *et al.*, 1961; Kovács, 1963). It has been shown that cortisone given in sufficient amounts to hypophysectomized rats restores renal blood flow and glomerular filtration rate to the normal level (Brunner, *et al.*, 1956b; Kovács, 1963; Kovács, *et al.*, 1965). Similar results have been obtained in other animal species and also in man (Burston and Garrod, 1952).

The cause of the reduction of the renal circulation in anterior pituitary insufficiency is not precisely known. There are several abnormalities in various cardiovascular parameters which develop following hypophysectomy and it is obvious that many of them are capable of influencing renal hemodynamics. Cardiac output, blood volume, and blood pressure are decreased after hypophysectomy. It is not clear, however, which of these alterations is the most important in affecting renal circulation. It seems very probable that all these changes play some role in causing a reduction of renal circulation.

In cases of diabetes insipidus, when only an isolated posterior pituitary failure exists, there is no significant decrease either in renal blood flow or

in the glomerular filtration rate (Leaf and Coggins, 1968). Under physiological conditions, the neurohypophyseal hormones do not markedly influence the circulatory parameters of the kidney. However, vasopressin, given in large amounts, causes striking renal vasoconstriction leading to a reduction of renal blood flow. When very large doses of vasopressin are administered, the constriction of the renal vessels is so extensive that ischemia develops, which subsequently results in tubular necrosis or, in more intense cases, in renal infarction (Byrom, 1937). It is interesting to note that estrogen pretreatment sensitizes the renal vessels to the constrictive action of vasopressin (Byrom, 1937, 1938; Kovács, *et al.*, 1964; Horváth and Kovacs, 1967). In estrogen-pretreated rats, smaller amounts of vasopressin are required to induce infarction in the renal cortex. Hypophysectomy protects the kidneys against the necrosis (Kovács, *et al.*, 1964). The preventive effect of prior hypophysectomy cannot be explained by abolition of the renal vasospasm, because the renal vessels are constricted in estrogen pretreated hypophysectomized rats following administration of vasopressin (László, *et al.*, 1966). It is more likely that the O₂ requirement of the kidneys is decreased in hypophysectomized animals and therefore the kidneys also survive a longer-lasting ischemia.

Renal Function

The main function of the kidneys, which consists of the excretion of water and electrolytes, is influenced by several processes. The role of the pituitary gland will only be discussed briefly here. It has to be emphasized, however, that in the regulation of urine production, the importance of extrahypophyseal factors such as renal circulation, neural stimuli etc., cannot be neglected.

The adenohypophyseal hormones with the exception of STH do not exert a direct influence on renal function; their effect is mediated via their target glands.

It is rather difficult to assess the effect of STH on water and electrolyte excretion. Several authors deal with this problem but their conclusions are contradictory. Differences in the experimental arrangements such as timing, doses, or animal species, may explain the divergent results. Another influencing factor might be the use in these experiments of various STH preparations and the possibility that sometimes impure substances were applied which, besides containing STH, were contaminated with other biological active substances capable of influencing water and electrolyte metabolism.

Despite reservations about the published results, it now seems fairly well established that STH does not play a major role in the regulation of water

and electrolyte excretion. Contrary to earlier findings (White, *et al.*, 1949a, b, 1951; de Bodo, *et al.*, 1951; Earle, *et al.*, 1951) it has been clearly shown that STH does not restore the diuretic reaction in hypophysectomized animals (Kovács, 1963). These animals retain water after an oral load.

ACTH plays a considerable role in the regulation of water and salt metabolism. Its effect is mediated via the hormones of the adrenal cortex. As is well known, two classes of corticoids which are produced by the adrenal cortex are important in affecting water and electrolyte excretion: the so-called mineralocorticoid and glucocorticoid hormones. These two classes have qualitatively and quantitatively separate actions and their secretions are also differently regulated. Aldosterone, the main representative of the mineralocorticoids, is produced in the zona glomerulosa. In the regulation of its secretion ACTH plays only a minor role. It is mainly stimulated by extrahypophyseal factors, principally by the renin-angiotensin system (Ganong and Mulrow, 1958; Genest, *et al.*, 1961; Davis, 1961; Davis, *et al.*, 1962; Gross, *et al.*, 1965). Cortisol, the most important glucocorticoid hormone (corticosterone in the rat) is produced in the inner cortical layers. Its secretion is almost entirely regulated by ACTH. Aldosterone increases cation exchange in the distal convoluted tubules resulting in a decreased urinary sodium and increased potassium excretion and, furthermore, expansion of plasma volume, hypopotassemia and hypernatremia (Bartter, 1956; Lütscher, 1956; Gross, *et al.*, 1965). Cortisol has a similar but milder effect on water and electrolyte metabolism. The situation is complicated by the fact that both mineralocorticoid and glucocorticoid hormones elevate glomerular filtration rate which also influences water and electrolyte excretion. Nevertheless, it is clear that in cases of diabetes insipidus polyuria and polydipsia strikingly decrease when adenohypophyseal deficiency develops or when both adrenal glands are removed (Chester-Jones, 1957; Kennedy and Crawford, 1961; László, *et al.*, 1962; László and Kovács, 1968). In hypophysectomized or bilaterally adrenalectomized rats, no diuretic reaction can be elicited by oral water loads (Brunner, *et al.*, 1956b; Kovács, 1963); it is also known that the administration of cortisone or even of ACTH is capable of restoring the diuretic reaction to a normal level in hypophysectomized rats (Kovács, 1963). Glucocorticoids also possess a diuretic action in normal rats, induce polyuria and polydipsia in hypophysectomized rats and enhance water excretion in pituitary stalk lesioned animals having diabetes insipidus (Kovács, 1963; László and Kovács, 1968).

The effect of TSH on water and electrolyte excretion is mediated via the secretion of the thyroid hormones. Thyroidectomy does not abolish completely the polyuria and polydipsia in animals suffering from diabetes insipidus. Nevertheless, it has been shown that in cases of diabetes insipidus

the urinary output is decreased when the thyroid gland is also removed (Biasotti, 1934; Ingram and Fisher, 1937; White, *et al.*, 1938; Heinbecker and White, 1939; Blotner and Cutler, 1941; Kovács, 1963). Administration of thyroxine causes some increase in water excretion but this effect is manifest only in cases of hypothyroidism. In cases of human hypothyroidism due to prior thyroidectomy or some organic disease of the thyroid gland leading to thyroid insufficiency, treatment with thyroxine ameliorates edema and water retention and induces polyuria and increased sodium excretion. Compared with the effect of corticoids, thyroid hormones are only of rather secondary importance. Thyroxine is not capable of restoring the diuretic reaction in hypophysectomized rats and it has no diuretic potency in normal rats loaded orally with water (Kovács, 1963).

Neither gonadotrophic hormones nor the gonadal steroids have any marked influence on water and electrolyte excretion. Several publications have appeared concerning this problem, but we will not discuss them here. It suffices to mention that progesterone and testosterone possess some anti-mineralocorticoid action by antagonizing the effect of aldosterone at the level of the renal tubules (Kagawa, 1958; Landau and Lugibihl, 1958; Kagawa and Jacobs, 1959). Gonadotrophic hormones, testosterone, estrogen hormones and progesterone given to hypophysectomized rats fail to restore the diuretic reaction after an oral water load (Dignam, *et al.*, 1956; Gillman and Gilbert, 1956; Kovács, 1963). Removal of the testes or the ovaries do not modify the polyuria and polydipsia in rats with diabetes insipidus (Kovács, 1963).

Regarding the two neurohypophyseal hormones the role of oxytocin will only be briefly mentioned. Some data show that under certain experimental conditions oxytocin can cause increased natriuresis and a rise in glomerular filtration rate (Croxatto, *et al.*, 1954; Berde and Cerletti, 1956; Brunner, *et al.*, 1956a, 1957; Ali, 1958; Brooks and Pickford, 1958; Horster, *et al.*, 1959; Barnafi, *et al.*, 1960; Wesson, 1961).

The other neurohypophyseal hormone, vasopressin (antidiuretic hormone) is of very great importance in water and salt homeostasis. It acts on the distal convoluted tubules and on the collecting ducts of the kidney by increasing water permeability of the lining epithelial cells (Berliner, *et al.*, 1958; Gottschalk, 1964; Orloff and Handler, 1964). When vasopressin is administered, water is conserved in the body; the quantity of urine decreases and its specific gravity increases. The retention of water leads to an expansion of the total body water compartment and to a dilution of extracellular and intercellular electrolytes. It was previously thought that vasopressin is synthesized in the pituicytes of the posterior lobe (Geiling and Oldham, 1937; Gersh, 1939). Contrary to this assumption, several observations support the theory put forward by Scharrer and Scharrer (1940, 1945,

1954) and by Bargmann (1954) that vasopressin is produced in the so-called hypophyseal nuclei of the hypothalamus (i.e. in the ganglion cells of the magnocellular portion of the paraventricular nucleus and mainly of the supraoptic nucleus) and transported via the supraoptico-hypophyseal tract, through the pituitary stalk, between the unmyelinated nerve fibres into the posterior lobe where it is stored; when there is a demand, it is released into circulation (Kovács, *et al.*, 1954; Sloper, 1958). An increase of extracellular osmolarity is the stimulus for vasopressin release (Verney, 1946, 1947). Osmoreceptors in the internal carotid artery are very susceptible to extracellular hypertonicity. A slight rise in the concentration of extracellular fluid caused by dehydration, water loss or sodium excess, leads to hypersecretion of vasopressin to conserve body water and restore water homeostasis. It has to be mentioned, however, that besides the osmotic impulses, other stimuli (nicotine, acetylcholine, various stressors, nervous influences, etc.) are capable of eliciting vasopressin release (Leaf and Coggins, 1968).

When vasopressin is not secreted in sufficient amounts, a well defined disorder of water metabolism, diabetes insipidus, characterized by polyuria and polydipsia develops. Deficiency of the anterior pituitary leads to a definite amelioration of the symptoms in man and also in experimental animals (von Hann, 1918; Mertens, 1955; Kovács, 1963). Total hypophysectomy does not result in diabetes insipidus (Brunner, *et al.*, 1956; Kovács, 1963). However, marked polyuria is induced in hypophysectomized man or animals by administering ACTH or cortisone (Brunner, *et al.*, 1956b; Kovács, 1963).

CONCLUSIONS

Disturbed secretion of the hypophyseal hormones or removal of the pituitary is followed by pronounced alterations in the function of the cardiovascular system and of the kidneys. Since the administration of a single pituitary hormone cannot completely restore cardiovascular and renal performance it is justified to conclude that more than one hypophyseal hormone is responsible for the changes found in hypopituitary states. The literature on the subject is very voluminous, sometimes contradictory and several problems still remain unsolved. The correlations between the actions of the individual hormones of the pituitary and that of the target glands have not yet been explored in detail. Our present knowledge permits no more than an oversimplification, so that a great deal of further work is needed to explore precisely the regulatory role of the pituitary gland in the control of cardiovascular and renal functions. The study of the hormonal interactions is very important because hormonal synergism or antagonism can markedly modify various responses. Research on the ac-

tion of pituitary hormones at the molecular level has only just started, and results greatly contributing to a better understanding of the underlying hormonal mechanisms can be anticipated.

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CHAPTER 14

CARDIOVASCULAR AGING AND THE PITUITARY

ARTHUR V. EVERITT

SUMMARY

WITH INCREASING AGE cardiac output falls, arteries become less "elastic," systolic blood pressure rises and the heart enlarges. The incidence and severity of atherosclerosis increases with age in man.

Pituitary and target gland hormones influence the aging of the cardiovascular system. In the hypophysectomized rat and in the hypopituitary patient a number of cardiovascular age changes are retarded, due to the lack of pituitary hormones. Both clinical and experimental studies show that atherosclerosis is promoted by ACTH (and cortisol) and growth hormone, and inhibited by thyroxine and estrogen.

INTRODUCTION

In the minds of young lovers the heart is the very symbol of life. To the physician treating the elderly patient this is also true in our present era, since diseases of the heart and blood vessels are the major reasons for loss of life in old age.

The functions of the cardiovascular system, like those of other organs, decline with advancing age (Strehler, 1959), except where disease has produced compensatory changes like cardiac hypertrophy and hypertension. The principal age changes in the cardiovascular system have been reviewed by Harris (1970), and the structural aspects surveyed by Lev and McMillan (1961) for the heart and by Abramson and Turman (1961) for the blood vessels.

This chapter will summarize present knowledge of cardiovascular aging in man and the laboratory rat, and attempt to assess the role of pituitary hormones in these changes.

CARDIAC AGING

Aging changes can be seen in the structure of the endocardium, valves, myocardium and the conduction system of the heart, as well as changes in function and the development of age-related pathology.

Gross Changes with Age

Heart size usually increases with age. Linzbach and Akuamoah-Boateng (1973) studied 7,112 human hearts from birth to 110 years and concluded

that heart weight increases up to the 9th decade of life, with a small reduction thereafter. These workers found that the increase in heart weight corresponds to the age-dependent rise in mean arterial blood pressure in both men and women aged between 30 and 80 years. However, in the absence of hypertension or clinical heart disease, heart size may remain unchanged between middle and old age or may even become smaller as a result of the reduced physical demands and activity in old age (Korenchevsky, 1961; Harris, 1970). In the rat, heart weight increases with age (Berg and Harmison, 1955; Everitt, 1958; Lee, *et al.*, 1972).

Structural age changes in the endocardium, valves, myocardium and conduction system have been described in detail by Lev and McMillan (1961). Hemodynamic stress causes thickening of the endocardium and valves of the human heart in old age (Harris, 1970).

Histological Changes with Age

Lev and McMillan (1961) have described the principal histological age changes in the heart. Associated with enlargement of the aging human heart is the greater size of muscle fibers and the increased amount of elastic tissue in the aged human heart (Korenchevsky, 1961). Sclerosis, fibrosis and calcification of mitral and aortic valves is commonly found in man (Harris, 1970). Thickening of the base of the aortic valve cusps is associated with the relatively high incidence of aortic systolic murmur developing with increasing age after 50 years (Bruns and van der Hauwaert, 1958). Collagen increases in the endocardium where hemodynamic stress causes thickening as it does in the left and right atria, papillary muscles and the apex of the left ventricle (Harris, 1970). In addition to deposition in specific regions, there is also a general increase in the collagen content of the left ventricle in the aging rat (Tomanek, *et al.*, 1972). Lipofuscin, an age pigment, accumulates in the myocardium in man (Strehler, *et al.*, 1959) and also in the rat (Reichel, 1968), and consequently the heart muscle becomes a deeper brown in old age.

Functional Changes with Age

In man, cardiac output as measured by the dye dilution technique decreases about 1 percent of the 50 year value per year between ages 20 and 90 years (Brandfonbrener, *et al.*, 1955). The reduction in cardiac output is the result of an agewise decrement in stroke volume per unit of body size, plus decreases in body size and heart rate (Brandfonbrener, *et al.*, 1955). Left ventricular work at rest declines with advancing age (0.5% per year), while the power of the heart (ratio of heart work per beat to systolic duration) shows a greater decline with age of 0.9 percent per year (Landowne, *et al.*, 1955).

There is a decrease in cardiac reserve with increasing age in man. From

the 6th decade there is a progressive reduction in the efficiency and range of adaptability of the heart to load, producing a latent cardiac insufficiency (Reindell, *et al.*, 1967), which in old age becomes manifest cardiac insufficiency. For example, the recovery of the heart from exercise is slower in old age (Norris, *et al.*, 1953). However, the existence of a physiological age insufficiency of the heart has been questioned by Linzbach and Akuamoa-Boateng (1973) because of the relatively high frequency of cardiac pathology in old age.

The electrocardiogram gives information about the aging heart, but the coexistence of cardiac disease in many elderly patients complicates the interpretation. One study (Fisch, *et al.*, 1957) of 347 patients over the age of 70 years with no overt clinical evidence of heart disease showed that 34 percent of electrocardiograms were abnormal. The commonest abnormalities are left ventricular hypertrophy, prolonged Q-T interval, low voltage, premature ventricular contractions and myocardial ischemia.

In the aged rat, the electrocardiogram shows increased incidence of arrhythmias, left axis deviation and prolongation of the PR and QRS intervals (Berg, 1955; Everitt, 1958; Jones, *et al.*, 1967). In anesthetized rats, heart rate decreases in old age (Berg, 1955; Everitt, 1957; Everitt, 1958; Lee, *et al.*, 1972) but in unanesthetized rats Rothbaum, *et al.* (1973) found a slight increase. In unanesthetized rats, the cardiac output and stroke volume decreased with age (Rothbaum, *et al.*, 1973) as in man. Cardiac performance (as measured by left ventricular work) in response to angiotensin II infusion was less in the 24-month-old rat than in the 6- and 12-month (Lee, *et al.*, 1972).

Age-Related Pathology

The most common diseases of the human heart in old age are disorders of cardiac rhythm, coronary heart disease and hypertensive heart disease (Harris, 1970). Aortic systolic murmurs have been recorded in 66 percent of patients over 70 years of age (Bruns and van der Hauwaert, 1958). Valvular disease may occur in old age without significant symptoms (Bedford and Caird, 1960). In the old rat the most frequently observed pathological changes are myocardial fibrosis, sclerosis of the coronary arteries and left ventricular hypertrophy (Wilens and Sproul, 1938).

VASCULAR AGING

The study of vascular aging in man is complicated by the lack of agreement on the role of atherosclerosis in the process. The rat, on the other hand, is virtually free of this disease and hence is a useful animal for investigating physiological aging of the vascular system.

The principal age changes in blood vessels have been reviewed by Abramson and Turman (1961) and Milch (1965).

Gross Changes with Age

The walls of human arteries progressively increase in thickness with age and become dilated (Abramson and Turman, 1961; Learoyd and Taylor, 1966). The peripheral arteries are more prone to thickening of the wall and the proximal arteries to dilatation (Learoyd and Taylor, 1966). In advanced old age, there may be a diffuse aneurysmal dilatation of the aorta, called senile ectasia, which may eventually rupture (Ruffin, *et al.*, 1941).

The muscular arteries of the head, neck and extremities become more prominent and tortuous in old age, due to the increased collagen content; the process is facilitated by the loss of surrounding subcutaneous fat (Harris, 1970). There is also gross medial calcification of the arteries of the extremity in old age as seen by X-ray (Lansbury and Brown, 1934).

Histological Changes with Age

The basic components of the arterial wall are collagen, elastin, smooth muscle and ground substance.

The elastic properties of arteries are determined principally by collagen and elastin, which together constitute over one-half of the dry weight of the arterial wall (Harkness, *et al.*, 1957). Both collagen and elastin accumulate in aging arteries (Milch, 1965; Wolinsky, 1972a) and there appears to be a greater increase in collagen than elastin for rat aorta (Wolinsky, 1971 and 1972a), although it may not be true for human arteries (Cleary, 1963). The loss of "elasticity" or distensibility of aging arteries has been attributed to changes in elastic fibers which fragment, become calcified and thickened (Hass, 1943; Roach and Burton, 1959), and also to the loss of smooth muscle and the increase in collagen (Milch, 1965; Gow, 1972).

Diffuse intimal thickening of arteries appears to be an aging phenomenon peculiar to man. It is due principally to an increase in subendothelial collagen and elastin in approximately equal proportions (Guard and Bhende, 1953). This diffuse intimal thickening of the aorta during the first three decades is regarded by Movat, *et al.* (1958) as physiological aging. Intimal thickening of the small muscular arteries, including the coronary arteries, narrows the lumen and so reduces the blood supply.

In the media of the aorta there is degeneration of the elastic laminae, which are replaced by collagen and become calcified (Guard and Bhende, 1953). Calcification occurs in the media of musculo-elastic arteries beginning in the left coronary artery in the second decade and the aorta in the third decade (Lansing, *et al.*, 1948).

In the old female rat, Cliff (1970) found thickening of the tunica media with a reduction in cellularity. In the intima of old rats Cliff observed a diffuse thickening in the absence of extracellular lipid, and a remarkably active endothelium both in young and old rats (Gerrity and Cliff, 1972).

Functional Changes with Age

The "elasticity" (distensibility) of medium and large arteries decreases with age (Hallock and Benson, 1937; Roach and Burton, 1959; Bader, 1963 and 1967; Nakashima and Tanikawa, 1971 and Fig. 14-1), and in consequence the pulse wave velocity increases (Hallock, 1934; Wezler, 1935; Schimmler, 1965). In the arterial tree of young human subjects (Learoyd and Taylor, 1966) as in other mammals (Taylor, 1964) wall stiffness in-

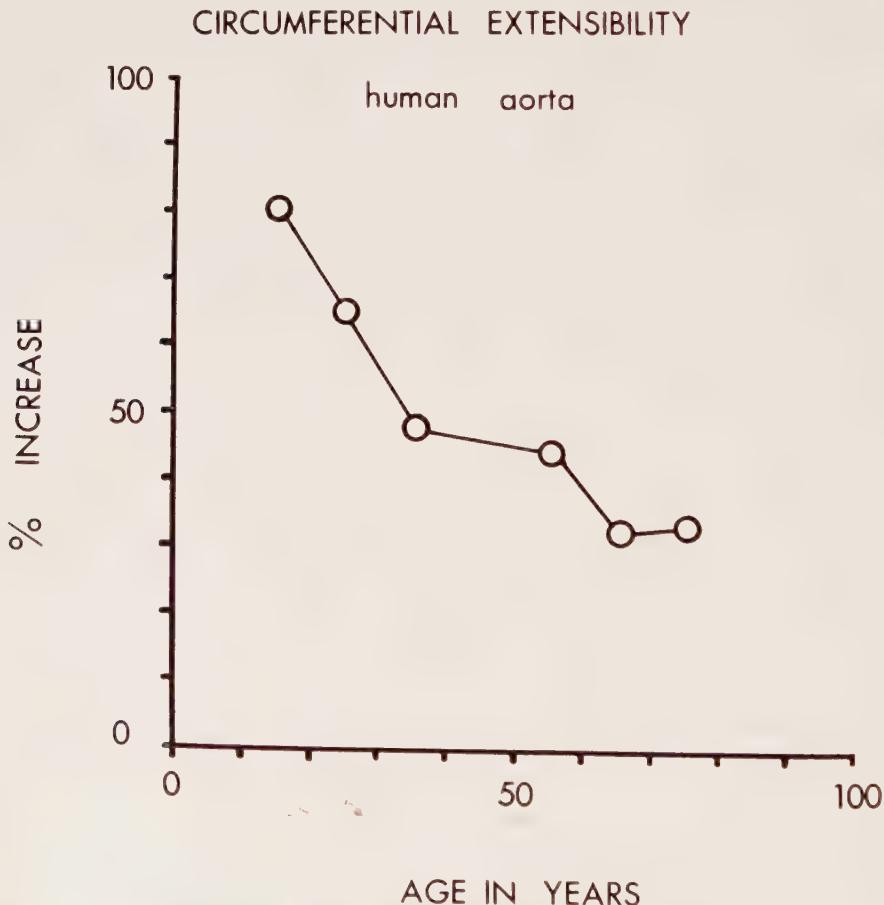


Figure 14-1. The decline with age in the circumferential extensibility of the abdominal aorta of Japanese subjects under a tension of 10^6 dynes cm^{-2} (from the data of Nakashima and Tanikawa, 1971).

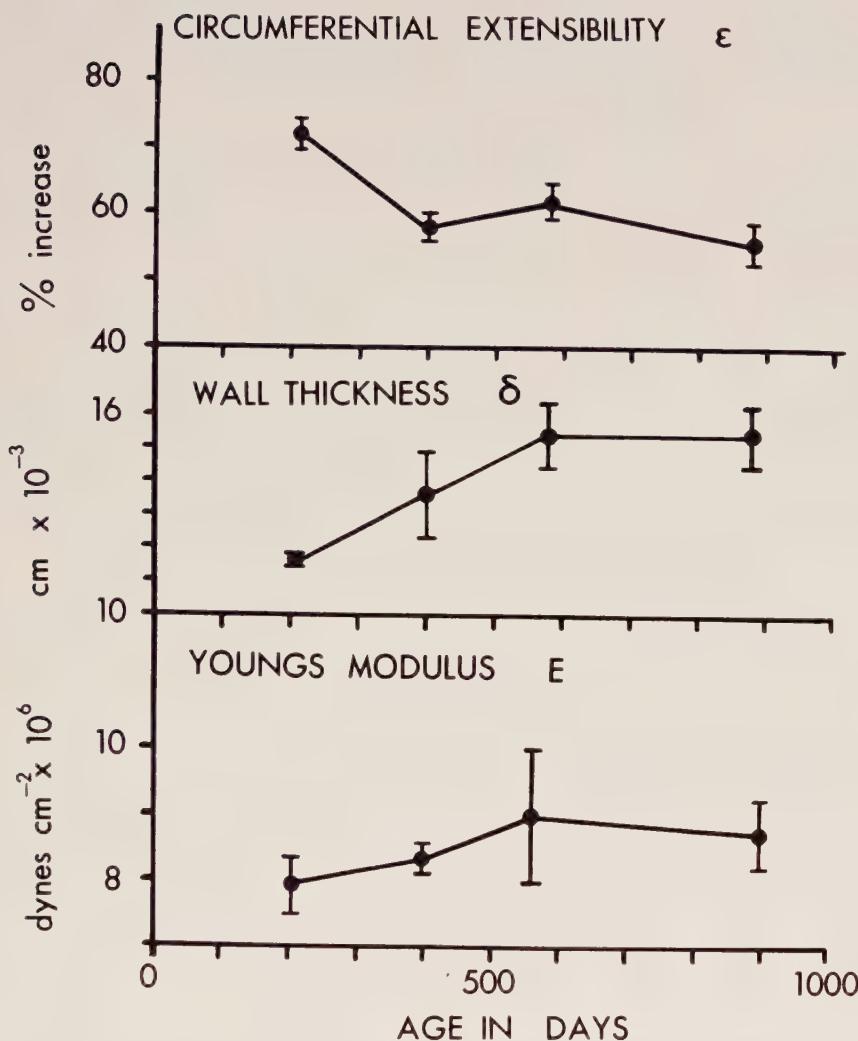


Figure 14-2. The effect of age on mechanical properties of rings of excised abdominal aorta (3 mm wide) from the male Wistar rat. Circumferential extensibility (ϵ), wall thickness (δ) and Youngs modulus (E) are plotted as means \pm S.E.M. for 5 rats at each age. Youngs moduli were calculated from the ratio of the change in stress ($d\sigma$) to the change in strain ($d\epsilon$). The change in stress was calculated from the slope of the descending limb of the load-circumference curve, the circumference (C) and the volume of the wall. Youngs moduli were compared at a wall stress corresponding to a physiological distending pressure (P) of 100 mm Hg, the stress (σ) being calculated using the expression $= \frac{PC}{2\pi\delta}$ (Data were kindly supplied by Dr. Barry Gow and Miss Denise Edwards).

creases towards the periphery, but in old subjects the opposite trend was found in the dynamic elastic modulus (Learoyd and Taylor, 1966).

The aorta of the rat, unlike that of man, shows only minimal changes in dynamic elastic properties with age (Fig. 14-2 and Band *et al.*, 1972). In our laboratory the extensibility of excised aortic rings of abdominal aorta was found to undergo a small reduction between youth (200 days) and middle age (400 days) probably due to the increase in wall thickness (Fig. 14-2). There was, however, no significant change in the stiffness of the aortic wall, as measured by Youngs modulus (Fig. 14-2).

Systolic blood pressure rises with age in apparently healthy subjects without known heart disease (Russek, *et al.*, 1946; Master, *et al.*, 1957; Miall and Lovell, 1967; Colandrea, *et al.*, 1970). This is thought to be due mainly to the stiffening and loss of distensibility of large arteries. There may also be age changes in vasopressor control (Gribbin, *et al.*, 1971) due to arterial wall changes affecting baroreceptor sensitivity, leading to peripheral resetting of the blood pressure control system (see Korner, 1971). Venous pressure does not appear to change with age in the recumbent subject (Ochsner, *et al.*, 1951).

The peripheral resistance in healthy human subjects increases with age about 1 percent per year between ages 20 and 90 years (Landowne, *et al.*, 1955). In the absence of arteriosclerosis the cerebral, coronary and skeletal circulations are minimally affected by age, but the splanchnic, renal and finger circulations are greatly reduced (Bender, 1965).

The velocity of blood flow decreases with age. The arm to tongue circulation time using magnesium sulphate increases with age about 1 second per decade in both sexes (Williams, *et al.*, 1971). The increased circulation time is due to the decrease in cardiac output and the increase in peripheral resistance with age. Blood volume, another factor in circulation time, does not change significantly with age in man (Yiengst and Shock, 1962).

In the rat under anesthesia, mean arterial blood pressure increases in old age (Lee, *et al.*, 1972), and the incidence of systolic hypertension (pressure greater than 140 mm Hg) rises with age (Medoff and Bongiovanni, 1945; Berg and Harmison, 1955). However, in the unanesthetized rat, Rothbaum, *et al.* (1973) failed to demonstrate any significant difference with age in systolic blood pressure. Aortic blood flow in anesthetized rats is lower at 24 months than at 12 months (Shreiner, *et al.*, 1969; Lee, *et al.*, 1972).

Age-Related Pathology

Atherosclerosis is the major arterial disease of old age in man and is the chief hazard to life. This disease causes focal thickening of the intima of

the large (elastic) and medium (muscular) size arteries. There is a focal accumulation of lipids, complex carbohydrates, blood, fibrous tissue and calcium. Intimal thickening narrows the lumen of the artery and so obstructs blood flow, producing ischemic injury to the heart, kidneys and brain.

In the rat, spontaneous atheromatous lesions are rarely seen (Humphreys, 1957), although spontaneous arteriosclerosis is found in the repeatedly bred rat (Wexler, 1964). Arteriosclerosis refers to intimal thickening of the artery in the absence of lipid deposition.

Conclusion

The principal age changes in the cardiovascular system are a fall in cardiac output and a rise in peripheral resistance. With increasing age, arteries become less distensible, systolic blood pressure rises and the heart enlarges. Atherosclerosis is the major disease of the cardiovascular system in man.

PITUITARY FUNCTION AND CARDIOVASCULAR AGING

There is little doubt that the hormones of the pituitary and its target glands influence the course of aging in the cardiovascular system.

Effect of Hypophysectomy

Studies on hypophysectomized animals show that most parameters of cardiovascular function are depressed (Kovacs and Horvath, Chap. 13). Decreases are seen in cardiac weight, cardiac output, cardiac work, blood volume and blood pressure. Because of the reduced load on the cardiovascular system in hypopituitarism a slower rate of aging might be expected.

Cardiac enlargement occurs in the old rat (Fig. 14-3) as in man. This age change develops at a significantly slower rate in the rat hypophysectomized early in life at 50 days (Fig. 14-3). Clearly a pituitary factor is accelerating this age change, but from our studies it does not appear to be either growth hormone or corticosteroid (cortisone) in physiological amounts (Fig. 14-3). However, the work of Beznák (1963) indicates that both growth hormone and thyroxine are necessary for experimental cardiac hypertrophy in the young rat (see Kovacs and Horvath, Chap. 13). Hypophysectomy also retards or may even abolish the small decline in heart rate (measured under pentobarbital anesthesia) between ages 150 and 800 days (Everitt, Chap. 4, Fig. 4-6). These two studies suggest that hypophysectomy at an early age may retard certain age changes in the heart of the rat.

The increase in aortic wall thickness and the small decrease in aortic distensibility with age (Fig. 14-2) are not found if rats are hypophysectomized at an early age. This probably occurs because there is no change in the ratio of wall thickness to vessel radius. It also suggests that there are no gross changes with age in the physical properties of wall components in hypophysectomized rats.

The activity of benzylamine oxidase in rat aorta is markedly diminished by hypophysectomy and partly restored by growth hormone and cortisone

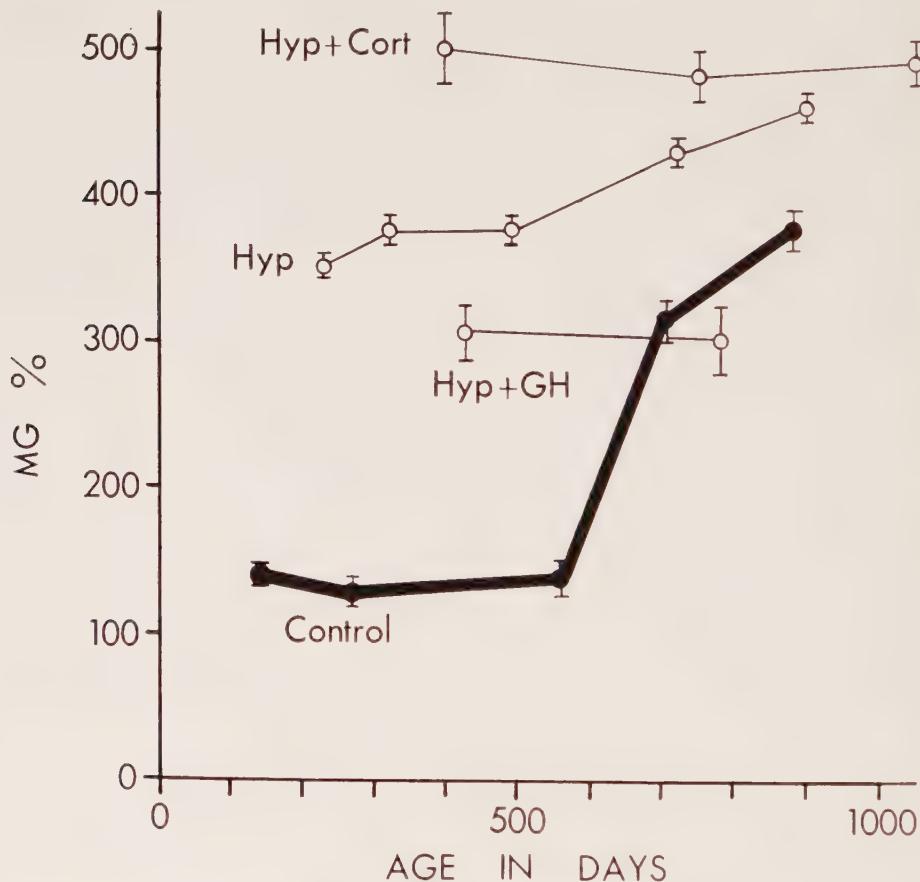


Figure 14-3. Heart weight (mg/100g of body weight as mean \pm S.E.) increases markedly with age in the old intact rat (Control) shown in the thick line, but the increase is only minimal in the hypophysectomized rat (Hyp). Cardiac hypertrophy in the old rat is not restored by long-term treatment of the hypophysectomized rat with physiological doses of either growth hormone (Hyp + GH) or corticosteroid (Hyp + Cort). Heart weight depends on body weight and endocrine factors; the body weight factor is eliminated in this graph.

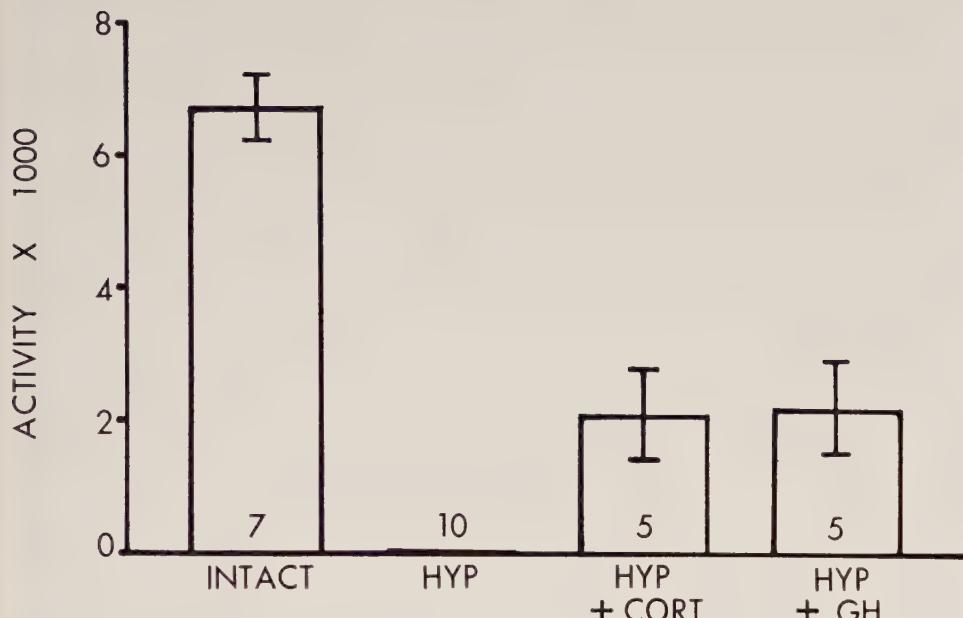


Figure 14-4. Effect of hypophysectomy (HYP) at 50 days, and cortisone (HYP + Cort) and the growth hormone (HYP + GH) replacement on amine (lysyl) oxidase activity (mean \pm S.E.) of rat aorta at 100 days. Amine oxidase was assayed using benzylamine as substrate by a variation of the technique of Tabor, *et al.* (1953). From Howarth and Everitt (1974), reproduced with permission of S. Karger, Basel.

treatment (Howarth and Everitt, 1974; Fig. 14-4). It is believed that this enzyme is identical with lysyl oxidase* the enzyme concerned with cross-linkage of collagen and elastin in the aorta. A reduction in the activity of lysyl oxidase after hypophysectomy would retard aging processes dependent on cross-linkage of collagen and elastin (Shoshan *et al.*, 1972). The elastin content of the aorta is found to be higher in the old hypophysectomized female rat than its control, probably due to a reduced activity of elastolytic enzymes in the hypophysectomized rat (Loeven, 1970).

A combined light and electron microscopic study of the changes that occur with aging in the aorta of hypophysectomized and control rats is currently in progress (Cliff and Everitt). So far, tissues have been examined from animals 210 and 1,012 days old. Qualitatively, the aorta of the hypophysectomized animals shows considerably less severe age changes (cf. Figs. 14-5 and 14-6). The aortic mediae from a 1,012-day-old hypoph-

* Lysyl oxidase from bovine aorta is able to catalyze the oxidation of benzylamine (Rucker, R. B., *et al.*: *Biochem Biophys Res Commun*, 40:1391, 1970).

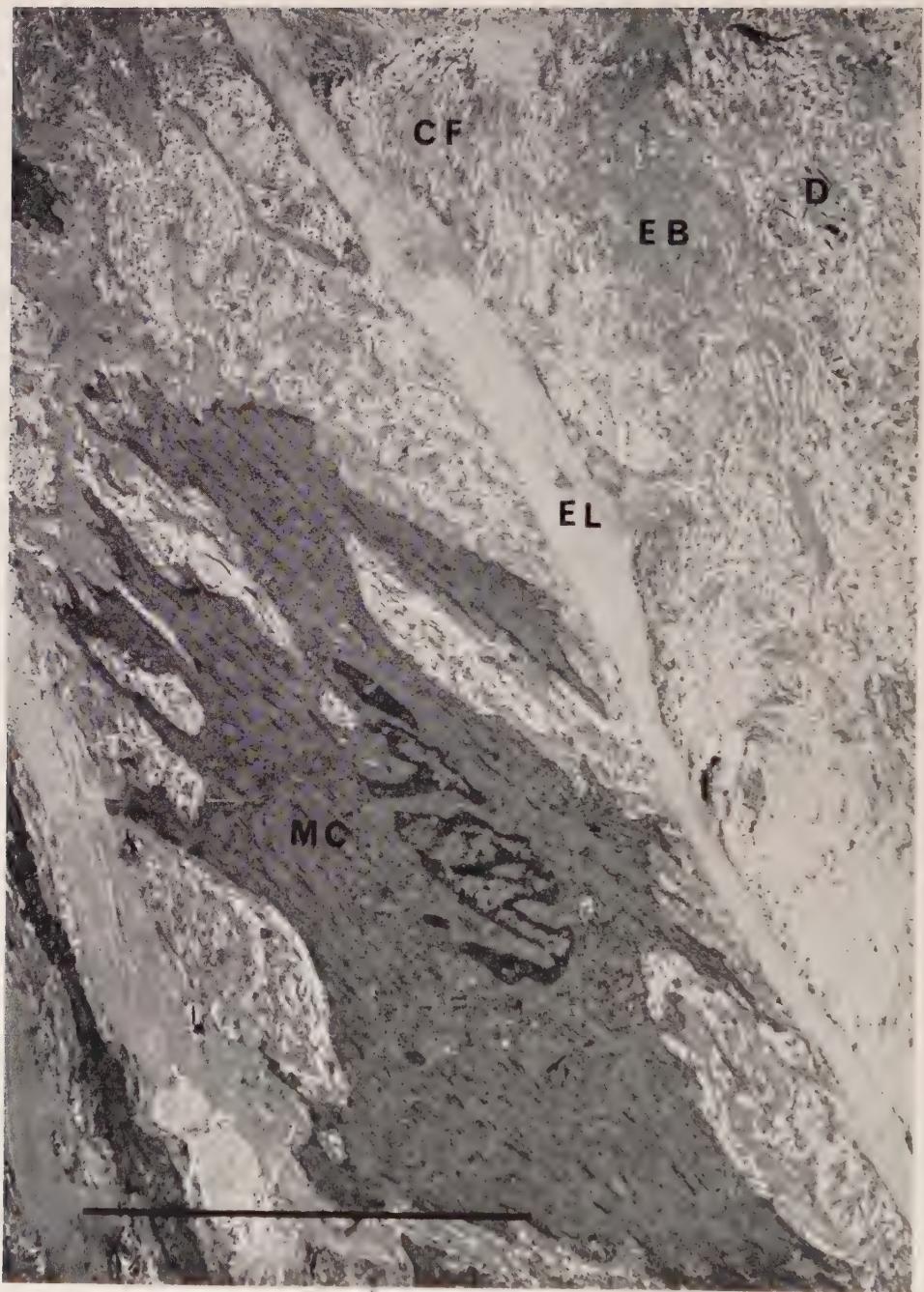


Figure 14-5. Aortic media of control rat 1,012 days old. The medial muscle cell (MC) is apparently hypertrophied and has an irregular outline. The elastic laminae (EL) are narrow and there is a large amount of elastic tissue distributed in branching fibers (EB). Collagen fibrils are plentiful (CF) and collections of debris (D) can be readily identified. Transverse section: bar indicates $10\mu\text{m}$ (The electron micrograph was supplied by Dr. Wally Cliff).



Figure 14-6. Aortic media of hypophysectomized rat 1,012 days old. The medial muscle cells (MC) are more numerous and show no evidence of hypertrophy cf. Figure 14-5. The elastic laminae (EL) are broad and of normal appearance whilst branch elastic fibers (EB) are less prominent cf. Figure 14-5. Collagen fibrils (CF) are well developed. No accumulations of debris are recognizable. Transverse section: bar indicates $10\mu\text{m}$ (The electron micrograph was supplied by Dr. Wally Cliff).

ysectomized and a control rat have been subjected to stereological analysis (Weibel, Kistler and Scherle, 1966). These preliminary results (Fig. 14-7) reveal that in the hypophysectomized rat the elastic laminae remain largely intact and that only a relatively small amount of elastic tissue becomes distributed as branching fibrils throughout the media, which is the predominant form of distribution of the elastic tissue in the aging control rat (Fig. 14-7 and Cliff, 1970). There is less collagen present per unit volume of media whilst the volume occupied by medial cells is greater in the hypophysectomized versus the control rat aorta. The control media contains a larger volume of debris, mainly derived from cell degeneration and necrosis, as compared to the hypophysectomized rat (Fig. 14-7). These preliminary observations indicate that the tunica intima does not show such clear cut differences in the severity of aging between the hypophysectomized and control rats.

In clinical studies of long standing cases of hypopituitarism, heart disease is infrequent (Escamilla and Lisser, 1942; Aloia and Field, 1971). There was no evidence of increased cardiac dysfunction in 22 patients observed for as long as 25 years (Bernart and de Andino, 1958). In another

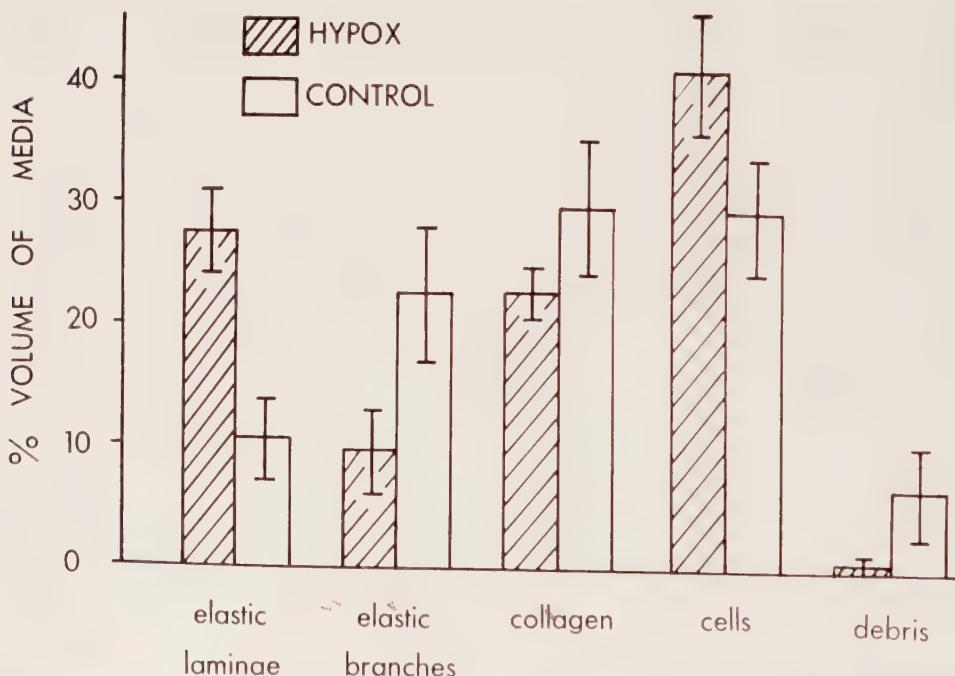


Figure 14.7. Histogram showing percentage of medial volume occupied by various components of media in control and hypophysectomized rats. Bars indicate \pm standard deviation for each component measured from six separate low power micrographs for each group ($n = 6$) (Data supplied by Dr. Wally Cliff).

series of 20 patients, Kosowicz and Roguska (1963) reported an absence of anginal symptoms in hypopituitary men past the age of 60 years. Kosowicz and Roguska suggest that coronary disease does not develop because the mode of life of the hypopituitary patient protects from myocardial infarction. These patients do little work, are slow and inactive, sleep much, are apathetic, inexcitable, have no sexual activity, and the adrenocortical hypofunction prevents the development of hypertension (Kosowicz and Roguska, 1963).

A small number of hypopituitary patients do develop heart disease (see Alois and Field, 1971). There are electrocardiographic changes in hypopituitarism, such as bradycardia, low QRS and changes in ST and T wave (Sheehan and Summers, 1949; Kosowicz and Roguska, 1963). Since these changes are reversible on therapy with thyroxine and cortisone (Kosowicz and Roguska, 1963) they are not true aging changes, which by definition are irreversible (Strehler, 1959a).

Thus the bulk of evidence suggests that cardiovascular aging proceeds at a slower rate in hypopituitary patients than in the normal person.

Effect of Growth Hormone

An excess of growth hormone appears to accelerate cardiovascular aging. In acromegaly, which is due to hypersecretion of pituitary growth hormone, there is a relatively high incidence of heart disease, and cardiac enlargement is a common finding at autopsy (Aloia and Field, 1971). The role of hormones in limiting or inducing cardiac hypertrophy has not been systematically studied in man, but in the rat there have been a number of investigations (see Kovacs and Horvath, Chap. 13). In our rats, cardiac enlargement develops slowly in hypophysectomized rats, and is not accelerated by physiological doses of growth hormone (Fig. 14-3) given daily since early life at 150 days. The studies of Beznák (1963) and Whitehorn (1971) emphasize the importance of thyroxine rather than GH in cardiac hypertrophy in the rat.

Premature atherosclerosis is common in acromegaly (Hamwi, *et al.*, 1960; Aloia and Field, 1971) and hypertension is frequently reported in this condition (Balzer and McCullagh, 1959; Aloia and Field, 1971). In sensitized rats, Selye (1970) showed that growth hormone produced nephrosclerosis and hypertension.

Although growth hormone accelerates the onset of cardiovascular disease, progress of the disease is not rapid in acromegaly (Hirsch, *et al.*, 1969). Hypophysectomy appears to retard the progression of cardiac disease in acromegaly, but it does not reverse the cardiac lesions. In fact many of the patients with acromegaly die as a result of their cardiac lesions, even after hypophysectomy (J. Hickie, personal communication).

Effect of Adrenocortical Hormones

Wexler (Chap. 17) reviews the evidence indicating that over-secretion of ACTH and the adrenocortical hormones accelerates the onset of age-related cardiovascular pathology. The patient with Cushing's syndrome (hypercortisolism) is usually found to have premature atherosclerosis (Bledsoe, 1971). In the rat ACTH has an aging effect on aortic collagen (Borkowski, *et al.*, 1966). Thus aging of arteries would appear to be increased by ACTH and the adrenocortical hormones.

Effect of Thyroid Hormones

Thyroid deficiency accelerates atherosclerosis. In hypothyroid patients, atherosclerosis and coronary heart disease are very common (Vanhaelst, *et al.*, 1967; Wren, 1968; Barnes, 1973). However, the majority of elderly people with coronary heart disease appear to have normal thyroid function (J. A. Burgess, personal communication). Therefore, thyroid deficiency is probably only one of several contributing factors.

An excess of thyroid hormones stimulates the heart, increasing heart size, heart rate, force of contraction and cardiac output. In thyrotoxicosis the heart is enlarged in 30 to 50 percent of patients, and systolic blood pressure is slightly or moderately raised (Harris, 1970). The detrimental action of thyroid hormones is that the metabolic burden may provoke cardiac failure in older subjects with existing coronary or hypertensive heart disease (Harris, 1970).

Gonadal Hormones

Coronary heart disease is more common in men than women, particularly in the younger age groups. Hamilton (1948) claimed that testosterone is responsible for the high incidence of cardiovascular disease in the male. Testosterone may augment atherosclerosis, since there is decreased atherogenesis in male hypogonadism (Furman and Howard, 1957). However, the sex difference is usually attributed to the protective action of estrogen against coronary heart disease (Katz and Pick, 1967). A protective action of estrogen has been demonstrated by a number of workers (Pick, *et al.*, 1968; Pelkonen, 1971), but is denied by others (Ritterband, *et al.*, 1963; Manchester, *et al.*, 1971). In various mammals and in the cockerel, estrogens have been shown to have a protective effect on cholesterol atherosclerosis of the coronary arteries, but not of the aorta (Selye, 1970). Estrogen has, however, an inhibitory action on the thickening of the aortic wall in response to experimental hypertension in the rat (Wolinsky, 1972b).

Hormones and Lipid Metabolism

One of the important factors in atherosclerosis is a derangement of lipid metabolism, one expression of which is hypercholesterolemia. This

subject has been reviewed by Katz and Pick (1967) and is discussed by Hruza in Chapter 24. The serum cholesterol level is lowered by thyroxine and estrogen and raised in hypercorticism and in acromegaly. Diabetes mellitus, a complication in many cases of acromegaly, is also associated with hypercholesterolemia and atherosclerosis.

CONCLUSION

Clinical and experimental studies show that a number of cardiovascular age changes proceed at a reduced rate in the absence of pituitary hormones. Most work has centered on the effects of hormones on atherosclerosis and coronary heart disease, which appear to be promoted by cortisol and growth hormone, and inhibited by thyroxine and estrogen. It is only possible to speculate on the mode of action of these hormones in atherosclerosis. For example, these hormones are known to regulate cholesterol metabolism, which is deranged in atherosclerosis. Although much is known about the effects of hormones on atherosclerosis, there is relatively little literature on the role of hormones in the physiological aging of the cardiovascular system. Our own studies suggest that hypophysectomy also retards physiological aging in the cardiovascular system.

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CHAPTER 15

AGING OF THE ADENOHYPOPHYSIS IN RELATION TO RENAL AGING

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SUMMARY

A LONG-STANDING HYPOTHESIS which attributes senescent changes in the kidney to primary senescence of the hypophysis is shown untenable in man. Stores of the renally active adenohypophysial hormones—adrenocorticotropic (ACTH) and growth hormone (GH) remain at constant level throughout life, as do stores of thyrotropin (TSH). The fasting plasma levels of GH are unchanged and plasma concentrations of ACTH are not grossly altered in old age. The adrenal cortex is well maintained throughout life and retains ability to respond to ACTH as in the young. There is, therefore, no evidence of functional deterioration of the hypophysis in old age.

Renal senescence has begun at 40 years of age and consists of a steady loss of whole nephrons, so that, by the age of 75 years only 60 percent of the nephrons remain; by 90 years of age less than 50 percent of the original numbers of nephrons are extant. The residual nephrons respond normally to renally active hypophysial and adrenal cortical hormones.

Renal senescence is a well-marked phenomenon which takes place in the absence of functional aging of the hypophysis.

INTRODUCTION

Inevitably, as the major importance of hypophysial function for the occurrence of normal growth, maturation, and homeostasis became known and the close functional association of the hypophysis with the hypothalamus was apprehended, it was suggested that this gland might also play a major part in the genesis of senescence (Smith, 1926). Hence, a link between the hypophysis and renal aging is to be sought, considered and judged in the light of present knowledge.

Senescent changes are all too easily confused with changes brought about slowly by advancing disease or with the sequelae of disease. Consequently, studies concerned with effects of age necessitate the very careful selection of suitable material. Lack of attention to this requirement amongst animal workers has well nigh confined the present discussion to a consideration of hypophysial and renal senescence in man.

The extent of the control of the hypophysis over renal function has been considered in a foregoing chapter of this book (Kovacs and Horvath, Chap. 13). The actions of pituitary, adrenal and renal hormones on the kidney have recently been reviewed elsewhere (Lockett, 1972).

The hypophysial hormones known to influence renal function directly are the antidiuretic hormone of the neurohypophysis (ADH) and the somatotropic or growth hormone (GH). The adrenocorticotrophic hormone (ACTH) has an important influence on renal function, mediated via the adrenal cortex. The thyrotropic hormone largely controls the synthesis and release of thyroid hormone: the latter probably has no greater influence on renal metabolism than on the metabolism of other organs and tissues. Change in the rate of secretion of one or more renally active hypophysial hormones should be demonstrable during aging of the hypophysis if the hypophysis is to be considered primarily responsible for renal aging.

AGING OF THE HYPOPHYSIS

Histological Changes

The mean weight of the human male pituitary is maximum (557 mg) in the fourth decade of life. Thereafter, the weight of this gland declines with age, gradually at first, but rapidly after the seventieth year (Rasmussen, 1938, 1947). The weight lost from the aging pituitary body is mainly from the anterior lobe (Shanklin, 1953). Cell counts made of the adenohypophysis of man at different ages have shown that there is a slight increase in the percentage of basophil cells in old age (Rasmussen, 1950; Parhon, Postelniou and Petrea, 1959) in man. The basophil cells are those which probably generate the gonadotropins (FSH and LH), the thyrotropic (TSH) and the adrenocorticotrophic (ACTH) hormones. The somatotropic or growth hormone (GH) and prolactin are probably produced by the eosinophils. Castration cells, also called postmenopausal cells, are found in senescent male and female adenohypophyses. These cells contain large granules, vacuoles and deeply staining nuclei and are sometimes found grouped together in small adenomata.

The Hormone Content of the Pituitary Body in the Aged

The content of GH in the adenohypophysis of man, expressed as μg per kg body weight (Gershberg, 1957) and of rats (Pindborg, Becks and Evans, 1957; Greenspan, Li, Simpson and Evans, 1949) changes little if at all with age.

The quantities of LH and FSH in the adenohypophyses of adult men and women of 30 to 40 years do not differ significantly per kg body weight but a threefold rise in FSH occurs in women after the menopause without alteration in the concentration of LH (Bahn, Lorenz, Bennett and Albert,

1953). It is probable that this rise in FSH is attributable to withdrawal of the inhibitory feed-back of ovarian hormones as a result of primary ovarian senescence. A similar rise in the concentration of FSH in the adenohypophysis results from castration in the male.

The quantity of TSH (mg/kg body weight) in the adenohypophysis appears to remain constant throughout life in men and women (Blumenthal, 1954) but may rise, in some individuals, during the later decades (Marine, Rosen and Spark, 1935; Blumenthal, 1954; Levey, 1963).

In 1955 Blumenthal investigated the ACTH content of the human pituitary between the ages of 8 and 71 years. This he did by implanting one quarter of each gland under the skin of a guinea pig on each of four successive days. The number of mitoses in the adrenal cortex of the pig were determined on the fifth day as a measure of the adrenal hyperplasia induced by the ACTH content of the transplanted gland. These experiments demonstrated the absence of gross change in the stores of ACTH during aging.

The Influence of Age on the Output of Hormones from the Adenohypophysis

No direct, *in vivo* measurements of the rates of secretion of hormones from the hypophysis were available until very recently. Great advance has, however, been made in methodology, particularly by development of the techniques of immunoassay and radioimmunoassay. These assays permit measurement of the plasma concentrations of peptide hormones and of changes in plasma concentrations of these hormones brought about by changes in the rates of hormone secretion and/or hormone metabolism, and/or hormone excretion.

Growth Hormone

The various methods available for the immunoassay and radioimmunoassay of GH are now yielding data which is in good accord (Hunter and Greenwood, 1962; Pearson, Stratman, Spector and Yen, 1964; Glick, Roth, Yallow and Berson, 1964; Lazarus and Young, 1966). Glick and his co-workers found that the plasma levels of 90 percent of adult men fell within the range of 0 to 3 μg ml after an overnight fast, irrespective of age. Lazarus and Young (1966) also found stability of the plasma levels of GH during adult life, and an absence of sex influence. However, a detailed study made by Finkelstein, Roffwarg, Boyer, Kream and Hellman (1972) draws attention to the intermittent nature of GH secretion. These workers determined plasma values of GH every 20 minutes for 24 hours in each of their human subjects. Adolescents secreted GH during wake and sleep periods alike. During the 24 hour period, the number of secretory episodes ranged from 2 to 8 and occupied 100 to 300 min; 328 to 1406 μg

GH were secreted during these episodes. In young adults (21 to 41 years) secretory episodes occupied an average of 133 min/24 hr during which 215 to 769 μg GH were secreted. In older adults (47 to 62 years) the secretory episodes were very much reduced or absent. The data demonstrated an age related change in the pattern of the spontaneous release of GH and most probably an overall reduction with age in the 24-hr output of GH from the adenohypophysis.

No evidence has yet indicated a positive correlation between the plasma level of GH and the body weight in man, although this correlation is demonstrable in pigs regardless of age, sex or strain (Siers and Swigger, 1971; Machlin, Horino, Hertelendy and Kipnis, 1968).

ACTH

Radio-immunoassays are now available for measurement of plasma levels of ACTH in man (Lipscomb and Nelson, 1962; Immura, 1967; Matsuyama, Harada, Ruhmann-Wennhold, Nelson and West, 1972) with which good correlation is obtained between the immuno- and biological assay figures. The slightly higher values given by the immunoassays are probably attributable to the presence of ACTH-breakdown products in the plasma which have retained their immunological specificity. These immunological assays could be used for the direct measurement of the effects of age on the secretion of ACTH from the adenohypophysis. Indirect evidence is all that is available at the present time.

Any marked decrease in the secretion of ACTH with age must necessarily be reflected by decrease in the secretion of glucocorticoid from zona fasciculata and zona reticulata of the adrenal and by atrophy of these cortical zones. Atrophy of the adrenal does not, however, occur in the aged. Histological senescence of the adrenal is characterized solely by reduction in the number of mitotic figures and by some vacuolization of the cortical cells (Townsend, 1946; Blumenthal, 1945). The collagen in the adrenal capsule and in the parenchyma increases (Dribben and Wolfe, 1947). Senescent changes in the adrenals of rats are completely reversed by estrogen, androgen and progesterone (Korenchevsky, Paris and Benjamin, 1953).

The influence of age on the spontaneous release of glucocorticoid from the adrenal has received study in man. The average 24-hour secretion of cortisol can be determined by measurement of the secretion of any one of its four major urinary metabolites (Romanoff, Morris, Welch, Rodriguez and Pincus, 1961) by methods developed by Romanoff, Rodriguez, Seelye, Parent and Pincus (1957a and b, 1958 and 1959). These experiments indicate that the output of glucocorticoid from the adrenals of elderly men and women is 70 to 75 percent that from the adrenals of young adults.

This difference disappeared however when these values were expressed in mg per g endogenous creatinine in the urine. Hence the age difference appeared related solely to the differences in the muscle masses of the subjects. The mean ratio of the 11-ketonic to the 11-hydroxylated metabolites was unaffected by age.

The response of the aged adenohypophysis to reduction in the plasma concentration of 11-hydroxylated glucocorticoid, and that of the aged adrenal to ACTH have been shown to resemble those of healthy young adults by the following experiments made in man. Metyrapone reduces the production of cortisol by inhibiting 11β -hydroxylation in the adrenal cortex. Hence this drug causes the plasma levels of cortisol to drop. Consequently, there is an outpouring of ACTH from the adenohypophysis. Since the formation of cortisol by the adrenal cortex is inhibited because 11β -hydroxylation is inhibited, 11-deoxycompounds are formed instead. Hence the plasma concentration of compound S (11β -deoxycortisol) rises markedly in the plasma. The rise in plasma compounds reached maximum in 4 hours after administration of 5 g metyrapone to man. The levels of compounds reached in the plasma were $11 \pm 1.27 \mu\text{g}/100 \text{ ml}$ for 37 healthy geriatric and 30 healthy young subjects. There was no significant difference between the responses of the young and the old to metyrapone (Blichert-Toft, Blichert-Toft and Kaalund-Jansen, 1970). Aged men also respond by eosinopenia to ACTH as do the young (Solomon and Shock, 1950), and the continued administration of ACTH to the aged (12 mg/day for 12 days) caused increases in body weight, retention of sodium chloride and raises the excretion of potassium and 17-ketosteroids (Duncan, Solomon, Rosenberg, Nichols and Shock, 1952) as in the young. Hence, large doses of ACTH appear to enhance the secretion of aldosterone by the aged, as by the young.

Release of ACTH by high concentrations of adrenaline persists in old age and there is no change with age in the adrenaline content of, or output from, the adrenal medulla (Salimbeni and Gery, 1912; Karki, 1956).

TSH

The advent of a very highly sensitive radio-immunoassay technique for the estimation of TSH (Odell, Wilbur and Utiger, 1967) enabled Mayberry, Gharib, Bilstad and Sizemore (1971) to study the influence of age on plasma levels of TSH. The relationship found between the plasma concentration of TSH and the age in years from 307 euthyroid subjects was parabolic. Maximum mean values of TSH ($10 \mu\text{U}$ of Human Research Standard/ml plasma) were found in the first year of life; the plasma values then declined parabolically; the minimum mean value of $5 \mu\text{U}/\text{ml}$ was reached at 50 years; thereafter the plasma values again rose, parabolically,

to mean at 7 μ U/ml (approx) at the age of 80 years. An age dependent decrease in the rate of uptake of iodide by the thyroid gland of man probably indicates a decrease in the rate of synthesis of thyroid hormone as age advances. Since the renal clearance of thyroxine decreases with age as does the rate of degradation of this hormone, plasma levels of thyroid hormone remain steady throughout life (Gregerman, Gaffney and Shock, 1962; Gaffney, Gregerman and Shock, 1962). Hence, the well known age-dependant decrease in the basal metabolic rate (Lewis, 1938) is not related to plasma levels of thyroid hormone.

The purification of TSH-releasing factor (Condliffe, 1963) and the availability of synthetic TSH-RF (Guillemin, Burgus and Vale, 1971) for clinical use (Fleischner, Lorente, Kirkland, Kirkland, Clayton and Calderon, 1972) has made measurement of any influence of age on adenohypophysial sensitivity to TSH-RF in man a further possibility.

Effects of Age on the Response of the Adrenal Cortex to ACTH

In 1951, Heller and Shipley studied the endocrine outputs of a group comprised of elderly men and elderly women, making comparisons with a younger group of subjects. Whereas the output of cortin was unchanged by age or sex, the 17 ketosteroid output declined in the elderly of both sexes, more in women than in men, probably as a result of primary gonadal senescence.

Injections of ACTH made into aged men caused both eosinopenia and lymphopenia (Solomon and Shock, 1950). Daily administration of 100 mg ACTH for 12 days to elderly men resulted, as in younger men, in salt and water retention, increase in body weight, and increase in the excretion of potassium and 17-ketosteroids (Duncan, Solomon, Rosenberg, Nichols and Shock, 1952). These observations have recently been confirmed and extended (Blichert-Toft, *et al.*, 1970). These latter workers found no quantitative difference between young and old men in the rises in plasma levels of cortisol and compound S caused by single injections or by infusions of ACTH, 0.25 μ g. The duration of the infusions was 4 hours.

RENAL SENESCENCE

In Man

The incidence of disease increases in the kidney (Kennedy, 1958; MacNider, 1952) with age as in the other organs (Simms, 1946). It is, therefore, certain that within any local population but few of the elderly will exhibit renal senescence uncomplicated by renal disease. However, a long series of observations is available which was made on aged men selected especially for the study of renal senescence because they had neither a history nor signs of renal disease and had systemic arterial pressures not ex-

ceeding 140/90 mm Hg (Shock, 1949, 1956, 1958). All measurements on these subjects were made under basal conditions and during sustained water diuresis.

The effective renal plasma flow (ERPF), measured either as the clearance of diodrast (Lewis and Alving, 1938; Howell and Piggott, 1948; Davies and Shock, 1950; Shock, 1952) or as the clearance of p-aminohippurate (PAH) (Miller, McDonald and Shock, 1951; Watkin and Shock, 1955) remained virtually unchanged from the age of 20 to 35 years and thereafter decreased to approximately 75 percent of the original values at 60 years and to approximately 42 percent at 90 years of age. This overall rate of decrease in ERPF exceeds the rate of decrease in cardiac output during aging (Lewis, 1938b; Brandfonbrener, Lansdowne and Shock, 1955). Thus the proportion of the cardiac output passing through the renal cortex decreases progressively after 35 years of age. It must, therefore, be concluded that the intrarenal resistance to blood flow, measured under basal conditions, undergoes a greater increase than does resistance in the majority of other organs and tissues as the years go by. This relative increase in intrarenal resistance reflects a functional state rather than the onset of intrarenal vascular rigidity since the proportionate increase in renal blood flow accompanying the pyrogenic effect of typhoid vaccine is as great in the old as in the young (McDonald, Solomon and Shock, 1951).

The decrease in the glomerular filtration rate during aging (measured as the clearance of inulin) is proportionately similar to the decrease in ERPF (Davies and Shock, 1950; Miller, McDonald and Shock, 1952; Watkin and Shock, 1955) for the filtration fraction shows only a very small increase with age. The maximum tubular masses (T_m values) for diodrast and for glucose decrease during aging in proportion to the GFR (Miller, McDonald and Shock, 1952). Hence, the mean inulin clearance per unit of T_m is constant from the age of 20 to 90 years, but the diodrast clearance per unit of T_m diodrast falls from 12.6 in the fourth decade to 9.7 in the ninth decade of life.

Overall, these findings suggest (1) a gradual decline with age in the number of functioning nephrons, (2) normal function in all surviving nephrons up to the time of their death (possibly as a result of glomerular infarction) and (3) the absence of compensatory hypertrophy and hyperplasia in response to the gradual spontaneous reduction of functioning nephrons during aging. This hypothesis has received histological support from the early work of Moore (1931) who found a steady decline in the number of glomeruli in the human kidney as age advanced. Additionally, Friedman, Raizner, Rosen, Solomon and Sye (1972) have scanned 35 pairs of elderly kidneys, mean age 75 years, which were of normal size. In 25, asymmetric areas of decreased uptake were observed. Planimetric examina-

tion of the scans showed inequality in the two organs: the mean single kidney area was about 50 percent that normal for young adults. Intimal fibrosis was observed in the intrarenal arteries: the glomeruli and tubules appeared normal: the total numbers of nephrons were markedly reduced. Although there was no evidence of pyelonephritis in these subjects (i.e. no blunting of the renal calyces was seen), the incidence of bacteria was high. Marketos, Papanayiotou and Donas (1969) have demonstrated that local multiple infarcts can arise in kidneys as a result of bacteria and nonobstructive vascular disease. The possible occurrence of amyloid deposits in the kidneys of elderly subjects, as in the hearts (Pomerance, 1965) was also suggested to account for the scanning defects.

Studies concerned with the ability of the senescent kidney to perform its homeostatic functions have also been made in man. The osmolality, the water content and the pH of the plasma do not change with age (Shock and Yiengst, 1950). The concentrations of sodium and chloride (de Bellis, 1954; Herbeuval, Cuny and Manciaux, 1954; Lippi and Malerba, 1955) and of potassium (Videback and Ackerman, 1953) in plasma do not alter. Plasma (Cohn and Shock, 1949) and extracellular fluid volumes (Shock, Watkin and Yiengst, 1954) undergo no significant change although total body water decreases (Shock, Yiengst and Watkin, 1954) from 54.8 percent to 50.9 percent body weight from the fifth to the eighth decade of life. This decrease is at the expense of the intracellular water (Parker, Olesen, McMurray and Hansen, 1958; Olbrich and Woodford-Williams, 1956). A small comparable decrease in the total exchangeable potassium found in the aged of both sexes (Salgid, 1956) is attributable to the reduction in intracellular water without change in the intracellular ratio of water : protein molecules.

A reduction in the total number of nephrons in the absence of change in renal mass (Friedman, *et al.*, 1972) should (Lockett, 1972) result in a decline with age in the transmedullary osmotic gradient. This prediction, if confirmed, can explain the decrease in the maximum ability to concentrate the urine which accompanies senescence (Shock, 1958). A reduced transmedullary osmotic gradient together with reduction in the number of functional nephrons can readily explain the decrease in sensitivity to the antidiuretic action of vasopressin observed in the senescent kidney (Miller and Shock, 1953). The remarkable absence of deterioration in the functional capacity of the residual nephrons in the kidneys of aged man is well evidenced by their ability, as in young nephrons, to double $T_{mp_{AH}}$ when provided with additional energy yielding substrate (McDonald, Shock and Yiengst, 1951).

Acid-base balance is well maintained in the aged under nonstressful sit-

inations, but the maximum loads of acid (and probably of base) that can be handled by the aged have decreased (Hilton, Goodbody and Kruesi, 1955) and the time required for re-establishment of acid-base equilibrium after 10 g NH₄Cl has more than trebled (Shock and Yiengst, 1948).

Over all, it must be conceded that the changes in renal function which are associated with progressive renal senescence in man cannot be attributed to the development of adenohypophysial dysfunction in old age. Attention is drawn to two factors which might be causative of age-related nephron loss in man: infection and vascular disease. Friedman, *et al.* (1972) found 37 percent of their 72 elderly subjects (mean age 75 years, all over 60 years) had significant pyuria and 25 percent of these patients had abnormal scans. These patients had however been selected for study because they had neither a history of renal disease, nor hypertension. Bacteriuria in man increases with age and is frequently associated with symptomless histological changes resembling those of pyelitis (MacDonald, Mallory and Kass, 1957). Additionally, hypertensive vascular disease in the young can produce renal scans resembling those seen in the elderly (Haynie, Stewart and Nofal, 1961), and afferent arteriolar atrophy is found, histologically and angiographically, both in the normotensive aged and in younger hypertensive man (Jungquist, 1963).

In Rats

Arataki (1926) found the left kidney of Wistar rats to contain 31,000 glomeruli at maturity but only 20,000 glomeruli at 500 days. Glomeruli were found to enlarge throughout life and showed mean diameters of 62 μ at birth, 111 μ at 250 days, 118 μ at 350 days and 124 μ at 500 days. Moore and Hellman (1930) also found a decrease in the number of glomeruli in the rat kidney as age increased. They observed 28,000 glomeruli per kidney at 230 days and 20,000 per kidney at 320 days.

Andrew and Pruett (1957) found fibrosis of the glomerular tufts and deposits of colloid in the tubules of senescent rats (868-983 days). Large aberrant tubular epithelial cells were frequent and these contained greatly enlarged and often hyperchromatic nuclei in which an enlarged prominent nucleolus was a common finding. The adventitia of many of the arteries and the adjacent connective tissue was heavily and patchily infiltrated with lymphocytes and plasma cells. There was evidence of hyperplasia, especially in the proximal nephron.

The senescent changes in the rat kidney, described above, may however be the consequence of disease. Many crowded rat colonies suffer from an endemic respiratory infection of a type which leads to extensive infiltration of the lungs by lymphocytes and plasma cells, destruction of lung tis-

sue, the formation of giant cells, and eventually, to somewhat similar infiltration of many organs, including the kidneys, by lymphocytes and plasma cells. It is moreover well known that lung disease reduces life-expectancy in rats by 100 to 160 days (Everitt and Cavanagh, 1963). Hence the commonly observed histological picture shown by the kidneys of rats described above is unlikely to be one of true senescence uncomplicated by disease. However, Simms and Berg (1957) examined the kidneys and other organs of male Sprague-Dawley rats from a colony in which respiratory infection had been reduced to a low incidence by routine elimination of all obviously infected animals and those whose weight curves showed plateaux. The minimal renal changes observed in these animals consisted of patchy proximal tubular dilation with epithelial flattening. In the lesions described as moderately severe, more tubules were dilated and the corresponding glomeruli were atrophied. Lesions described as severe demonstrated extensive destruction of the parenchyma which contained cyst-like dilated tubules of varying size. Most of the glomeruli had either disappeared, were atrophic or had hyalinized and there was relative increase in connective tissue. Lymphocytic infiltration was evidenced by some but not by all these severely lesioned kidneys.

In vitro measurements of age-related renal functional changes were made by Barrows and his colleagues in female Wistar rats from a colony with a low incidence of infections. Senescence was accompanied by a small (10%) cellular loss, as determined by decrements in deoxyribose nucleic acid (Barrows, Roeder and Falzone, 1962) and marked decrease in the proximal tubular active transport of PAH and α -aminoisobutyrate in kidney slices (Adams and Barrows, 1963; Beauchene, Fanestil and Barrows, 1965). More recently, and *in vivo*, Gregory and Barrows (1971) found no changes in the clearances of inulin in these rats between the ages of 12 and 30 months, but T_{maxPAH} was, at 30 months, 25 percent lower than at 12 or 24 months. The functional changes found *in vivo* accord with those predictable from the minimal histological lesions of Simms and Berg (1957), nephrotic in type, associated with marked increase in the urinary excretion of protein (Simms and Berg, 1957; Everitt and Cavanagh, 1963). The discovery by Pollard (1971) that the kidneys of germ-free senescent rats are free of nephrotic lesions and show no age-related increase in the urinary excretion of protein is crucial: senescent pathology of the rat kidney is germ-dependent. These germs need not necessarily be associated with respiratory disease for Everitt and Cavanagh (1963) have shown that the age-related rise in urinary protein does not differ in male rats with and without respiratory infections.

CONCLUSION

In the judgment of the author, the renal functional changes characteristic of senescence in man and in rats cannot be attributed to changes in adenohypophysial activity during aging. The stores of ACTH, GH, and TSH remain plentiful in the senescent adenohypophysis of man. Despite changes in the 24-hour outputs of GH and of TSH with age, the plasma concentrations of renally active corticoids, GH and protein-bound iodine (Kountz, *et al.*, 1949) remain constant throughout adult life. Age-related changes in the rates of secretion of adenohypophysial hormones therefore appear linked, directly or indirectly, to tissue requirements.

Since no senescent changes occur in the kidneys of germ-free laboratory rats, infection is causative of renal senescence in this species. Moreover, evidence has already accumulated to indicate that senescent changes in the kidneys of man may prove largely attributable to silent infection and to undiagnosed vascular disease. Man is certainly more prone to arteriosclerosis than the rat.

Verzár (1956) has drawn attention to marked species differences in patterns of senescence. It is possible, in the future, that differences in pathogenic environment and in susceptibility to the various germs, may be found largely determinant of the patterns of senescence characteristic of different species.

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CHAPTER 16

ANTERIOR PITUITARY IN RELATION TO RENAL DISEASE

JOHN J. CHRISTIAN

SUMMARY

THE EFFECTS OF PITUITARY HORMONES, particularly adrenocorticotropin and growth hormone, have been studied extensively, but their roles in the pathogenesis of natural renal disease is obscure. Growth hormone augments experimental renal hypertension by virtue of its sodium-retaining action and possibly by its renotropic action in the presence of a renal metabolic load. The adrenals, or one of the mineralocorticoids, are necessary for the nephrosclerotic action of growth hormone. ACTH also requires the adrenals and presumably growth hormone to be effective in rats. On the other hand, there is evidence that crowding, possibly the rate of growth of cities, and a number of other stressful stimuli either produce or augment the production of hypertensive disease. Presumably ACTH, adrenal corticoids, renin, and possibly other factors are involved in this hypertensive response.

ACTH does not produce persistent renal lesions in rats, voles, white-footed mice, and rabbits, but it does so in other species. The effect of ACTH on the kidneys of house mice does not require the adrenals, although an unidentified adrenal factor augments its effect. The lesions are primarily glomerular and consist of a marked increase in the mesangium. There is leakage of serum protein in the juxtaglomerular region and distal tubules. Basement membranes are unaffected. The glomerular lesions are unlike any common human glomerular lesions or experimental immunologic glomerular lesions in animals.

A glomerulosclerosis accompanying aging in rats, mice, hamsters, and guinea pigs resembles the renal lesions produced by ACTH in mice. These lesions of aging may reflect repeated or chronic increases in endogenous ACTH secretion. Electron micrographs of the lesions of aging are required for firm conclusions.

A natural glomerular disease of woodchucks resembles the ACTH-produced disease in mice and can be duplicated with ACTH. The prevalence, incidence, and severity of this glomerular disease respond to changes in

the level of social strife and generally increase with age, suggesting that ACTH may be involved in its production.

The glomerular lesions associated with aging, induced by ACTH, or occurring naturally in woodchucks, and other species, seem to be limited to rodents. Renal growth may be a factor in their genesis. They may not be valid models for human disease.

INTRODUCTION*

Hormones of the anterior pituitary have been implicated, either directly or indirectly via their target organs, in a number of renal diseases, including those associated with senescence. However, in most investigations of the pathogenesis of diseases such as hypertension and nephrosclerosis in experimental animals the hormones of the target organs were shown to be primarily important and those of the anterior pituitary only secondarily so. In fact as late as 1961 it was concluded that of the adenohypophyseal hormones only growth hormone has a direct effect on renal function (Wesson, 1961). This conclusion certainly reflects the use of rats and rabbits as experimental animals, because in them adrenocorticotropin (ACTH), growth hormone (GH), and thyrotropic hormone (TSH) do not produce renal lesions in the absence of their target organs. Similarly the decline of renal function with age in man and experimental animals has been attributed to antidiuretic hormone and adrenocortical steroids (Shock, 1958; Friedman, *et al.*, 1967) and not directly to anterior pituitary hormones.

Since hypertension and nephrosclerosis are important diseases of aging in man, the discovery that apparently similar diseases could be produced by salt-retaining corticoids such as desoxycorticosterone (DOC) or aldosterone in rats "sensitized" by unilateral nephrectomy and a high salt intake or by adrenal regeneration resulted in emphasis of research on the cortical steroids and relative neglect of the anterior pituitary hormones. Further emphasis on the adrenal cortex was given by discovery of the relationships between the renal juxtaglomerular apparatus, renin, angiotension, and the control of aldosterone secretion and their possible roles in the pathogenesis of hypertension.

Despite the negative evidence, the possibility of a direct relationship between renal disease and hormones of the anterior pituitary still existed. For example a spontaneous renal glomerular disease in woodchucks (*Marmota monax*) that generally increased in severity with age and which responded to changes in population density in a way suggesting that ACTH

* Most references are omitted in the introduction since they will be discussed in more detail later.

and the adrenals might be involved could not be duplicated with corticoids. However, it was duplicated with ACTH. Similar renal glomerular lesions were produced in mice by ACTH. The interest of these lesions with respect to aging lies in the fact that they closely resemble the glomerulopathies associated with aging in a number of species of experimental animals. Senescence in rats is accompanied by characteristic renal lesions, variously called "nephrosis," "glomerulosclerosis," "pyelonephritis" and "glomerulonephritis" (Berg, 1967), which have been correlated with adrenocortical overactivity (Kennedy, 1958) and therefore might reflect to some degree a direct effect of ACTH. This renal disease was found to be the most frequent primary cause of death in aged rats. It is characterized by dilation of many convoluted and collecting tubules into cystic structures by proteinaceous casts, hyalinization of many glomeruli, fibrosis of the supporting stroma and parietal Bowman's capsule, and infiltration of the intertubular spaces by lymphocytes (Berg, 1967). The severity and frequency of these lesions increased with age.

Anterior pituitary hormones may have important, but less obvious, effects on other renal diseases. A discussion of the possible relationships between these hormones and renal disease comprises the balance of this chapter.

TERMINOLOGY

The terminology of renal disease in experimental animals leaves much to be desired. It has been borrowed largely from human pathology and applied to lesions that may not be identical to those of the human disease.

The names of most renal diseases of lower mammals, especially of the glomeruli, have been based on appearances with light microscopy, sometimes with only hematoxylin and eosin staining. Only a relatively small number of such entities have received critical study by electron microscopy and immunofluorescent procedures. Among these are aleutian disease of mink, lesions of LCM nephritis in mice, experimental immunologically-induced glomerulonephritis in several species of laboratory animals, experimental diabetic nephropathies, and a few others. "Nephrosclerosis," "glomerulosclerosis" and "glomerulonephritis" have been used as diagnostic terms for lesions that may differ more or less from their presumed human counterparts, and therefore, may be misleading. For example, "glomerulosclerosis" is used generically to describe a type of glomerular lesion seen in aging hamsters and mice which appears to be different from human diabetic glomerulosclerosis, especially if the former lesions are similar to the glomerular lesions produced by adrenocorticotropicin (ACTH) in mice, as suggested below. However, to avoid further confusion, the

name used by the original authors in describing a particular entity will be used in this account.

ACTH AND THE KIDNEYS

Indirect Effects of ACTH on the Kidneys

Experimental Hypertension

STEROID NEPHROSIS AND HYPERTENSION. Steroid biosynthesis and secretion by the adrenal glands are controlled by ACTH from the pituitary. In rats, and possibly in other rodents, the secretion of aldosterone also is largely under the control of ACTH and growth hormones (GH) (Palmore, *et al.*, 1969, 1970). Therefore, the production of nephrosclerosis and hypertension by certain natural and synthetic mineralocorticoids is germane to the present topic, although historically this fact served to divert attention from the effects of the pituitary itself.

Desoxycorticosterone (DOC or DOCA), desoxocortisone, and other synthetic mineralocorticoids, as well as aldosterone, the powerful natural compound, produce nephrosclerosis and hypertension in sensitized (unilaterally nephrectomized and high NaCl intake) experimental animals, usually rats (Selye, 1950a, b; Friedman, *et al.*, 1951; Skelton, 1953; Selye and Bois, 1956a; Hall and Hall, 1965a, b, 1967). These effects may be due, in part, to the sodium-retaining action of mineralocorticoids, since sodium chloride augments their action (Selye, 1950a; Hall and Hall, 1965a, b) and a high salt diet alone will produce nephrosclerosis and hypertension in sensitized adrenalectomized rats (Meneely, *et al.*, 1953; Wilgram, *et al.*, 1963). However, hormonally or sodium-induced hypertension is unaccompanied by gross accumulation of sodium or decrease in potassium in the tissues (Schackow and Dahl, 1966). A significant increase in aldosterone, but not corticosterone secretion, accompanies experimental renal hypertension (Singer, *et al.*, 1963).

The nephrosclerotic changes in the kidneys of rats include enlargement, hyalinization, and sometimes focal necrosis of the glomerular tufts, and glomerular basement membranes may be swollen and frayed (Selye, 1950a; Meneely, *et al.*, 1953; Skelton, 1955; Anderson, 1963). Foot-processes of the glomerular epithelium may be abnormal, and proteinuria is common. Visible arteriolar changes are minimal and scattered (Kaley, *et al.*, 1960; Anderson, 1963), although hyperplastic arteriolosclerosis with hyalinization and fibrinoid changes in smaller arterioles may accompany hypertension in rats (Spiro, *et al.*, 1965; Wiener, *et al.*, 1965). The arteriolar lesions apparently result from elevated blood pressure of sufficient degree and duration and not from the direct action of the steroids or other method used

to produce hypertension (Wiener, *et al.*, 1965; Heptinstall and Hill, 1967). The renal lesions of aging in rats duplicate in many respects these of experimental hypertension (Berg, 1967).

Crude extracts of anterior pituitary (APE) also produce nephrosclerosis in sensitized rats (Selye, 1950a). The nephrosclerotic effect of APE is augmented by concurrent administration of ACTH in the presence of the adrenals, but is prevented by adrenalectomy (Selye and Bois, 1956a; Selye, 1958). However, ACTH in doses up to 12 mg per day failed to produce nephrosclerosis in sensitized rats with intact adrenals (Selye, 1950a). Similarly ACTH will produce nephrosclerosis in intact, but not adrenalectomized rats, when given doses of hydrocortisone (F) and DOCA that together produced minimal nephrosclerosis (Selye and Bois, 1956b). In general these results suggest that any effect ACTH may have on experimental nephrosclerosis and hypertension in rats is mediated by the adrenals. However, other investigators found that 3 IU of ACTH decreases the severity of pathologic changes produced by DOCA, and that ACTH and cortisone (E), when given in large doses concurrently with DOCA, prevents the production of pathologic changes (Rosenberg, *et al.*, 1952). In smaller doses they have had no effect. These results are difficult to reconcile with those above; however, aldosterone seems to lessen the effect of ACTH in producing glomerular lesions in mice (Pasley and Christian, 1972b), which may reflect a similar action. Exposure to cold, and possibly other stressful agents, enhances the nephrosclerosis and hypertension in sensitized rats produced by DOCA (Selye, 1957; Ingle and Baker, 1957; Crane and Ingle, 1958; Crane, *et al.*, 1958; Hall and Hall, 1959; Chaffee, *et al.*, 1963). Cold apparently is the only stressor tested that clearly and consistently produces hypertensive cardio-renal disease in sensitized rats (cf. Hall and Hall, 1959a).

In none of the above experiments is there good evidence that ACTH acts directly on the kidneys of the animals.

EFFECT OF ACTH ON THE JUXTAGLOMERULAR APPARATUS (JGA). Renin, which can produce hypertension via angiotension II, comes from the renal juxtaglomerular (JG) cells and is specifically associated with the granules in the JG cells and afferent arteries (Cook, 1963; Brown, *et al.*, 1967; Davis, *et al.*, 1967; DeJong, *et al.*, 1969; Hartroft, 1966). Therefore, any effect ACTH has on the JG granules, could be a factor in hypertensive renal disease. ACTH causes hypergranulation of the JG cells and a rise in plasma renin activity (Dougherty, 1948; Hauger-Klevene, 1969). These effects of ACTH are countered by glucocorticoids (Hauger-Klevene, *et al.*, 1969) and the importance in this action of ACTH is unknown.

ACTH apparently has little direct effect on the development of experi-

mental renal hypertension. However, adrenal corticoids may be important in its genesis; therefore ACTH may be indirectly important.

Effects of Glucocorticoids

Hydrocortisone (F) or cortisone (E) (or their synthetic analogues) produce characteristic renal changes; so one would expect ACTH to have similar effects when given to intact animals.

In rabbits cortisone, hydrocortisone, prednisone, and other potent glucocorticoids produce dilation and congestion of the glomerular capillaries, glycosuria, and albuminuria, and sometimes large deposits of homogeneous eosinophilic material in the glomeruli, usually in capillary lumens (Rich, *et al.*, 1951; Bloodworth and Hamwi, 1955, 1956; Wilens and Stumpf, 1955; Wilson, *et al.*, 1962; Ogilvie, *et al.*, 1965). Hydrocortisone produces similar glomerular changes in mice (Christian, *et al.*, 1965). These changes begin to disappear by the 40th day of continued treatment or as soon as treatment ceases. EM shows that these corticoids produce two types of lesions in the glomeruli: one results from proteinaceous occlusion of one or more degenerate capillaries, which are either normal in shape and size or are dilated and locally collapsed; the other from an axial intermingling of basement-membrane-like material with unusually eosinophilic endothelial cytoplasm. The glomerular epithelium may have hyaline droplets, vacuoles and intense osmophilia. Endothelial cells are sometimes abnormally electron-dense and vacuolated. The cells lining Bowman's capsule may show similar changes and there may be plasma-like material in Bowman's space. Four months of cortisone treatment in rabbits produces arteriolosclerosis, focal pyelonephritis, and diffuse thickening of intercapillary septa (Bloodworth and Hamwi, 1955).

Lesions apparently identical to the large eosinophilic masses in the glomeruli of rabbits occur naturally in feral woodchucks.

In house mice, voles (*Microtus pennsylvanicus*), and white-footed mice (*Peromyscus leucopus*), hydrocortisone produces apical vacuolation of the tubular epithelial cells, especially in the proximal tubules (Christian, *et al.*, 1965; Pasley and Christian, 1971, 1972a).

Direct Effects of ACTH on the Kidneys

House Mice and Woodchucks

ACTH apparently has little or no direct effect on the kidneys of rats other than on JG granulation. However, ACTH produces severe renal lesions in mice (*Mus musculus*) and several other species (Christian, 1967a, b; Christian, *et al.*, 1965; Vilar and Christian, 1967). The similarity be-

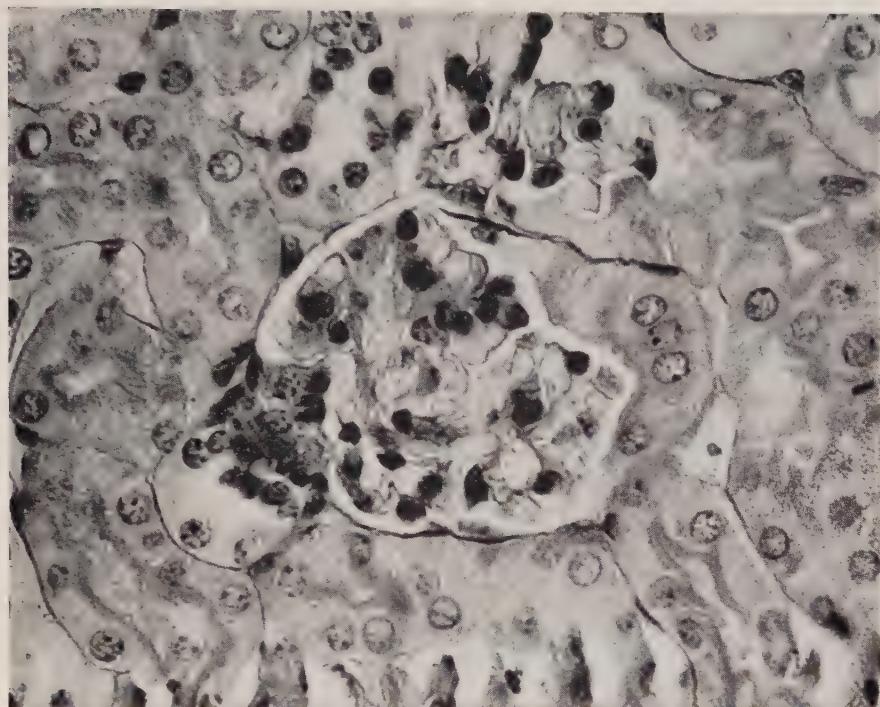


Figure 16-1. Normal glomerulus of a house mouse (*Mus musculus*). PAS—allochrome. $\times 664$

tween the glomerular lesions associated with aging in mice or hamsters and those produced by chronic treatment with ACTH make the latter particularly relevant to the present topic. It might be of interest to look for comparable changes in patients subjected to prolonged ACTH treatment.

Injections of from one to eight units of ACTH daily for ten days results in characteristic renal lesions in mice, the severity of which is dose dependent (Christian, *et al.*, 1965; Christian, 1967b). The changes are diffuse and fairly uniform. Eight units of ACTH daily produces massive deposition of intensely PAS-positive homogeneous material, presumably fibrin and plasma proteins, in glomerular capillaries, mesangium and juxtaglomerular region (Fig. 16-2); compare with the normal glomerulus in Figure 16-1. There is an increase in mesangial matrix material and a moderate increase in mesangial cells. The marked increases in mesangial mass partially or completely occludes most glomerular capillaries. Some capillaries of the tuft, especially peripheral, may be occluded by intensely PAS-positive material (Fig. 16-2), while other peripheral capillaries may be dilated and congested (Figs. 16-3, 16-6, 16-7). Generally there is deposition of intensely PAS-positive material, usually as droplets, in and prolifera-

tion of cells at the base of the glomerular stalk. Similar droplets may be found in the epithelial cells of the tuft and in the angle between the visceral and parietal the basement membranes. Four units daily of either natural ACTH or synthetic β^{1-24} corticotropin produced similar changes except that the deposits were less conspicuous (Christian, *et al.*, 1965; Christian, 1967a). If anything, there was an even greater increase in mesangial material and dilation of the peripheral capillaries. Distal convoluted tubules were dilated and usually contained precipitated protein. Often there is a similar precipitate in Bowman's space and in the interstitium be-



Figure 16-2. Renal glomerulus from a mouse (*Mus*) given 8 units of ACTH daily for 10 days. There is a marked increase in mesangial material. Some peripheral capillaries are greatly dilated and filled with intensely PAS-positive material. Several smaller deposits of similarly PAS-positive material may be seen imbedded in the mesangial matrix. Also note the fine apical vacuolation of the epithelial cells of the proximal convoluted tubules which is presumably due to glucocorticoids. PAS—allochrome stain. $\times 640$

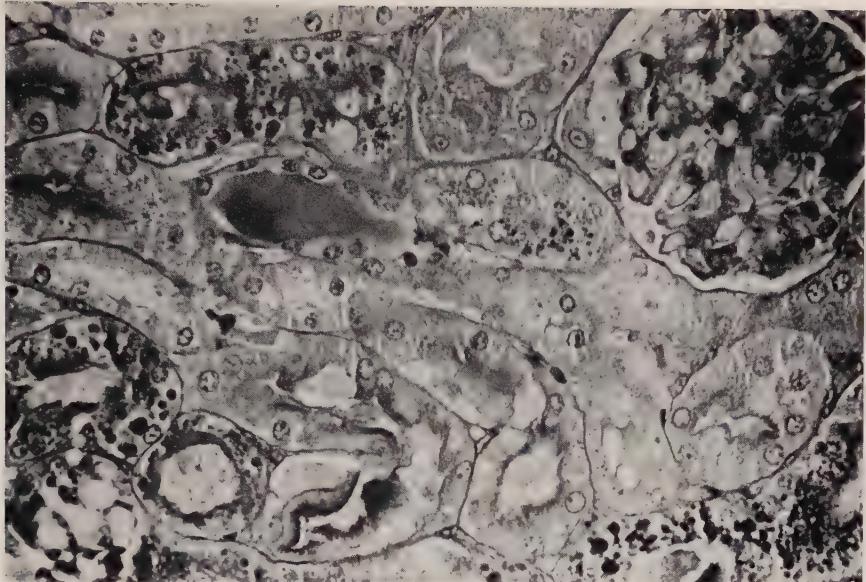


Figure 16-3. Kidney of another mouse given 8 units of ACTH daily for 10 days with PAS-positive droplets of resorbed protein in the proximal tubular epithelial cells. Similar, but smaller droplets are seen in the epithelial cells of the glomerular tuft. Distal tubules contain casts. PAS—allochrome. $\times 335$

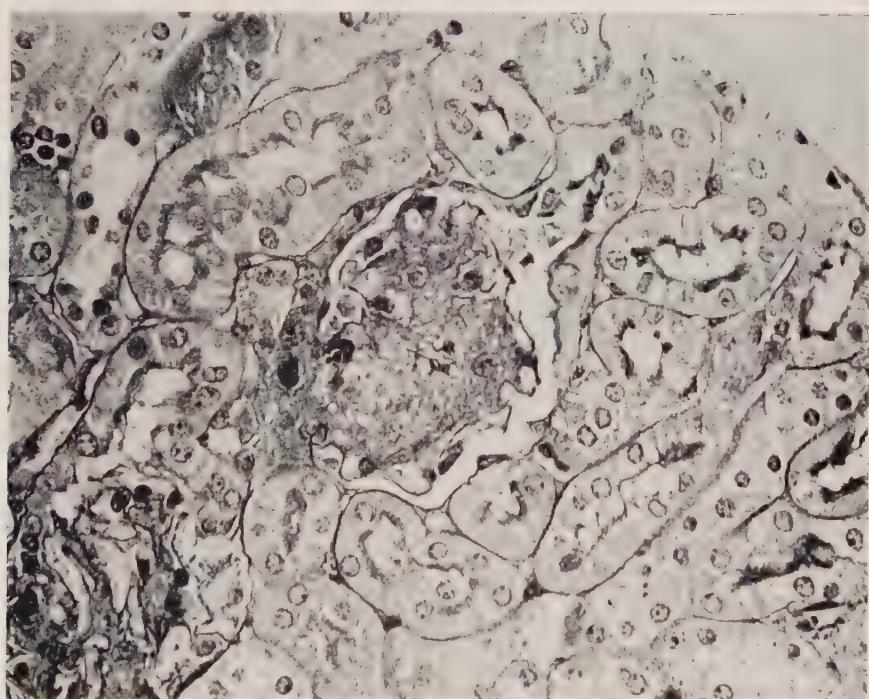


Figure 16-4. Glomeruli from a mouse given 4 units of ACTH daily for 27 days. Glomerular structure is replaced by mesangial, moderately PAS-positive, fibrillar material. The lumens of many capillaries are occluded. Only a few peripheral capillaries remain patent, and the lumens of these are greatly reduced. With H & E the tuft appears to be "hyalinized." The moderately large, strongly PAS-positive deposits of intensely PAS-positive material seen in the tuft earlier are now absent. Juxtaglomerular architecture is destroyed. PAS—allochrome. $\times 400$

tween tubules (Fig. 16-8). There is no PAS-positive deposit between the epithelial cells and the basement membrane of the distal convoluted tubules. Fine vacuolation of the apical portions of proximal epithelial cells is characteristic and resembles that produced by hydrocortisone, and is therefore, presumably a result of increased secretion of adrenal corticoids (Fig. 16-2). Also there often are PAS + droplets, presumably resorbed protein in the epithelial cells of the convoluted tubules, mainly proximal, and a PAS + precipitate in the distal and collecting tubules (Fig. 16-3). Intact and adrenalectomized mice treated with ACTH developed proteinuria that progressed for two weeks and then remained persistent and severe (Vilar and Christian, 1967). Proteinuria was greater in intact than in adrenalectomized ACTH-treated mice. Kidneys were negative for amyloid.

With more prolonged treatment with 4 units of ACTH daily the mesangial fibrillar material is greatly increased, but the number of nuclei are decreased (Fig. 16-4). Sometimes only nuclear remnants are seen in the great-

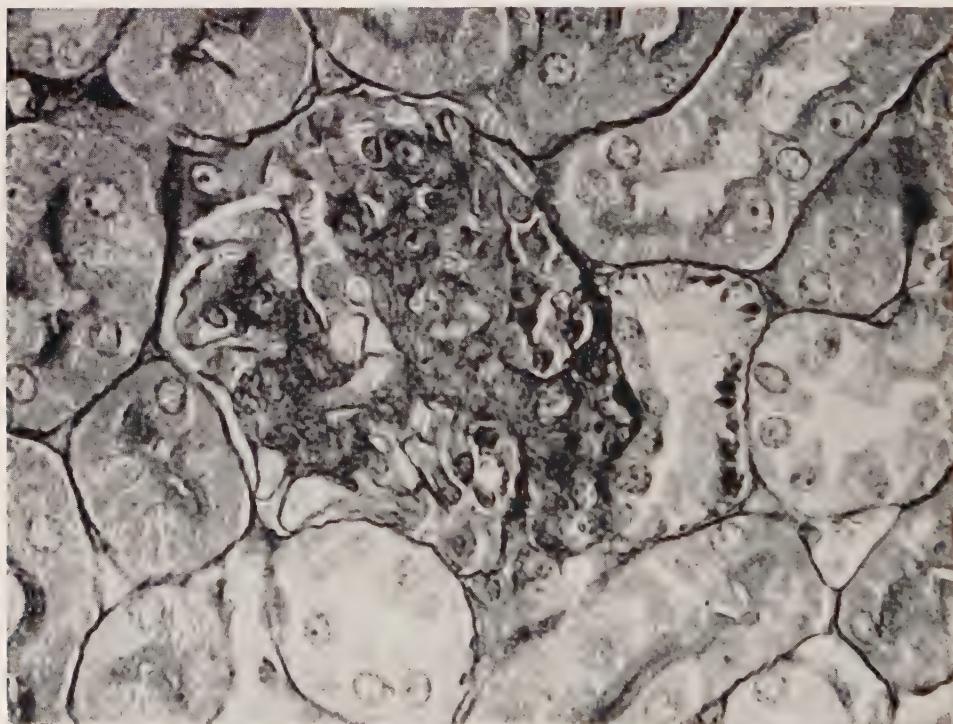


Figure 16-5. Section from a kidney of a mouse given 4 units of ACTH daily for 27 days. The distal convoluted to the right of the right glomerulus contains intensely PAS-positive "pegs" that are a hallmark of ACTH-induced renal lesions (see text). PAS—alochrome.



Figure 16-6. Section of a kidney of a mouse given 4 units of ACTH daily for 27 days. Mesangial matrix is increased and contains intensely PAS-positive deposits; its cellularity is decreased, and capillaries are dilated. However, the most striking lesion is the massive deposition of intensely PAS-positive material, presumably pooled blood constituents, *between* the distal tubular basement membrane and the epithelium. These lesions certainly represent massive leakage into the tubules, but the exact source is unknown. PAS—allochrome. $\times 640$

ly enlarged mesangium. Peripheral glomerular capillaries often are widely dilated, but may be occluded in some instances (Fig. 16-4). The most characteristic feature at this time are intensely PAS+ deposits in the basal portions of the epithelium of distal tubules adjacent to glomeruli (Fig. 16-5). These have been seen in the laboratory only following ACTH-treatment. However, they have been found in some cases of natural glomerular disease and may reflect prolonged hypersecretion of ACTH. These deposits are more or less triangular "wedges" or "pegs" with their bases on the tubular basement membrane and lying in or between epithelial cells. Their development can be traced in the kidneys of mice receiving 4 units of ACTH daily for 4 weeks. The initial lesion seems to be a massive deposit of strongly PAS-positive plasma proteins between the epithelial cells and basement membranes (Fig. 16-6). Subsequently there is condensation and

concentration of this extracellular exudate with loss of fluid and an accompanying increase in the intensity of PAS staining (Fig. 16-7). The deposits ultimately are reduced to the characteristic "pegs" or "wedges" (Fig. 16-5). They remain thus for at least 12 weeks and probably considerably longer. The presence of plasma proteins and fluid in the tubules and interstitial spaces of the kidney appears to be due to local changes in permeability. The scarcity of precipitated protein in Bowman's spaces and proximal convoluted tubules is inconsistent with leakage of proteins through the glomerulus. The leakage must be in the region of the juxtaglomerular—arteriole—distal tubule complex.

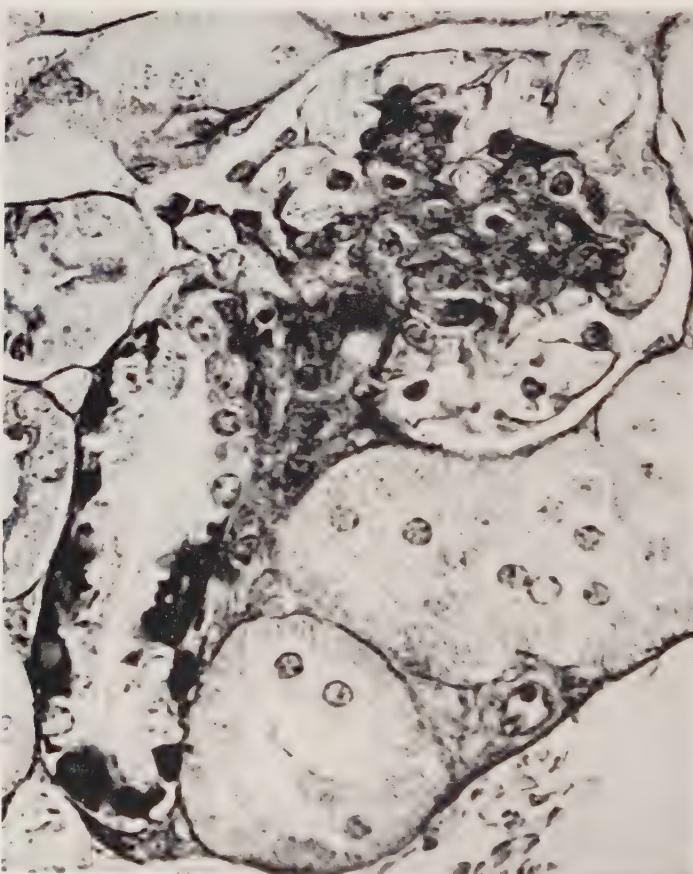


Figure 16-7. Another section from the same kidney as in Figure 16-6 showing partial consolidation of the deposits beneath the distal tubular epithelium. The deposits are smaller. Continued reduction in size logically would lead to the "pegs" shown in Figure 16-5. The glomerular changes are characteristic for this period and dose of treatment. In this instance the remaining peripheral capillaries are dilated. PAS—allochrome. $\times 640$

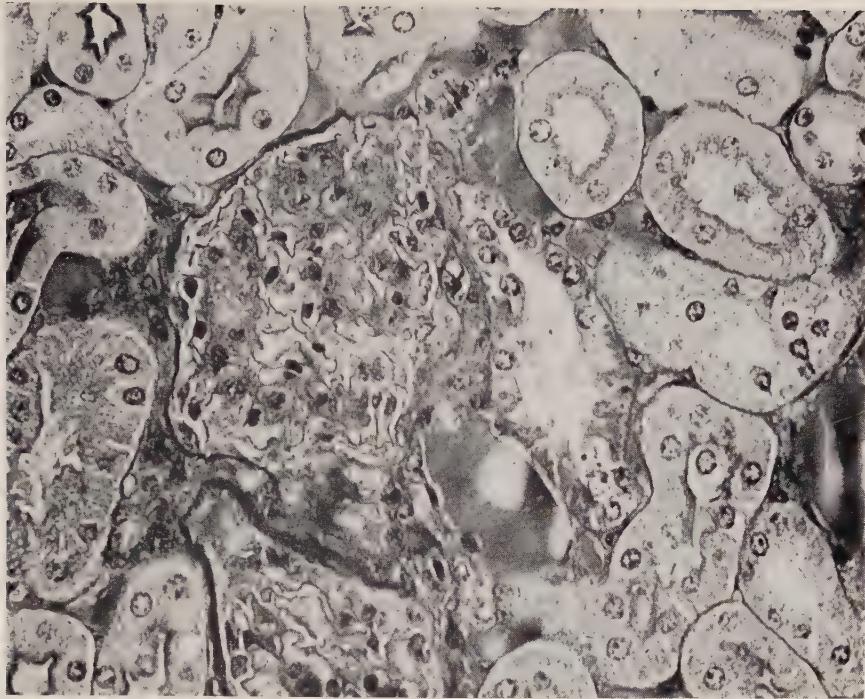


Figure 16-8. Kidney of a mouse given 4 units ACTH daily for 27 days. This figure depicts essentially complete replacement of glomerular tuft by increased mesangium as well as marked leakage of blood proteins into the intertubular spaces. PAS—allochrome. $\times 400$

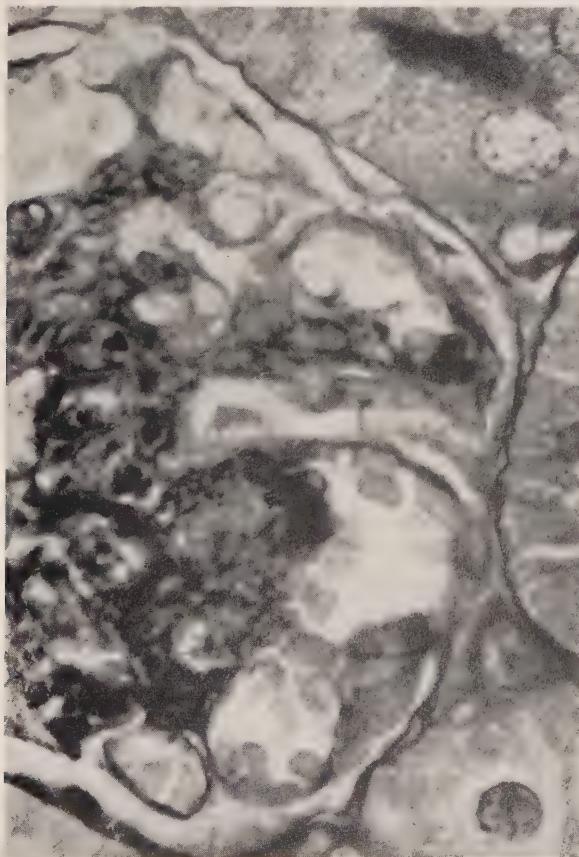


Figure 16-9. Portion of a glomerulus of a mouse given 4 units of ACTH daily for 6 weeks. Note small, homogeneous, strongly PAS-positive deposits in the mesangium as well as the increase in mesangial matrix. The deposits coincide with the electron-dense material shown in Figure 16-10. Peripheral capillaries are dilated. PAS—allochrome. $\times 1478$

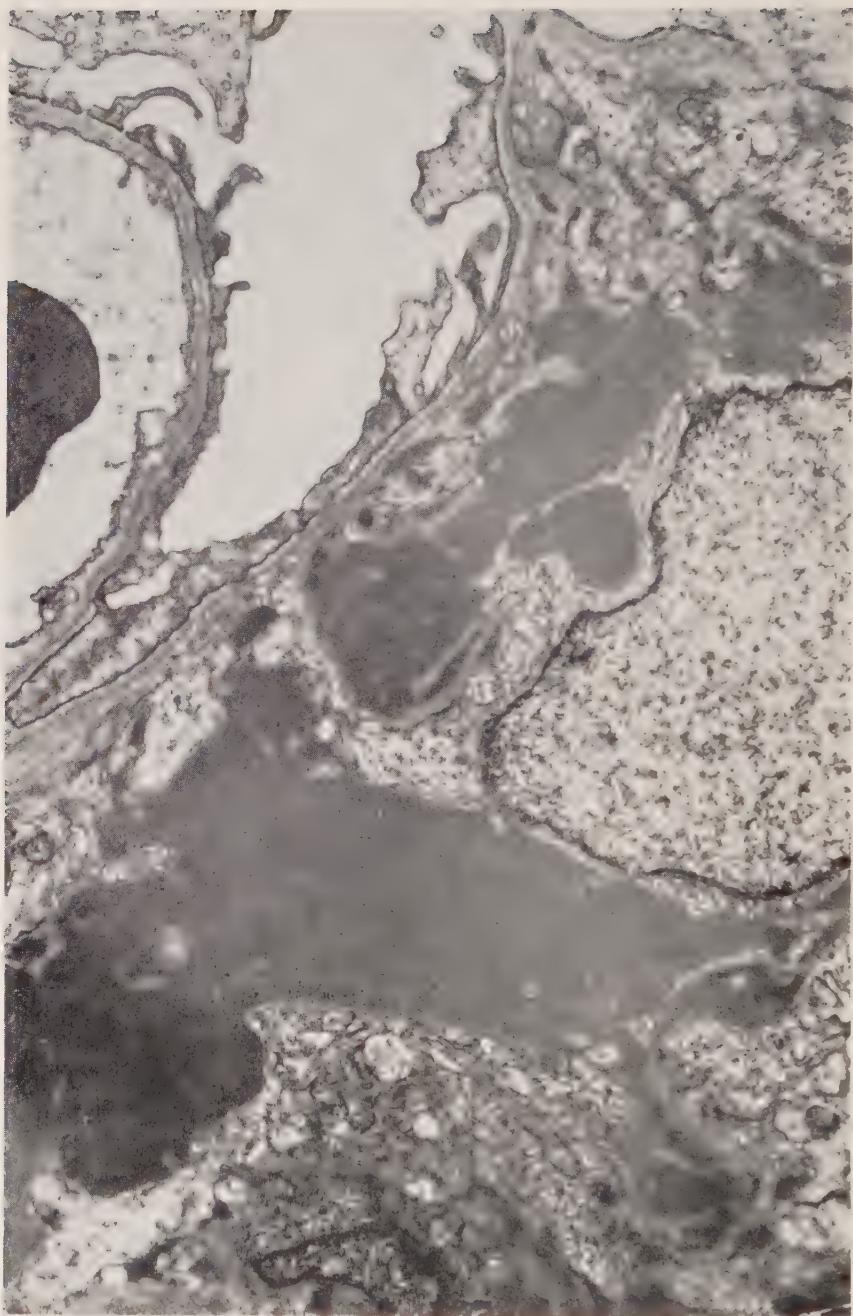


Figure 16-10. Electron photomicrograph of a portion of glomerulus from a mouse given 4 units of ACTH daily for 3 weeks followed by 6 units of ACTH for 2 weeks. There is deposition of a homogeneous, very electron-dense material within the mesangium. These deposits may become massive enough to occupy most of the mesangium and they correspond to the intensely PAS-positive material noted in the mesangium with light microscopy. The basement membranes are normal and in general the epithelial cells and their foot processes are normal. In this illustration there may be focal and minimal fusion of foot processes, but this is not the usual situation (Vilar and Christian, 1967). PAS -allochrome. $\times 22,000$. (Reproduced from Vilar and Christian, 1967, through courtesy of *Laboratory Investigation*.)

These glomerular changes are more pronounced after 4 to 12 weeks of treatment with ACTH, although the intensely PAS-positive deposits usually are reduced in size (Fig. 16-8, 16-9). They are seen by EM to be massive deposits of electron-dense material surrounding mesangial nuclei and interdigitating with mesangial fibers (Vilar and Christian, 1967) (Fig. 16-10). They compress the mesangial cells and contribute to the mesangial enlargement noted with light microscopy. There also were deposits of this material in basement membrane-like mesangial trabeculae and occasionally in axial portions of capillary basement membranes. The fibrillar basement membrane-like mesangial matrix also is considerably increased. ACTH had little or no observable effect on peripheral portions of the capillary loops. There may be a few small foci of fusion of the epithelial foot-processes, but in general the peripheral capillary basement membrane is normal. There are progressively more glomeruli involved with increasingly larger deposits of electron-dense material in each glomerulus as dose of ACTH or duration of treatment increased.

The severity of the glomerular lesions in mice (*Mus*) increased with increasing doses of ACTH. However, the most severe glomerular lesions were in intact mice receiving 4 units of ACTH daily for six weeks or the same amount given daily for 3 or 4 weeks followed by six units daily for one or two additional weeks (Christian, *et al.*, 1965; Vilar and Christian, 1967).

An outstanding feature of the ACTH-induced renal disease is the virtual absence of interstitial inflammation and thickening of the basement membrane of Bowman's capsule. PAS-positive granules of potassium deficiency are not seen in the renal papillae.

ACTH produces renal lesions in adrenalectomized mice maintained on glucocorticoids similar to those in intact mice (Christian, *et al.*, 1965; Christian, 1967a, b), but the lesions are less severe. The severity of the glomerular lesions is unaffected by the type or dose of glucocorticoid (Christian, *et al.*, 1965). The augmenting agent might be DOC, or an unidentified adrenal secretory product. The first is unlikely because the nephrosclerotic lesions are unlike those produced by ACTH. Aldosterone, 25 or 50 µg daily for 21 days, does not augment the renal lesions in mice induced by 4 units of ACTH daily, and may have slightly counteracted the effect of ACTH (Pasley and Christian, 1972b).

ACTH in equivalent doses has the same effect in mature and immature mice of both sexes (Christian, *et al.*, 1965). There is no difference with respect to sex in the severity of glomerular lesions, and castration of males was without effect (Christian, 1967b). Dehydroepiandrosterone had no effect on ACTH-induced renal lesions of female mice (Christian, *et al.*, 1965). B^{1-24} synthetic corticotrophin, with little antigenic activity, behaved like natural ACTH in approximately equivalent doses; therefore,

it is unlikely that an immune response was involved (Christian, 1967a). The absence of interstitial inflammation and only minimal leucocytosis in the glomerular tufts also suggests that there was no immunologic process involved.

Conceivably ACTH or corticoids unmasked latent infections of lymphocytic choriomeningitis (LCM), despite the dissimilarity of the renal lesions (Hotchin and Collins, 1964; Oldstone and Dixon, 1969). However, there was no focal or general chronic interstitial inflammation in the kidneys, and LCM virus was not found[†] in spleens, brains, and kidneys from 2 albino and 2 brown house mice from the same colony. LCM-free NCR mice, completely isolated from other mice, developed typical renal lesions when treated with ACTH. There was little or none of the thickening of capillary basement membranes in ACTH-induced glomerular lesions which distinguishes these lesions from those produced by slow virus disease, such as those of spontaneous renal lesions of NZB BL mice (Chaining, *et al.*, 1965; Mellors, 1965; Mellors, *et al.*, 1969; Oldstone and Dixon, 1969).

The glomerular lesions produced in mice by 4 units of ACTH daily for 10 days persisted for at least a month after injections ceased, although mean severity declined from 3.38 ± 0.24 (std. error) to 2.61 ± 0.11 , based on a scale of 0 to 5, the latter being complete glomerular destruction. The untreated control value was 1.75 ± 0.09 . All differences are significant at $P < 0.02$ or better. Thus there is some resolution of ACTH-induced glomerular lesions, but it is slow.

It is clear that ACTH produces renal glomerular lesions in mice, but they differ from those of experimental or natural immunogenic nephropathies or from those produced by DOC or aldosterone in rats.

Similar lesions are produced by ACTH in the kidneys of woodchucks (*Marmota monax*) and brush-tailed possums (*Trichosurus vulpecula*), but not in laboratory rats or hairy rats (*Rattus villosissimus*), white-footed mice, voles, or rabbits (*Oryctolagus cuniculus*) (Pasley and Christian, 1971, 1972a, b; Christian, Unpubl.).

Relationships of ACTH Renal Lesions to Those of Aging and Other Renal Lesions

While ACTH has marked pathogenic, nonadrenal mediated effects on the kidneys of house mice and some other species, its mode of action is unknown. The glomerular lesions superficially resemble some of those observed in immunogenic glomerulonephritis, particularly with respect to increase in mesangial matrix and cellularity; for example, those seen in

[†] Courtesy of Dr. Rudolf Deibel, Laboratories of the New York State Department of Health, Albany, New York.

NZB/BL mice (Mellors, 1965) or in chronic glomerulonephritis (Steiner, *et al.*, 1962a, b). However, the massive deposits of electron-dense material in the mesangium, the general lack of thickening of the capillary basement membranes, and only focal and limited fusion of epithelial foot-processes comprise a distinctly different entity. The absence of electron-dense material on either side of the capillary basement membranes of the glomeruli distinguish these lesions from those of immunologic origin in mice, rats, humans, or other species (Reid, 1956; Farquhar, *et al.*, 1959; Churg, *et al.*, 1960; Anderson, 1963; Feldman, *et al.*, 1963; Bloodworth, 1965; Henson, *et al.*, 1967; Kimmelstiel, *et al.*, 1966; Spargo and Forland, 1967; McGiven and Lynraven, 1968; Pollak, *et al.*, 1968; Salinas-Madrigal, *et al.*, 1970). Similarly the glomerular lesions produced by ACTH also differ from those of either natural or experimental diabetes in the absence of thickening of the capillary basement membranes, as well as in the characteristic pattern of deposition of intercapillary hyaline material (Farquhar, *et al.*, 1959; Beaser, *et al.*, 1963; Lannigan, *et al.*, 1964; Bloodworth, 1965; Orskov, *et al.*, 1965; Gibbs, *et al.*, 1966; Schindler and Sommers, 1966; Salinas-Madrigal, *et al.*, 1970; Mostofi, *et al.*, 1971). The vascular changes associated with diabetic or hypertensive nephropathies also are absent.

With light microscopy the glomerulosclerosis associated with hepatic disease in some instances closely resembles the ACTH-induced lesions (Bloodworth and Sommers, 1959; Sakaguchi, *et al.*, 1965; Salomon, *et al.*, 1965), and marked adrenal hypertrophy is a prominent accompaniment of hepatic glomerulosclerosis. However, the changes in the capillary basement membranes, the nature of the mesangial deposits and the absence of proteinuria distinguish the hepatic glomerulosclerosis from the ACTH-induced nephropathy in mice.

There are two additional kinds of glomerulosclerosis that might be related to the ACTH-induced disease. The first is the glomerulosclerosis associated with aging in mice, rats, and hamsters (Guttman and Kohn, 1960; Gude and Upton, 1962). Unfortunately electron microscopic studies of these lesions have not been published and interpretation of many of the published light photomicrographs is difficult. The glomerular lesions in aging mice are characterized by increases in the number and size of the mesangial cells. In advanced cases the mesangium occupies most of the glomerular tuft at the expense of capillary patency, only peripheral capillaries remaining patent. Intercapillary deposits of hyalin or fibrin were not found. However, Gude and Upton (1962) state that homogeneous eosinophilic material accumulates in the mesangium as the sclerotic process advances. With continuing sclerosis, the cellularity of the tuft decreases (Guttman and Kohn, 1960). Capillary basement membranes are not thickened, although they were embedded in PAS-positive material. The

latter feature is not seen in the glomeruli from ACTH-treated mice. The basement membranes of Bowman's capsule appear thickened in aging mice, but not always in concert with the degree of glomerular involvement. There is no splitting of the basement membrane and arteriolar lesions are rare. Cystic tubular atrophy is common, but it is rare in ACTH-treated mice, even after three months of treatment. Aneurysmal dilation of the peripheral capillaries of the tuft is common (Gude and Upton, 1962). Eventually the entire tuft becomes hyalinized, as may those in ACTH-treated mice if the dose and duration of treatment are sufficient (Fig. 16-8). Amyloid was not detected in these glomerular tufts (Gude and Upton, 1962). The glomerulosclerosis is unrelated to inflammatory disease (Guttman and Kohn, 1960; Kohn and Guttman, 1964; Guttman and Bailey, 1965). Very similar glomerulosclerosis occurs in aging hamsters and rats (Guttman and Kohn, 1960; Kohn and Guttman, 1964). Ashworth, *et al.* (1960) also reported increased prominence of the PAS-positive component of the glomerular basement membranes of aging rats.

So far the description of the glomerular lesions found in aging mice is remarkably like that for ACTH-induced glomerular lesions. However, the massive mesangial deposits of strongly PAS-positive electron-dense material following ACTH treatment apparently are not found in the aging mice. These deposits may reflect a much more acute development of the lesions with ACTH, since with lower doses of ACTH, or after much more prolonged treatment with higher doses, the intensely PAS-positive deposits are not seen. They seem either not to appear or to disappear later on. Instead there is only a marked increase in mildly PAS-positive fibrillar material in the mesangium which replaces capillaries and obscures normal architecture of the glomerular tuft. Also the massive exudate of plasma in the distal tubules and fibrin seen acutely with higher doses of ACTH in the juxtaglomerular region, and sometimes in the tuft and Bowman's space, apparently is absent in the more slowly developing glomerular lesions of aging. The pathogenesis of lesions associated with aging is not understood (Guttman and Kohn, 1960). The characteristic, irregular grouping in clusters in the mesangial cells seen in advanced lesions of glomerulosclerosis in aging mice is absent in ACTH-induced lesions, although similar clustering was produced by ACTH in possums (*Trichosurus*). Nevertheless, the lesions associated with aging resemble those resulting from ACTH treatment sufficiently to speculate that the changes with aging may result from chronic, frequent, or continuous secretion of elevated levels of endogenous ACTH. In particular they may reflect the cumulative effects of repeated episodes of increased ACTH secretion following a variety of stressful experiences. This possibility is strengthened by the fact that caging in groups, which increases ACTH secretion, augments

the effect of ACTH on the glomeruli (Christian, *et al.*, 1965). X-radiation of the kidneys accelerates the development of glomerulosclerotic changes with age (Guttman and Kohn, 1960; Rosen, *et al.*, 1961; Kohn and Guttman, 1964; Wachtel, *et al.*, 1966). The difference between the lesions of aging and those following X-radiation of the kidneys are quantitative rather than qualitative (Guttman and Kohn, 1960; Kohn and Guttman, 1964). When the remaining kidney of uninephrectomized weaning rats is irradiated, the development of glomerular lesions similar to those seen in aging mice is accelerated and there are deposits of electron-dense material in the mesangium (Rosen, *et al.*, 1968). These deposits may be similar to those seen with ACTH treatment.

Thus the glomerular lesions following ACTH treatment, aging, and X-radiation of the kidney in the several species that have been studied are sufficiently alike to suggest that ACTH may be involved in the pathogenesis of all three conditions.

The glomerular lesions of guinea pigs made anemic on a 1 percent cholesterol diet also closely resemble those produced by ACTH in mice, except that the striking hyalinization of the juxtaglomerular apparatus sometimes seen in the guinea pigs was not seen in mice (French, *et al.*, 1967). Possibly ACTH plays a role in this condition. The renal pathology seen in aging rats apparently more closely resembles nephrosclerosis or pyelonephritis than they do the ACTH lesions (Berg, 1967), although ACTH could play a partial role in their pathogenesis.

Natural Glomerular Disease in Woodchucks

Woodchucks are subject to a natural diffuse, proliferative glomerulonephropathy that may approach a prevalence of 100 percent in certain circumstances. This disease was studied to determine if there was a correlation between its incidence and severity and population density.

In many respects the renal glomerular lesions are similar to those in aging mice or due to treatment with ACTH. The sequence of development of the glomerular lesions is presumptive, as repeated studies on an animal were not possible. In the majority of animals, its severity increases with age. The principal lesion is a progressive increase in mesangial PAS-positive fibrillar material accompanied by an increase in the number of mesangial cells (Figs. 16-11 to 16-16) and often by edematous swelling of tuft epithelial cells (Fig. 16-12). These changes result in a marked increase in mesangial mass which impinges on the glomerular capillary lumens with occlusion of some (Figs. 16-12 to 16-16). A few neutrophils may be present when glomerular changes are first evident. One of the first changes is hyperchromatism and contraction of endothelial nuclei (Fig. 16-12).

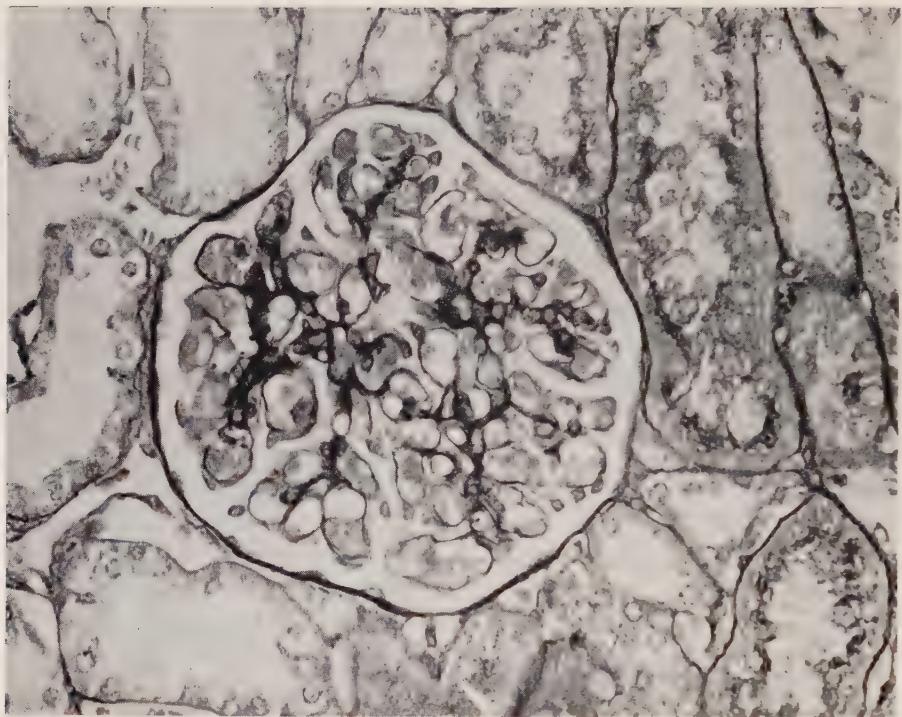


Figure 16-11. A normal glomerulus of a woodchuck. PAS—allochrome. $\times 400$

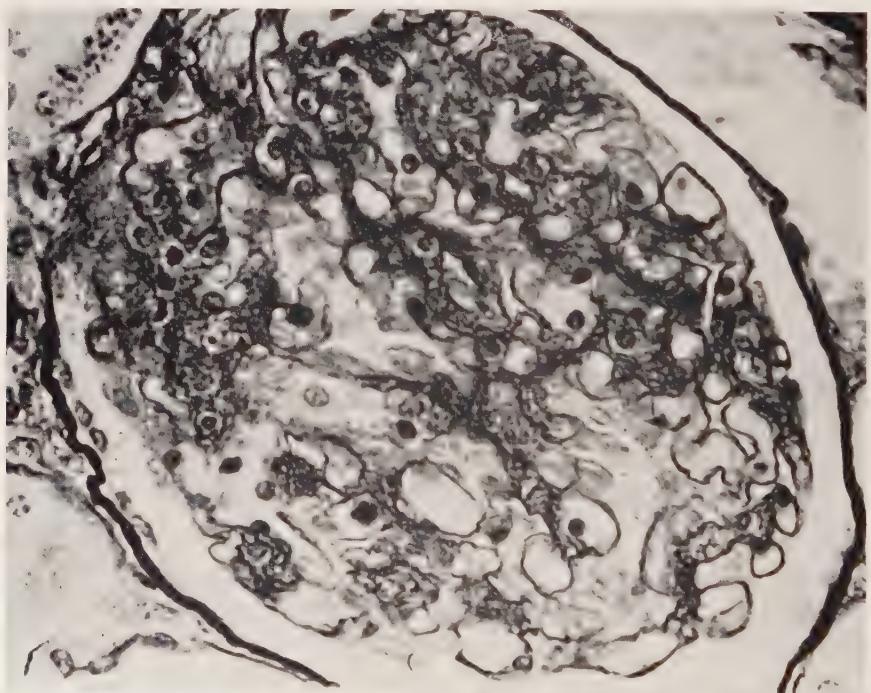


Figure 16-12. Glomerulus of a woodchuck with presumably acute glomerular lesions. Mesangial cellularity and mesangial matrix are increased. Tuft epithelial cells are swollen and "edematous." Nuclei in the base of the stalk are hyperchromatic. There are no detectable changes in the capillary basement membranes. PAS—allochrome. $\times 400$

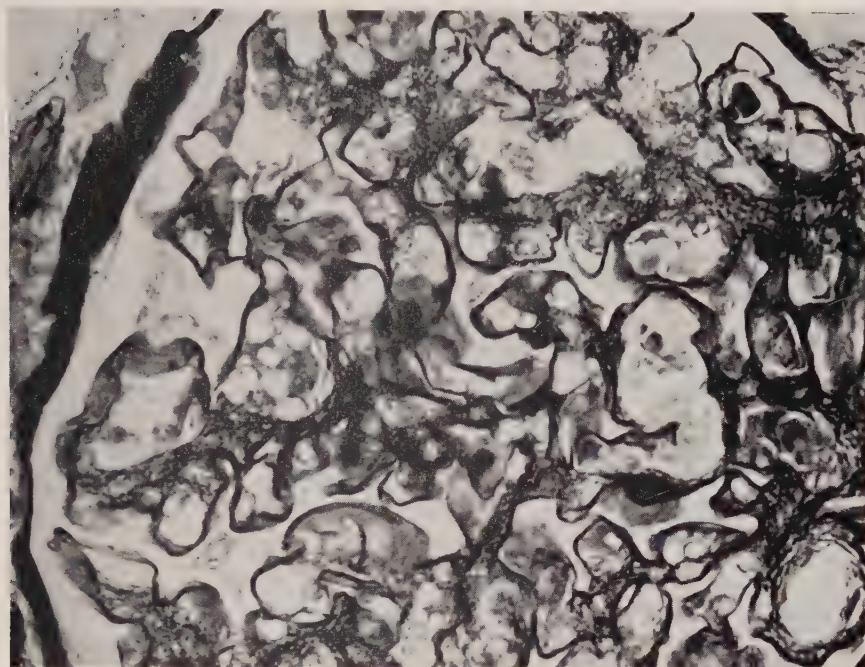


Figure 16-13. Portion of a glomerulus of a woodchuck with moderate chronic glomerular involvement. Mesangial matrix is increased and capillary lumens are reduced. The capillary loop at the lower right margin is lined by a layer of fibrillar mesangial basement-membrane-like material; the material producing the thickening of the capillary loops clearly is mesangial. The capillary loop appears to be evenly hyalinized with hematoxylin eosin staining. There is a marked increase in cellularity of the glomerulus. PAS—allochrome. $\times 640$

These nuclei are generally surrounded by a clear halo. Moderate proteinuria, edema of the feet, and ascites are associated with these lesions. At first there is no thickening of the capillary basement membranes (Figs. 16-12, 16-13) but with more advanced chronic lesions there may be irregular thickening, splitting, and reduplication of basement membranes (Figs. 16-14 to 16-16). Eventually the glomerular lesions include crescent formation, adhesions, and replacement of the tuft by mesangial matricial material (Figs. 16-14 to 16-16). The disease in woodchucks is unique in that it generally proceeds to total uniform destruction of the glomeruli with remarkably little interstitial inflammatory infiltration (Figs. 16-14, 16-15). Inflammation outside of the glomerulus, when it occurs, appears to be secondary to the glomerular disease (Fig. 16-16). Thickening of the parietal layer of Bowman's capsule and adhesions to the tuft appear to occur only with some degree of periglomerular inflammation (Figs. 16-14 to 16-16).

However, the glomerular lesions in such kidneys may resemble closely rapidly progressive subacute and chronic glomerulonephritis in humans (Figs. 16-15, 16-16). Hyperplasia and scarring of the glomerular stalk and juxtaglomerular region are constant and striking features of the disease (Figs. 16-14, 16-16). Intensely PAS-positive droplets frequently occupy the juxtaglomerular region, mesangium and visceral epithelial cells. Sclerosis of the arterioles adjacent to the glomerulus or in the base of the stalk often is conspicuous in advanced disease (Fig. 16-16), although sclerosis of



Figure 16-14. A glomerulus with a more advanced glomerular disease than that in the preceding figure. A striking increase in PAS-positive mesangial material is obvious. There is an adhesion of the tuft to the parietal layer of Bowman's capsule at the right. Most capillaries are either reduced in caliber or occluded. Sclerosis at the base of the stalk is especially noteworthy. Sclerotic arterioles were common in this kidney. A few chronic inflammatory cells are present around the thickened basement membrane of the parietal layer of Bowman's capsule, but their general paucity is notable. However, as in this disease in general, there is no thickening of the PAS-positive basement membrane proper of the glomerular capillaries, although in many instances mesangial material lines all or part of the glomerular capillary loops. The involvement of the glomeruli throughout both kidneys is relatively uniform, as is the case in this glomerular disease in general. PAS—allochrome. $\times 400$

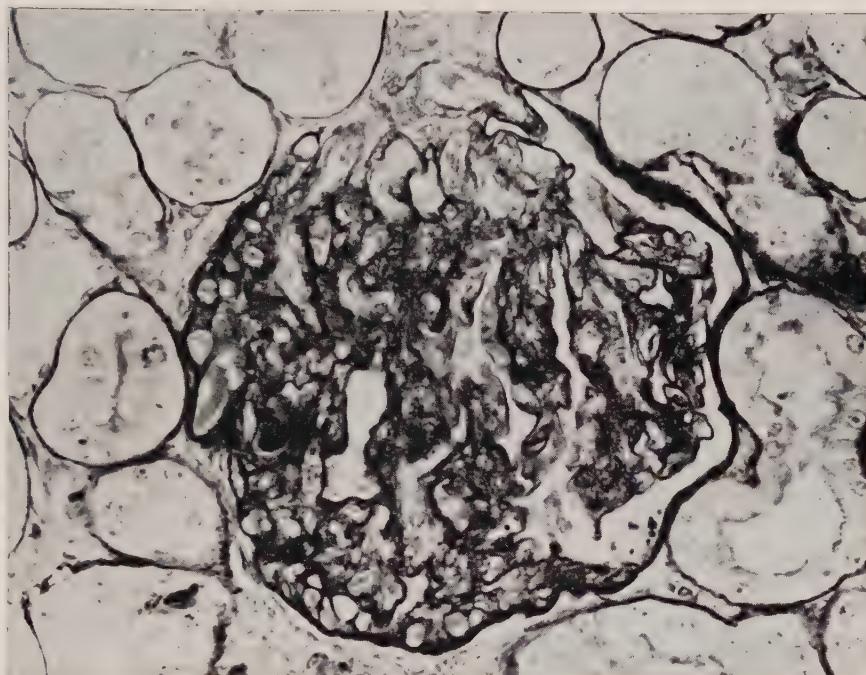


Figure 16-15. Glomerulus from a woodchuck with terminal chronic diffuse glomerulonephritis with ascites, hypertension and gross cardiac enlargement. The glomerular tuft is adherent to Bowman's capsule over much of its circumference. Few capillaries remain patent and red blood cells are rare. All glomeruli in these kidneys are equally and evenly involved. There is a slight interstitial inflammatory response, but far less than comparable human chronic glomerular disease. Tubules are not widely or markedly affected. There is sclerosis of the small arterioles. PAS—allochrome. $\times 400$

scattered arterioles may be observed earlier. This disease may be accompanied terminally by cardiac enlargement, hypertension, ascites, hydrothorax, hydropericardium, edema, and marked proteinuria. Lesions of the larger renal arteries generally are not observed.

The convoluted tubules generally are unchanged. PAS + hyaline droplets may occur in the epithelial cells of the proximal tubules and sometimes in the distal. Tubular atrophy is rare unless there is interstitial inflammation, in which case there may be atrophy or dilation. The most interesting feature of the tubules is occasional presence of intensely PAS-positive "pegs" basally located in or beneath the epithelium in segments of the distal tubules adjacent to glomeruli and similar to those produced in mice by ACTH. A few also were found in the kidneys of ACTH-treated woodchucks.

A lesion of the glomeruli, apparently identical to that produced in rab-

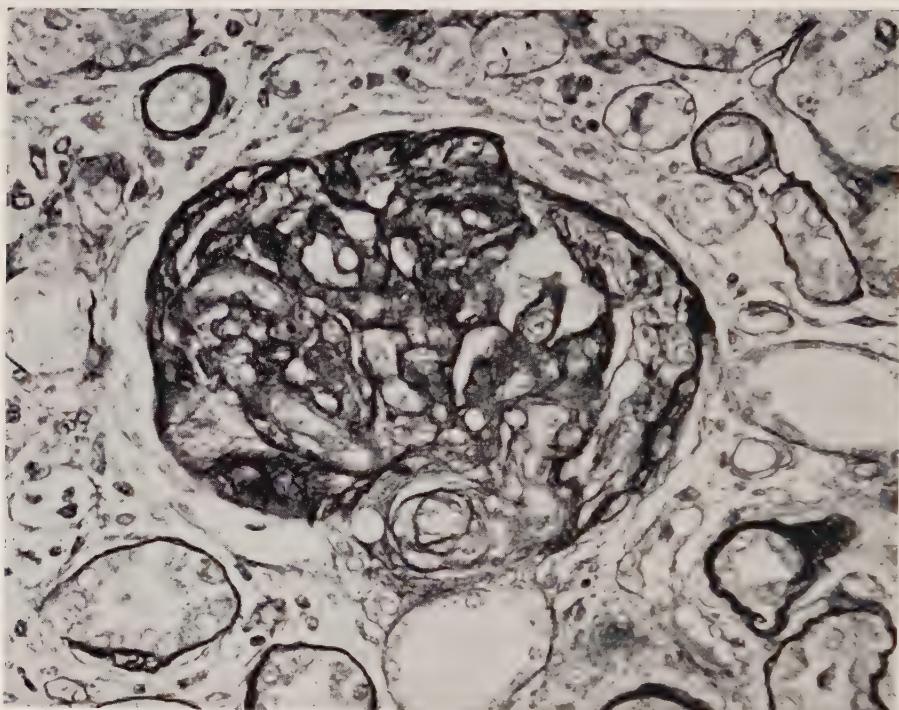


Figure 16-16. Kidney of a woodchuck presumably with rapidly progressive plus chronic glomerulonephritis. All glomeruli are about equally involved and exhibit epithelial proliferation with formation of crescents. In this instance there is marked diffuse interstitial inflammation with fibrosis and tubular atrophy. Basement membranes of most tubules are thickened. This is a relatively unusual degree of interstitial inflammation. Also there is arteriolar sclerosis with occlusion of the arteriole at the base of the glomerular stalk. The heart was nearly twice normal weight in this woodchuck. PAS—allochrome. $\times 400$

bits by cortisone or hydrocortisone, is occasionally seen. It consists of a large eosinophilic, PAS-positive homogeneous mass that fills a grossly dilated capillary and may compress the rest of the glomerular tuft. Since woodchucks secrete hydrocortisone F B ratio = .69, Salomon, Christian and Lloyd, unpubl.), these lesions may result from high circulating levels of endogenous hydrocortisone accompanying a high level of social strife (cf. Christian, 1962; Lloyd, *et al.*, 1964).

The glomerular lesions in woodchucks superficially resemble those of glomerulosclerosis produced by long-term unregulated alloxan diabetes (Beaser, *et al.*, 1963), although nodular or focal thickening of capillary basement membranes and other changes accompanying alloxan diabetes in rats (Greenberg, 1962) were not observed. Blood glucose levels in 80 animals with various degrees of renal disease, were not correlated with the se-

verity of renal lesions ($r = .04$). Diabetes appears to be an unlikely cause of the lesions in most animals. Also serum cholesterol levels in 319 animals were uncorrelated with the severity of renal disease.

Attempts to cultivate bacteria from the kidneys were negative. A variety of blood antibody determinations were negative.

Glomerular disease in over 2,500 woodchucks from this area was graded on a scale of 1 for normal to 6 for total destruction of the glomeruli. On this basis 85 percent of the population had definite renal disease with the severity averaging about $3\frac{1}{2}$ for 1955 through 1957. There were also animals that appeared perfectly healthy, but whose kidneys showed evidence of past disease with subsequent resolution and return to normal function (Christian, 1963; Christian, *et al.*, 1965). This consisted of somewhat increased mesangial cellularity in contrast to normal, an increase in mesangial intercapillary material, and lobulation of the tuft (Fig. 16-17). Practically all of the young developed glomerular disease within the first few months of life.

Reversal of the age composition of the population by trapping and removal of adults, with a probable reduction in population size, was accompanied by a significant decline in the mean severity of renal disease from about 3.5 to about 2.5. The incidence of renal disease also decreased. The

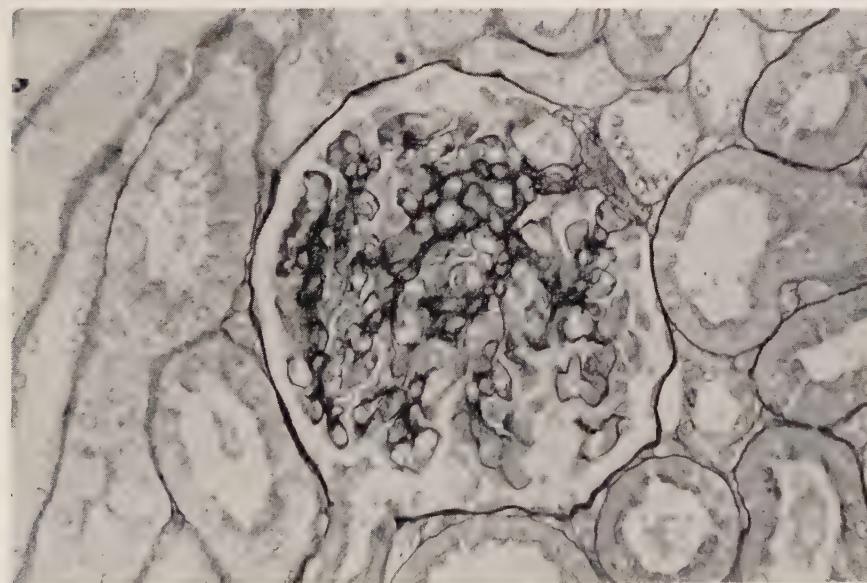


Figure 16-17. Some woodchucks evidently recover from their initial bout of glomerular disease and subsequently appear healthy and normal in all respects. This is a section of kidney from such an animal. There is residual axial scarring and distortion of normal glomerular architecture in an otherwise normal kidney. PAS—allochrome. $\times 335$

mean severity rose again when there was an influx of woodchucks from surrounding areas (Christian, *et al.*, 1965).

Reductions in social strife were accompanied by decreases in adrenal weight, presumably with decreased secretion of glucocorticoids and increased ACTH (Christian, 1962; Davis, *et al.*, 1964; Lloyd, *et al.*, 1964). Attempts to duplicate this renal disease in captive woodchucks by daily injections of corticoids were unsuccessful. However, injections of ACTH produced glomerular lesions that appeared identical to those of the naturally occurring disease, to the ACTH-induced renal lesions of house mice, and to those accompanying ageing in house mice and hamsters (Christian, *et al.*, 1965).

The handicaps inherent in a field study prevent firmer conclusions regarding the role of ACTH in producing the natural renal disease in woodchucks, but the evidence suggests that it may be important.

Amyloidosis and Group Caging of Mice

Teilum (1956, 1966) reported that the production of amyloid was accelerated by ACTH or cortisone, and the kidney is, of course, a site of amyloid deposition. Amyloidosis also develops in old mice when subjected to conditions which increase secretion of ACTH and corticoids, such as periodic electric shocks (Hall and Hall, 1960) or caging male mice in groups (Ebbesen and Rask-Nielsen, 1967). Survival of grouped males is prolonged by reserpine. In both instances amyloidosis was attributed to "stress," in the latter case due to psychologic stress resulting from aggressiveness and social strife. Both situations increase the secretion of ACTH and corticoids, but what relationship the secretion of these hormones has to deposition of amyloid is unknown. The lesions produced in mice by injected ACTH are quite distinct from renal amyloidosis (William and Peach, 1967).

Social Factors in Hypertension

As noted, caging male mice in groups enhances the effect of injected ACTH in producing renal disease and augments the development of experimental hypertension (Bernardis and Skelton, 1963). These effects probably are related to the psychosocially-induced increased secretion of ACTH and adrenal steroids in the group situation (Christian, *et al.*, 1965). Henry and Cassel (1969) suggest that short-acting psychosocial factors resulting in repeated responses of the "defense alarm reaction" may be causally important in human essential hypertension. They also present intriguing preliminary data suggesting that the rate of growth of a city, with its increasingly prevalent perturbations of the social status quo, may be related

to the incidence of hypertension in the human populations. Therefore, such factors might also play a role in some diseases of ageing.

OTHER ANTERIOR PITUITARY HORMONES

Growth Hormone and Related Problems

Pituitary growth hormone apparently may have a pathologic effect on the kidneys, but the trail leading to this conclusion has been tortuous. Crude anterior pituitary extracts (APE) are renotropic in sensitized rats, and thyroxine enhances this renotropism (Selye, 1950a). Chronic treatment with high doses of APE produced lesions resembling those produced by DOCA in similarly sensitized rats, including nephrosclerosis, dilation of convoluted tubules, and formation of casts (Selye, 1950a), although Masson, *et al.* (1949) found similar effects in only a small proportion of similarly treated rats. Adrenalectomy prevents the production of experimental nephrosclerosis and hypertension in rats by anterior pituitary preparations (Hall, *et al.*, 1946), or by pituitary growth hormone (Selye, 1951; Selye and Bois, 1956b). Combined treatment with GH and DOCA produced greater nephrosclerosis and proteinuria in sensitized rats than either agent alone (Selye, 1951; Selye and Bois, 1957), but GH did not augment nephrotoxic effects of DOCA in adrenalectomized rats (Selye and Bois, 1957). From these experiments it was concluded that the effects of APE and growth hormone were mediated by the adrenals. Cortisone prevents hypertensive cardiovascular changes associated with treatment with GH (Selye, 1951). However the stress of repeated electric shocks augments this action of GH (Hall and Hall, 1959a), just as it augments the hypertension and nephrosclerosis produced by DOCA (Hall and Hall, 1959b). ACTH or GH alone produces some degree of nephrosclerosis in sensitized nonadrenalectomized rats, but together they synergize in producing nephrosclerosis (Selye and Bois, 1956b). However, another factor must be considered at this point, namely the ability of a high salt-load alone to produce nephrosclerosis and hypertension (Wilgram, *et al.*, 1963). The production of hypertensive renal disease in rats may depend on the ability of GH and ACTH to effect sodium retention. This possibility becomes especially cogent in view of their synergism with mineralocorticoids in the production of nephrosclerosis and hypertension. GH can cause sodium retention (Biglieri, *et al.*, 1961). Furthermore, it has been shown that GH alone has some stimulatory effect on aldosterone secretion in rats (Lee and De-Wied, 1968; Palmore, *et al.*, 1969, 1970). Likewise, ACTH can stimulate aldosterone secretion and therefore sodium retention in rats (Palmore, *et al.*, 1969, 1970). However, either one alone can only partially restore to normal the secretion of aldosterone in response to sodium deprivation; but when given together, ACTH and GH fully restore it to normal (Pal-

more, *et al.*, 1970). Thus the ability of these hormones to synergize with each other in producing nephrosclerosis, as well as with DOCA and aldosterone, can be readily understood in terms of sodium retention as can the prevention of these effects by adrenalectomy.

Masson, *et al.* (1949), Rosen, *et al.* (1968) and others have suggested that increased functional demand on the remaining kidney of unilaterally nephrectomized rats at the time when hormonal factors are stimulating its growth may be a factor in producing experimental nephrosclerosis and hypertension. For example, the severity of glomerulosclerosis produced by X-irradiation, and its rate of development is much greater in weanling than older rats (Wachtel, *et al.*, 1966). The ability of food restriction to retard the development of glomerular lesions in the radiated kidneys of uninephrectomized rats supports this suggestion (Wachtel, *et al.*, 1966; Rosen, *et al.*, 1968). Starvation inhibits the normal compensatory mitotic response of the remaining kidney in unilaterally nephrectomized rats (Williams, 1962). Similarly, a restricted diet delayed the onset and reduced the severity of renal lesions associated with senescence in rats (Kennedy, 1958; Berg, 1960a, b, 1967; Berg, *et al.*, 1963; Simms, 1967). The glomerular changes present in old rats are greatly augmented by unilateral nephrectomy which produces a marked hyperplasia of mesangial cells and increase in mesangial matrix (Striker, *et al.*, 1969). This hypothesis does not seem to apply to ACTH-induced glomerular lesions of mice or the glomerulosclerosis of aging in mice, hamsters, and guinea pigs. A difficulty with this interpretation of renal lesions in the case of rats, as described earlier, is the difference between the glomerular and vascular lesions associated with various forms of experimental hypertension and those produced by ACTH or aging in other species. However, electron microscopy shows that the glomerular lesions resulting from irradiation of the remaining kidney of uninephrectomized rats, are in many respects similar to those produced by aging (Rosen, *et al.*, 1968), although there appears to be more changes in the glomerular basement membranes. For example, there is focal splitting of the basement membranes and crescent formation accompanying the glomerulosclerosis of aging in rats, particularly after unilateral nephrectomy (Striker, *et al.*, 1969). Perhaps related to this problem is the fact that the excretion of protein in the urine of rats increases with age, but its onset is greatly delayed by hypophysectomy (Everitt and Duvall, 1965). The fact that the lamina densa of the glomerular capillary basement membranes in rats increases in width with age while the laminae rarae decrease in width (Ashworth, *et al.*, 1960) may be a morphological counterpart to the increase in protein leakage.

The renotropic effects of growth hormone must be considered in the pathogenesis of the "glomerulosclerosis" associated with aging in rodents,

since they grow indefinitely, although at a reduced rate after reaching a certain size, usually at the time of, or soon after, maturation. Since renal growth continues allometrically with body growth, it means that the kidneys never escape from the threat of added loading despite the fact that their kidneys are less susceptible to damage by a variety of agents (cf. above). This may in part account for the absence of age-dependent "glomerulosclerosis" in humans. It does not, however, alter the fact that ACTH can produce renal lesions independently of the adrenals in mice and possibly other species, although GH may enter into the process.

Growth hormone has another action that bears importantly on renal disease. GH administration, 10 to 25 units daily for one to five years, produces diabetes in dogs accompanied by diffuse glomerulosclerosis and occasionally the nodular glomerular lesions of Kimmelstiel and Wilson (Bloodworth, 1965). Insudative glomerular lesions, thickening of the basement membranes of the glomerular capillaries and of Bowman's capsule, arteriolosclerosis and other features typical of diabetic nephropathy were present.

Thus GH can contribute to or cause experimental renal disease in several ways, but its role in pathogenesis of natural renal disease is unclear and complicated by the fact that nearly all of the experimental investigation of its effects, especially in hypertensive nephrosclerosis, has been in rodents, which must be considered with respect to continuous growth.

Luteotropin (LTH) or Prolactin

GH or LTH alone cause a rapid increase in body weight and a proportional increase in renal weight in rats (Bates, *et al.*, 1964). Therefore, luteotropin and growth hormone cannot be considered specifically renotropic, although both hormones stimulate general growth and with it renal growth. Nevertheless, it has been reported that high doses of LTH, which alone had no pathologic effect on the kidneys of rats, produced nephrosclerosis when given in conjunction with a dose of GH which alone produced only minimal nephrosclerosis (Selye, 1958). The significance of this effect of LTH with respect to renal disease is obscure, its possible pertinence to renal disease in aging is still more so.

Gonadotropins: Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)

The effects, if any, of these pituitary hormones on the kidney have not been investigated specifically, although they affect the kidney indirectly through the hormones of their target organs. For example, it is well established that natural androgens are renotropic (Selye, 1950a). Testosterone also increases the resistance of the kidneys of mice to sublimate poisoning

(Selye, 1950a) and methyl-testosterone to DOCA-induced nephrosclerosis (Selye and Rowley, 1944).

Thyrotropin (TSH)

It has been mentioned in several instances that thyroxine aggravates nephrosclerosis and other experimentally-produced renal lesions. It also is renotropic and alters the effects of pituitary hormones (Selye, *et al.*, 1945; Milkovic, *et al.*, 1964). These are, of course, indirect effects of TSH. There is little, if any, information on the direct actions of TSH with respect to renal disease.

CONCLUSIONS

Since hypertension is a widespread disease of aging it has resulted in many attempts to duplicate it in experimental animals. The kidneys have long been thought to be involved in the pathogenesis of hypertension; consequently, it is not surprising that manipulation of the kidneys, particularly production of renal ischemia, has been a conspicuous experimental approach to the problem. The production of nephrosclerosis in rats by growth hormone and ACTH was also investigated. However, since the adrenal glands were necessary for the nephrosclerotic action of these anterior pituitary hormones, attention soon shifted to sodium-retaining adrenal steroids and their analogues. With these it was possible to produce hypertension and nephrosclerosis in unilaterally nephrectomized animals on a high salt intake. The discovery of the very potent natural mineralocorticoid aldosterone was followed by intense interest in the regulation of its secretion by the adrenal and led ultimately to the discovery that the renin-angiotension system was an important factor in controlling its secretion. More recently it has been found that GH and ACTH are both necessary for the full response of aldosterone to sodium depletion in rats. This seems to bring us full circle back to the original experiments with these pituitary hormones and probably explains the important role of sodium chloride in experimental production of hypertension by hormonal means. However, whether or not the hypertension produced in animals by these means is applicable to the human disease is still uncertain. Nevertheless, there is very high incidence of hypertensive disease in Cushing's disease (Heptinstall, 1966). Furthermore, there is evidence that at least some kinds of hypertensive disease in humans are accompanied by elevated levels of angiotension and that the kidney is involved at least to the extent that it produces renin. There are other well known relationships between the kidney, its vasculature, and hypertensive disease. However, to what extent the pituitary might be involved in the natural pathogenesis of renal disease and hypertension in humans is as yet unknown. Even the validity of hormonal experimental hypertension in rats as a model of human disease is

questionable because, among other differences, of the apparent difference in the control of aldosterone secretion between rats and man. Nevertheless, the anterior pituitary has been directly implicated in the pathogenesis of renal glomerular disease in some species since ACTH produces severe glomerular lesions, involving mainly an increase in mesangium, in the absence of the adrenals. These lesions are remarkably similar to those accompanying aging in mice and hamsters. Therefore, it is possible that renal changes with aging in these animals might be due to ACTH. This possibility is supported by the fact that radiation accelerates and augments such changes. They are, nevertheless, similar to those occurring widely in a natural population of woodchucks and that appear to be related to social status, density, and to ACTH secretion. However, the lesions induced by ACTH in mice are unlike those of any well characterized human renal lesions. Therefore, it remains questionable whether or not the glomerular lesions associated with aging in rodents have a counterpart in human renal disease associated with aging.

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CHAPTER 17

COMPARATIVE ASPECTS OF HYPERADRENOCORTICISM AND AGING

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SUMMARY

HYPERADRENOCORTICISM has been observed in man, rats and fish associated with their reproductive efforts. Various metabolic and patho-physiologic derangements such as hyperglycemia, hyperlipidemia, hypertension and such degenerative changes as kidney stones, diabetes, arteriosclerosis, myocardial infarction, cerebrovascular accidents, decreased resistance to infection, premature aging and death are concomitants of the hyperadrenocorticism. This Cushing's disease-like spectrum of degenerative diseases and accelerated aging is believed to be due to the hormonal excesses induced by the reproductive effort via the hypothalamic-pituitary-adrenal-gonadal axis.

INTRODUCTION

Pregnancy, in the human, is often accompanied by aberrant changes in lipid metabolism, e.g. hypercholesterolemia and hypertriglyceridemia; in carbohydrate metabolism, e.g. diabetes; in protein metabolism, e.g. muscle wasting and other catabolic changes; as well as alterations in fibrinolytic and blood clotting mechanisms, e.g. a hypercoagulable state. In addition, hypertension and other vascular complications may compromise the course of pregnancy. Most of these degenerative patho-physiologic events have been ascribed to the abnormal hormone production associated with the reproductive effort. Particularly implicated with these patho-physiologic events is the increased production of the adrenocortical and gonadal steroids. These potent hormones increase progressively during each of the trimesters of gestation, reaching a peak at parturition and decreasing, almost precipitously, in the early postpartum period. With this decrease in the circulating levels of these dynamic anabolic-catabolic steroids the ab-

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normal degenerative changes, e.g. hyperglycemia, hyperlipidemia, hypertension, etc., also disappear. If these increased circulating steroid levels should persist considerably beyond parturition, then the abnormalities such as diabetes and hypertension also persist or become fixed, e.g. hyperadrenocorticism or Cushing's syndrome.

We have been intrigued with the possibility that hormones, particularly the adrenocortical and gonadal steroids, may play a larger role in the pathogenesis of cardiovascular disease than is currently appreciated. Special emphasis has been given to the role of the hypothalamic-pituitary-adrenal-gonadal axis and to the dynamic alterations which activation of this axis may express through morphologic, physiologic and biochemical vectors, which occur during the reproductive cycle and the relevance these changes would have to vascular disease. The adage that, "one is no older than one's arteries" is very apropos and therefore, we also relate our investigations to the problem of aging. These investigations, during the course of many years, have encompassed this problem on a broad, comparative basis.

The patho-physiologic changes which may complicate human pregnancy and which also occur in Cushing's disease or hyperadrenocorticism are well known. Robertson and Wexler (1957) have described the development of arteriosclerosis, hypercholesterolemia, hyperglycemia, accelerated aging and other dramatic degenerative changes in spawning Pacific salmon and rainbow trout. These patho-physiologic changes have been correlated with the concomitant hyperadrenocorticism which becomes progressively more intense as the fish fight their way upstream to the spawning grounds, i.e. piscine Cushing's disease. A strikingly similar "syndrome" of hyperadrenocorticism associated with intense or active reproductive effort—similarly complicated by the development of arteriosclerosis, hypertension, hyperglycemia, hyperlipidemia and accelerated aging has also been described in repeatedly bred male and female rats by Wexler, *et al.* (1967).

Thus, in the spawning fish, the repeatedly bred rat and in man himself there is a rich amount of bio-medical evidence which would suggest that the reproductive effort and its associated dynamic physiologic adjustments may contribute to the pre-mature appearance of vascular and degenerative disease changes and accelerated aging.

HYPERADRENOCORTICISM IN THE HUMAN

Because of the great strides made in modern medicine classical, full-blown Cushing's syndrome is seldom seen these days. Although these cases are relatively rare, when they do appear, one can observe, "all of the diseases that flesh is heir to," all embodied in one unfortunate patient. The abnormality responsible for this manifestation of such a broad spectrum

of diseases may reside in the hypothalamus, pituitary or the adrenal glands but the end result is the over-production of adrenal steroids with devastating metabolic and degenerative changes (Cushing, 1932; Heinbecker, 1944; Liddle, *et al.*, 1962; Williams, 1968).

Patients with full-blown Cushing's disease develop hyperglycemia or "steroid diabetes" which is not responsive to insulin. Under the aegis of the excessive quantities of glucocorticoids their islets of Langerhans often become hyperplastic and the insulin-producing beta cells degranulated indicative of decreased, "beta cell reserve." At the same time, because of the dynamic lipid-mobilizing effects of the adrenal steroids there is also a definite tendency toward the development of hyperlipidemia, e.g. hypercholesterolemia and fatty steatosis of the liver concomitant with an unusual anatomic distribution of fat. The Cushingoid patient appears to be obese but the "obesity" is truncal and is confined to the upper torso (Fig. 17-1). The breasts are often pendulous accompanied by an unusual accumulation of inter-scapular fat, i.e. the cervicodorsal fat pad or "buffalo hump." However, this appearance of obesity is deceiving since the extreme rounding of the face or "moon facies" and other distortions of normal body contour are largely due to heightened sodium retention and edema.



Figure 17-1. Patient with well-advanced Cushing's syndrome. Notice the moon facies, hypertrichosis, kyphotic posture, pendulous breasts and truncal obesity. This 18-year-old negress appears to be 40 years old.

Actually, catabolic activity is rampant and there is great wasting of the muscles and adipose tissue of the lower extremities and thinning of the skin. This rarification of the dermis is conducive to easy bruising, ulceration and poor wound healing. The red or purple striae which appear about the breasts and abdomen are more than stretch lines as the striae gravidarum of pregnancy. Rather, the striae reflect the abnormal carbohydrate metabolism induced by the gluconeogenic effects of excess corticosteroids produced by hyperplastic adrenal glands, deranged intercapillary ground substance or mucopolysaccharide and subsequent elastic tissue disruption.

Cushing's disease patients also manifest other cosmetic problems. Often times, there is generalized or patchy deep pigmentation of the skin relative to the excess production of adrenocorticotrophic or melanocyte stimulating hormone. Acne about the face as well as hirsutism or hypertrichosis about the upper lip, chin, sideburns and about the arms, breasts and legs are due to the excess production of adrenocortical and gonadal steroids, i.e. 17-ketosteroids.

Skeletal muscle is particularly liable to the catabolic inroads of excess adrenal steroids. As the adrenal steroids convert muscle protein into sugar the muscles waste and the patient complains of easy fatigability—one of the earliest manifestations of Cushing's disease. A similar catabolic process occurs in the bones so that these patients often develop generalized osteoporosis. Osteoporosis of the vertebrae is conducive to the kyphosis or to the humped-back appearance which is so characteristic of the patient with Cushing's disease. In addition to osteoporosis these patients develop hypercalcemia and there is a high incidence of renal calculi. Nephrosclerosis contributes to the early demise of patients with Cushing's disease. Sodium retention concomitant with excessive loss of potassium is quite prevalent. There is a corresponding electrolyte shift in the smooth muscle cells of the arterial wall, vasoconstriction or vasospasm with ensuing hypertension. Polycythemia and alkalosis further compromise the burgeoning array of physiologic derailments. Proper function of the gastrointestinal tract is also compromised, e.g. gastric ulceration. Function of the reproductive system may be interrupted with loss of libido in males and irregular or scant menses in the female. The ovaries of Cushingoid patients are often reduced in size, of a white rather than a normal yellow color, there is disappearance of primordial follicles, loss of follicular activity, patchy thickening and fibrosis of the ovarian stroma (Iannaccone, *et al.*, 1959). All of these changes, when found in young patients with Cushing's disease are akin to the usual menopausal changes and are indicative of premature aging.

Because glucocorticoids are lymphopenic or leukopenic they will increase antibody titers through their lytic effects on white blood cells and the consequent release of gamma globulin. However, unrelenting hyperadrenocorticism causes unabated destruction of white blood cells and anti-mitotic effects on thymocytes and other reticulo-endothelial cell elements which culminates with involution of the reticulo-endothelial system. This leads to loss of resistance to infection or a state of increased susceptibility to the inception and spread of infection. The invasiveness or noninvasiveness of tissue is controlled by hyaluronidase or ground substance spreading factors which are under the dynamic influence of adrenal steroids (Asboe-Hansen, 1966). Many Cushingoid patients succumb to broncho-pneumonia or intercurrent infectious disease.

The excitability of the central nervous system is greatly influenced by the adrenal steroids; Cushing's disease patients manifest bizarre personality or mood swings ranging from frank euphoria to severe manic depression. In fact, one of the hallmarks of Cushing's disease is the aberrant personality changes which often precede the other, more devastating, metabolic degenerative changes. Paramount to all of these psychic and metabolic changes is the hypothalamus and its control of the normal circadian or diurnal rhythm of adrenal steroidogenesis. The normal diurnal rhythm of

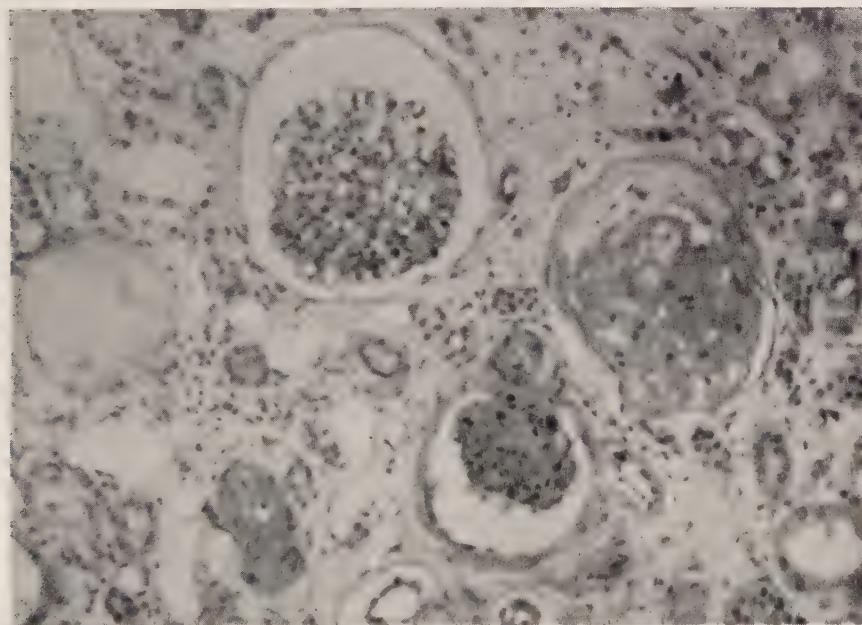


Figure 17-2. Advanced arteriolo- and nephrosclerosis in kidney of a patient with full-blown Cushing's syndrome. Glomerulus (on right side of photo) is completely hyalinized. Note extensive sclerosis of the renal parenchyma. H&E, $\times 175$.

high cortical secretory levels in the early morning tapering off toward nightfall is virtually lost in Cushing's patients with constant production of these potent catabolic hormones.

In addition to generalized connective tissue and ground substance degenerative changes, the elastic tissue, in particular, undergoes dissolution and there is widespread arteriosclerosis (Fig. 17-2) with considerable calcific complications, e.g. kidney stones as well as calcification of the arterial lesions. Cushing's disease patients often manifest marked EKG abnormalities, cardiac enlargement, tachycardia and many succumb due to myocardial infarction or to a cerebrovascular accident. Pertinent to this presentation, the chronic hyperadrenocorticoid state and its attendant metabolic sequelae of diabetes, hyperlipidemia, hypertension, osteoporosis, arteriosclerosis and muscle wasting are often attended by an unusual acceleration of the normal aging process so that the patient appears prematurely aged. This is particularly apparent in young patients suffering from Cushing's disease, e.g. a young patient of 18 years may appear to be 40 years old.

HYPERADRENOCORTICISM OF PREGNANCY IN THE HUMAN

During the several trimesters of human gestation there are also profound alterations in hormone production, e.g. adrenal and gonadal steroids. The adrenal glands undergo progressive hyperplasia particularly during the second and third trimester of gestation. Following parturition there is a marked involution of the adrenal cortex and an abrupt cessation of the previously active steroid production. This sudden cessation of corticosteroid production is often the cause of the bizarre post-gestational withdrawal symptoms which some women exhibit. If labor is difficult and prolonged some women may manifest symptoms of Addisonian crisis due to the stress of labor and the postpartum involution of the adrenal cortices.

Because of the relative hyperadrenocorticism of pregnancy there are profound metabolic and physiologic alterations during gestation which could be conducive to the induction of cardiovascular disease. For example, during pregnancy there is a progressive fatty infiltration of the liver, i.e. fatty liver of pregnancy, hypercholesterolemia, hyperlipidemia, islet hyperplasia and beta cell depletion with the appearance of overt diabetes in some cases (Baird, 1969). There is a definite tendency toward obesity which is often difficult to contain by dietary restriction. Sodium retention, edema, hypertension and toxemia are not infrequent concomitants of pregnancy. Dental caries, hypercalcemia, osteoporosis, varicosities, thrombo-phlebitis, hypercoagulability, arterial ground substance alterations, myocarditis and other cardiovascular complications also appear during gestation.

The alterations in adrenocorticoid production during the several trimesters of pregnancy bear particular relevance to, and are largely responsible for the spectrum of degenerative and metabolic changes described. Women who may be suffering from allergies or skin dyscrasias and a variety of other ailments will show progressive improvement in their symptoms when the adrenal glands begin to produce steroids in greater quantities during the second trimester. Symptoms may disappear completely during the third trimester only to reappear with renewed vigor during the immediate post-gestational phase of adrenal cortical involution. Women suffering from arthritis, if they should be fortunate enough to conceive, are often relieved of their symptoms during their pregnancy only to have a prompt recurrence of symptoms immediately postpartum. Increased adrenal steroidogenesis during pregnancy affords these women sufficient steroids to affect effective anti-inflammatory and anti-rheumatic relief.

Particularly germane to the theme presented here is the fact that many female patients with Cushing's disease can trace their symptoms of hyperadrenocorticism to a preceding pregnancy. That is, the gestational over-activity of the hypothalamic-pituitary-adrenal-gonadal axis is maintained postpartum with consequent persistence of the Cushingoid status. A corollary of this would be Sheehan's or Simmonds' disease where infarction of the once hyperemic pituitary gland during parturition precipitates the opposite spectrum of metabolic and physiologic symptoms, e.g. protracted Addisonian problems or crisis. A variation of this theme is the pregnant, grossly overweight and diabetic mother, who is late to deliver and eventually gives birth to a stillborn child or one weighing 10 to 11 pounds. These overweight babies often have Cushingoid facies, are temporarily diabetic and their islets of Langerhans are hyperplastic with degranulated beta cells. In time, this diabetic condition, imparted to the child by the mother, disappears. Usually, the maternal diabetes recedes promptly after parturition (Baird, 1969). However, if the diabetes-prone mother conceives a second or third time, with each pregnancy spaced closely upon the other, the once labile diabetes can become fixed or persistent. Similar problems connected with repeated pregnancies can occur involving other dynamic metabolic parameters.

Because man is such a heterogeneous animal it is difficult to put this concept of parity, hyperadrenocorticism and degenerative physiologic changes to proper test. However, there are several reasonably "pure" ethnic groups where problems clearly related to repeated pregnancies do provide some credence to our thesis. One such example may be found in the case of Natal Indians living in and about Durban, South Africa. These Indians originally migrated from India to Africa because of unfavorable economic conditions. In time, their affluence increased greatly and their badge of

affluence is demonstrated by the number of offspring in each family. Natal girls often marry at the relatively early age of thirteen. Because of strong religious and other ethnic ties marriages are often between closely related partners. Families of twelve or thirteen closely-spaced children are common. The Natal women become obese, hyperlipidemic, hyperglycemic, hypertensive, hyperuricemic, hirsute, age prematurely and often die due to a cerebrovascular complication or myocardial infarction (Campbell, 1963). Incidentally, their spouses also develop diabetes, apparently on a parity basis, and many marriages occur between Natales widowed by the demise of a spouse due to the cardiovascular problems which attend diabetes. There are other examples, throughout the world which would suggest that repeated breeding and consequent hyperadrenocorticism due to overactivity of the hypothalamic-pituitary axis may lead to abnormal metabolic changes, degenerative disease, premature aging and death.

Clinical endocrinologists have been aware of the Cushingoid habitus characteristic of some pregnant women and of very obese individuals. The fact that more overt degenerative Cushingoid changes are not seen is due to various compensating mechanisms pertaining to the absolute and relative level of circulating steroids, total body mass, renal clearance and other considerations. For example, a truly obese patient will exhibit hyperlipidemia, hyperinsulinemia and will have relative hyperadrenocorticism. However, if effective reducing measures are taken and total body mass is reduced the Cushingoid status will be alleviated (Wood, *et al.*, 1960). A similar condition may be operative during gestation. Further, it has been shown that during gestation extra circulating steroids are transported or bound to proteins, i.e. albumin or globulin or transcortin (Slaunwhite and Sandberg, 1959). It is believed that in the unbound or free form the adrenal steroids can exert their dynamic metabolic effects. However, when bound to protein they are relatively innocuous. As pregnancy progresses, the adrenal cortex quickens its production of steroids, however, the protein-binding or transcortin level also increases. Theoretically, if it were not for this protective protein-binding of the adrenal steroids many more pregnant women would manifest the untoward effects of Cushing's disease. Interestingly, the circulating levels of estrogen also increase dramatically as gestation progresses and estrogens effect increased binding of steroids to protein. Some investigators theorize that the reason why some pregnant women do develop overt signs of Cushing's disease is due to their failure to produce adequate levels of estrogen or due to some intrinsic defect of protein-binding of steroids. A similar mechanism is believed to be involved when women using contraceptive drugs evince Cushingoid levels of protein-bound steroids (Streeten, *et al.*, 1969). This falsely elevated level of steroids is ascribed to the pseudo-pregnancy state induced by con-

traceptive drugs or more specifically to the estrogen moiety contained in most contraceptive drugs currently in vogue. Again, the worrisome untoward changes encountered by some women taking contraceptive drugs may be due to their inability to affect proper binding of the elevated adrenal steroids occasioned by their pseudo-pregnant state, e.g. hyperlipidemia, hyperglycemia, hypercoagulability, bizarre psychic changes, headache, migraine and even "strokes" (Salhanick, *et al.*, 1969).

The recent findings of specific nerve centers in the hypothalamus controlling the release of pituitary tropic hormones, e.g. adrenocorticotropic, gonadotropic, thyrotropic and somatotropic hormones, has opened up tremendous vistas concerning neuro-endocrine regulation of homeostasis. New insight is being gathered concerning psychosomatic aspects of reproduction, degenerative disease processes and particularly, the regulation of diurnal or circadian rhythm (see below). Patients with brain tumors or compression fractures which impinge upon hypothalamic nuclei governing the various pituitary tropic hormone releasing factors will exhibit alterations in their normal circadian steroid secretory rhythm. Similarly, patients suffering from cerebrovascular hemorrhage or thrombosis will show bizarre alterations in their steroid secretory rates and it is not uncommon to find acute ulceration of the gastro-intestinal tract in such patients which can be ascribed to the unusually high corticoid production (Oka, 1956; Dalgaard, 1960). Evidently, this hypothalamic "biological time clock" is a built-in or evolutionary mechanism since blind individuals do not exhibit variations from normal circadian patterns despite the absence of light stimuli. This fascinating information concerning physiologic rhythms and the ebb and tide in the human body is most intriguing and provocative when one considers the myriad varieties of bio-medical inter-relationships, e.g. the migration of birds, jet air travel, the aging process and a multitude of other implications.

DEGENERATIVE CHANGES AND PREMATURE AGING RESEMBLING CUSHING'S DISEASE IN REPEATEDLY BRED MALE AND FEMALE RATS

As our interest in the interrelationships of reproductive physiology, hyperadrenocorticism, Cushing's disease and aging increased we continued to search for experimental models which would help us to explore our thesis further. Although there are several experimental models which do have applicability to our hypothesis of hyperadrenocorticism and premature aging, no one model was sufficiently adequate to expend any unusual amounts of investigative effort. At this time, Hench and Kendall (1949) made their astute observation concerning the alleviation of pain in arthritic female patients who succeeded in becoming pregnant, i.e. increased

adrenal secretory activity of pregnancy and related steroid anti-rheumatic or anti-inflammatory effects. Added impetus was also provided to the author's central research theme when patients receiving the then, newly-available adrenocorticotrophic hormone (ACTH) or adrenal steroids, e.g. cortisone, developed the untoward effects of steroid overdose and became Cushingoid, i.e. iatrogenic Cushing's disease. At the same time, the author had the good fortune to be working under the auspices of Drs. H. M. Evans, M. E. Simpson and C. H. Li at the University of California and was able to observe and participate in the early investigations concerning the isolation of ACTH and to delve into the manifestations of pituitary-adrenal stimulation. While at Stanford University, the author and several associates were investigating a tropic substance extracted from the pituitary gland which unlike conventional ACTH preparations had unusual adrenal weight or growth promoting activity (Liddle, *et al.*, 1954; Jailer, *et al.*, 1957). This material, in animals and patients, would cause adrenal hyperplasia and lipid storage and would make the adrenal gland unusually sensitive to a challenging dose of conventional ACTH so that the patient or animal would respond with an inordinately high production of adrenal steroids (Liddle, *et al.*, 1954; Jailer, *et al.*, 1957). The author speculated that such a truly tropic hormone might account for the adrenal hyperplasia of pregnancy or the unusual sensitivity of the adrenal glands of Cushing's patients to relatively modest doses of ACTH.

Quite fortuitously, a unique animal model became available to us which would enable us to experiment and probe into our thesis of hyperactivity of the hypothalamic-pituitary axis, degenerative diseases and aging. In the early 1950's the author was searching for a suitable source of "older" animals for purposes of gerontological research. No truly old animals were available but the author did learn that commercial suppliers of rodents for biomedical research discard their breeder animals when they have outlived their effective reproductive capacity. Keeping our central hypothesis in mind that pregnancy is a state of relative hyperadrenocorticism and that repeatedly bred animals might display evidences of extra adrenal activity, we began to experiment with repeatedly bred rats. In order to make breeder rats even more hyperadrenocorticoid we gave them long-acting ACTH over a period of time. Just prior to sacrifice, we found that virgin animals given ACTH were normotensive but the breeder females given the same ACTH had severe hypertension. At autopsy, we were surprised to find that the breeder females had severe, lurid-appearing, calcific arteriosclerosis extending from the arch of the aorta distal to the bifurcation of the iliac arteries. Multiple saccular and fusiform aneurysms were also found along the length of the aorta. In addition to the severe arteriosclerosis these ani-

mals also displayed other evidence of degenerative changes, i.e. kidney stones, gastric ulcers, polyarteritis nodosa and particularly, gross signs of aging such as kyphotic posture, lethargy and constantly shedding and disheveled fur (Figs. 17-3 to 17-6). These animals were fed a commercial rat diet which has a relatively low fat content (4%). Also, the rat is ordinarily resistant to the induction of hypercholesterolemia and arteriosclerosis. Therefore, we reasoned that the excess release of adrenal steroids caused by the chronic injection of ACTH had broken through this resistance and had induced the degenerative syndrome of arteriosclerosis and premature aging. However, we subsequently discovered that repeatedly bred females already have established arteriosclerosis *prior* to their being treated with ACTH. Thereupon, we began an epidemiological survey of repeatedly



Figure 17-3. Photo of a repeatedly bred Sprague-Dawley rat (left) after six successive pregnancies compared with a virgin female rat of comparable age. Note the rounded contour of the head (breeder rat), kyphotic posture, the matted and disheveled fur. Light plucking on this animal's left flank led to virtually complete removal of fur. The breeder animal appears much older than the virgin control.

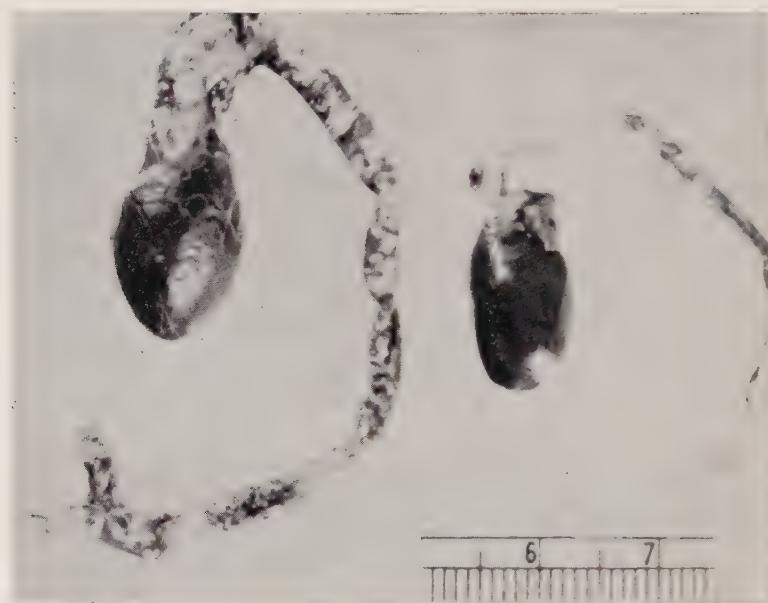


Figure 17-4. Cardiovascular system of the two animals shown in Figure 17-3 removed for purposes of comparison. The heart of the breeder rat is enlarged and exhibits marked left ventricular compensatory hypertrophy. The breeder rats also have advanced hypertension. The aorta is severely ectatic and calcific plaques extend throughout the length of the aorta, up into the carotid (upper edge of photo) and into the iliac arteries. Oftentimes, the aortae of female breeder rats have single or multiple saccular or fusiform aneurysms.

bred rats gathered from all parts of the United States. We found that the appearance of arteriosclerosis, premature aging and other degenerative changes is a naturally-occurring phenomenon in all of the strains of breeder rats we examined irrespective of geographical location, diet, drinking water or climatic or seasonal variations (Wexler, 1964a). Since the severity of the spectrum of degenerative changes varied considerably between the various strains of breeder rats we then began to examine whether the frequency with which the commercial dealers bred their animals might be of significant import. It soon became apparent that this was a likely possibility. Thereupon, we designed an extensive series of scientifically-controlled breeding experiments in our own Research Breeding Colony in which a careful history was kept of the number of litters sired by male breeders, the number and frequency of pregnancies, the number of young nursed and other pertinent information. A large colony of breeder animals was provided for so that a sufficiently large number of animals could be sampled after each successive pregnancy or litter sired, i.e. after 1, 2, 3, 4, 5,

6, 7, 8 etc., breedings. In this way, we hoped to be able to reconstruct the pathogenesis of this naturally-occurring spectrum of degenerative diseases and accelerated aging.

Particularly intriguing and provocative, but very puzzling, was our discovery that the male breeder rats used repeatedly as studs developed essentially the same syndrome of degenerative diseases as the repeatedly pregnant female rats. However, there is an unusual sex dichotomy in the severity and anatomical distribution of the arterial lesions. Most outstanding, however, is the fact that the repeatedly bred males are unable to withstand stressful procedures and they age and die significantly earlier than the breeder females.

One of the first changes we observe is that the islets of Langerhans in the pancreas become progressively hyperplastic with each successive pregnancy or breeding (males). Similarly, the insulin-producing beta cells become progressively degranulated and by the time of the fifth reproductive



Figure 17-5. Photomicrograph of the aortic arch of a Sprague-Dawley female breeder rat (5 pregnancies). The raised intima contains copious quantities of mucopolysaccharides and is interlaced by many strands of collagen. The internal elastica is eroded and many of the elastic laminae are covered by granular material (deep black in photo). This material is an admixture of calcium and mucopolysaccharide. When these elastic fibers fragment the area either becomes repaired by calcification or a protruding aneurysm will herniate its way outward. H&E, $\times 150$.



Figure 17-6. Severe gastric ulceration of a repeatedly bred female rat (9 pregnancies). There is extensive mucosal necrosis underlined by fibrinoid. H&E, $\times 10$.

cycle the beta cells begin to show hydropic degeneration (Wexler and Fischer, 1963a). Subsequent glucose tolerance tests of male and female breeder rats demonstrated that there is a definite and progressive development of abnormal glucose tolerance becoming worse with each successive, closely-spaced breeding until these animals become definitely diabetic (Wexler and Fischer, 1963b). Their diabetes is reminiscent of steroid diabetes since the degree of hyperglycemia is not too severe. Concomitant with the development of diabetes there is a progressive fatty metamorphosis of the liver so that by the fifth or sixth breeding or pregnancy there is marked fatty infiltration of the liver (Wexler, *et al.*, 1964; Wexler and Kittinger, 1967). This is accompanied by progressively increasing hypercholesterolemia, hypertriglyceridemia and an atherogenic type of beta:alpha lipoprotein ratio (Wexler, *et al.*, 1964). With each succeeding pregnancy or breeding there is progressively increasing hypertension, hypercalcemia and nephrolithiasis (Wexler, 1963a, b), particularly in the male breeder rat. One of the most salient features of the untoward changes associated with repeated breeding is the progressive hyperplasia of the adrenal cortex (Wexler, 1964c). The adrenal glands become increasingly hyperemic with each successive breeding. There is extensive lipid depletion from the zona

glomerulosa, and eventually, by the fifth or sixth pregnancy or breeding a major number of breeder rats manifest adrenocortical hemorrhage, thrombosis and infarction. In the female breeder, as many as 10 percent of the successively pregnant females will manifest pheochromocytoma-like impingement of the adrenal medulla upon the adrenal cortex causing compression of the cortex against the capsule. All of this histopathologic evidence of markedly increased adrenocortical activity is accompanied by progressive and severe involution of the thymus gland. The adrenocortical hyperplasia and thymic involution in both repeatedly bred male and female rats strengthened our growing suspicion that increased adrenocortical secretory activity may be playing a large role in the pathogenesis of the naturally-occurring degenerative changes.

Of particular import to us was the pathogenesis of the arteriosclerosis. As early as the first or second reproductive cycles microscopic lesions appear in the abdominal aortic segment of both male and female breeder rats (Wexler, 1964a). With each succeeding reproductive cycle the arterial lesions grow in complexity, morphologically and biochemically, and at the same time the lesions begin to spread anatomically. Like man, the lesions appear first in the abdominal aorta appearing subsequently in the arch and thoracic aortic segments and radiating outward into the coronary (Wexler, 1964b), carotid (Wexler and True, 1963), renal, mesenteric and peripheral arteries (Wexler, 1964d). By the third or fourth reproductive cycle an interesting sex dichotomy becomes apparent between male and female breeder rats. The lesions in the male breeder rats persist to be of microscopic proportions, within the aorta, but become grossly visible in the common iliac arteries. In the female breeder, grossly visible arterial lesions become detectable in the abdominal aortic segment and eventually in the aortic arch and thoracic aortic segment with each successive pregnancy, i.e. fourth, fifth and sixth pregnancy. Despite the comparatively milder form of arterial disease in the male breeder rats they, nonetheless, die significantly earlier and in much greater numbers than the female breeders. Their demise is usually due to an intercurrent infection, complications of their diabetes or hypertension and most often due to myocardial infarction (Wexler and Kittinger, 1965).

Of special interest is the fact that although the spontaneously-occurring arterial lesions are accompanied by hyperglycemia and hyperlipidemia which all become progressively worse with each successive reproductive cycle, morphologically the arterial lesions contain very little lipid but consist of intimal mucopolysaccharide accumulations followed by deposition of collagen or fibrosis (Wexler, *et al.*, 1964). This intimal ground substance and connective tissue change is apparently related to smooth muscle or mesenchymal cell activity of the aortic wall. This is pertinent because

adrenal steroids have profound effects on mesenchymal cells and connective tissue and all the more so in view of our finding of abnormal adrenocortical activity in breeder rats (please see below). With continued breeding the intimal lesions become more complex and extensive and ground substance and connective tissue degenerative changes now appear in the media accompanied by elastolytic degeneration, calcification, cartilaginous metaplasia and even bone formation. For example, in the repeatedly bred female rat the carotid arteries are particularly prone toward calcification and bony metaplasia so that they appear markedly ectatic, kinked and tortuous (Wexler and True, 1963).

We have made breeder rats severely hyperlipidemic by feeding them high fat diets (Wexler and Kittinger, 1967), or severely diabetic by means of alloxan or both hyperlipidemic and hyperglycemic by chronic treatment with adrenal steroids or ACTH. Although the arterial lesions become definitely exacerbated as do the other degenerative changes, the morphologic characteristics of the arterial lesions remained unchanged, i.e. relatively little lipid and copious accumulations of mucopolysaccharides and collagen. Because of these findings we felt that the hyperlipidemia and hyperglycemia of repeated breeding was probably due to the endogenous mobilization of lipid and glucose occasioned by excess adrenocorticoids released during repeated stimulation of the hypothalamic-pituitary-adrenal-gonadal axis during the stress of repeated breeding. Therefore, we began to focus our attention on the nature of adrenal steroid production of repeatedly bred rats.

Since the rat has so little circulating steroid at any one time we were compelled to resort to *in vitro* analyses of the steroidogenic capacity of the adrenal glands of repeatedly bred rats. First, by paper electrophoresis and later, by gas chromatographic methods we were able to separate the alpha-ketolic spectrum of steroids produced by rat adrenal glands incubated *in vitro* (Wexler and Kittinger, 1965). The adrenal glands of breeder rats, when challenged by ACTH were unable to produce normal quantities of total steroid when similarly compared with the adrenal glands removed from virgin rats of comparable age. When radioactive progesterone was added to adrenal incubates the adrenal glands of virgin rats were able to convert this steroid precursor, quite readily, into corticosterone (Compound B), the definitive steroid produced by the rat adrenal cortex. However, the adrenal glands of breeder rats were unable to effect such conversion (Wexler and Kittinger, 1965). This inability to respond to a challenging dose of ACTH and to convert progesterone and other adrenal steroid precursors into definitive adrenal steroids became even more meaningful when we found that the adrenal glands of breeder rats contained super-

abundant quantities of cholesterol which is the starting material for all corticoid steroidogenesis. This suggested that repeated breeding caused progressive "exhaustion" of the steroidogenic capacity of breeder rat adrenals and that this was probably due to some progressively worsening cortical enzymatic defect or to a progressive refractoriness to ACTH. In this same connection we found that breeder rats produce unusual quantities of 18-hydroxy-deoxy-corticosterone which is a precursor of aldosterone. This would again indicate that the enzymes responsible for the conversion of 18-hydroxy-deoxy-corticosterone into definitive aldosterone, are missing or defective. This would also conform with our finding of progressively increasing hypertension and lipid depletion from the zona glomerulosa of the adrenal cortex of breeder rats. That is, the mineralocorticoids influence electrolyte metabolism and vascular contractibility and blood pressure levels and the histopathologic demonstration of adrenal zonal lipid depletion would conform with the physiologic and biochemical evidence of impaired synthesis and release of adrenal steroids.

As indicated above and from our demonstration of actual decreased production of Compound B with continuous breeding it appears that after a period of temporary hyperadrenocorticism the adrenal glands of breeder rats eventually become unable to produce steroids. This we have confirmed with our most recent *in vivo* adrenal steroid studies and by transplanting pituitary glands from repeatedly bred rats into hypophysectomized virgin recipients (Wexler and Saroff, 1968). In keeping with this alternating increased then decreased adrenal secretory activity in breeder rats we have also found progressively increasing basophilia in their pituitary glands. In this connection, the basophilia can be interpreted to be indicative of increased synthesis of ACTH followed by adrenocortical refractoriness to ACTH. Therefore, we believe that repeated breeding causes increased stimulation of the hypothalamic-pituitary-adrenal-gonadal axis which causes increased stimulation of the adrenal cortex and steroid production. This state of hyperadrenocorticism induces the various patho-physiologic changes, e.g. hyperlipidemia, arteriosclerosis, etc., or Cushingoid changes. Eventually, however, the adrenal cortices lose their steroidogenic capacity and exhibit thromboses and vascular collapse which would account for the unusual susceptibility of breeder rats toward infection or stress and the concomitant induction of premature aging and death. Virgin rats of comparable age do not develop any of the above stigmata. Senile rats, 2 to 3 years old, will develop these degenerative changes, however. Since breeder rats develop these changes, spontaneously, when they are less than 7 months old we consider their Cushingoid habitus, arteriosclerosis and premature death to be indicative of an acceleration of their normal aging process.

PISCINE CUSHING'S DISEASE

As we continue to gather information concerning hyperadrenocorticism and its possible interrelationships with reproductive physiology in the human and in repeatedly bred rats we also had an on-going, long-term project concerning the post-spawning explosive aging and death of the Pacific salmon. To our amazement and delight it became apparent to us, over the years, that much of the basic pathophysiology operative in spawning salmon was fundamentally similar to that which occurs in hyperadrenocorticoid humans and in repeatedly bred rats. In fact, my esteemed colleague Dr. O. H. Robertson, partly facetiously, referred to the breeder rats as "repeat spawners" because of the great similarity of the degenerative changes in breeder rats to those found in spawning salmon.

The Pacific salmon are born in quiet little streams and, as fingerlings, begin their downstream migration to the Pacific Ocean. The young salmon disperse over wide ranges of the Pacific. After a maturation period of four to five years these salmon grow in length and girth eventually becoming 80 to 90 pounds in weight. While in their ocean habitat the salmon feed and store-up energy, particularly in the form of hepatic lipid. At this time there is little or no lipid in their sera. Governed by a similar seasonal biological time clock located in the hypothalamus, the salmon pituitary gland begins to release a spawning reflex hormone as well as other pituitary tropic hormones and the fish begin their long migratory journey back to the river of their birth. Salmon, separated by thousands of miles, have been known to appear at the mouth of the river of their birth within one-half hour of one another. As the salmon begin to swim upstream they stop feeding and begin to use their hepatic reserves for energy. Hepatic lipid decreases and concomitantly, serum cholesterol begins to rise. As the fish leaves the salinity of the ocean and enters the "sweet" or brackish waters of its native river the cardinal veins in the head kidney display clusters of adrenocortical cells which are arranged in glomules resembling the zona glomerulosa of the mammalian adrenal gland. The fish produce aldosterone and it would seem logical, teleologically, that the increased aldosterone production permits the transition of the fish from the salt water of the ocean to the sweet water of the river (Robertson and Wexler, 1957; Robertson and Wexler, 1959).

The salmon begin to fight their way upstream and at this time their pituitary glands become hyperplastic and cytoplasmic stains indicate that there is active production of tropic hormones (Robertson and Wexler, 1962a & b). In the Pacific Northwest, many salmon beat themselves to death against the concrete dams in their frenzied migration to the spawning grounds. Those which avail themselves of the "fish ladders" must then

escape the many predators, animal and human, which await their annual upstream migration. Within an incredibly short space of time, one to two months, the surviving salmon find their way to their original stream in which they were born 4 to 5 years earlier, age explosively and die. During this short time, the male and female have built up vast stores of sperm and eggs. Having used up their hepatic stores of lipid their livers are now completely devoid of lipid droplets but their sera is milky, containing as much as 1000 mg% of cholesterol. A devastating progeria-like or accelerated aging transpires during this brief upstream migration (Figs. 17-7 to 17-13). The once lush-looking pituitary gland shows complete degranulation of all of its cell types, these cells become "ghost-like" as cellular detail is lost and finally, the pituitary gland becomes transposed into a gliotic mass

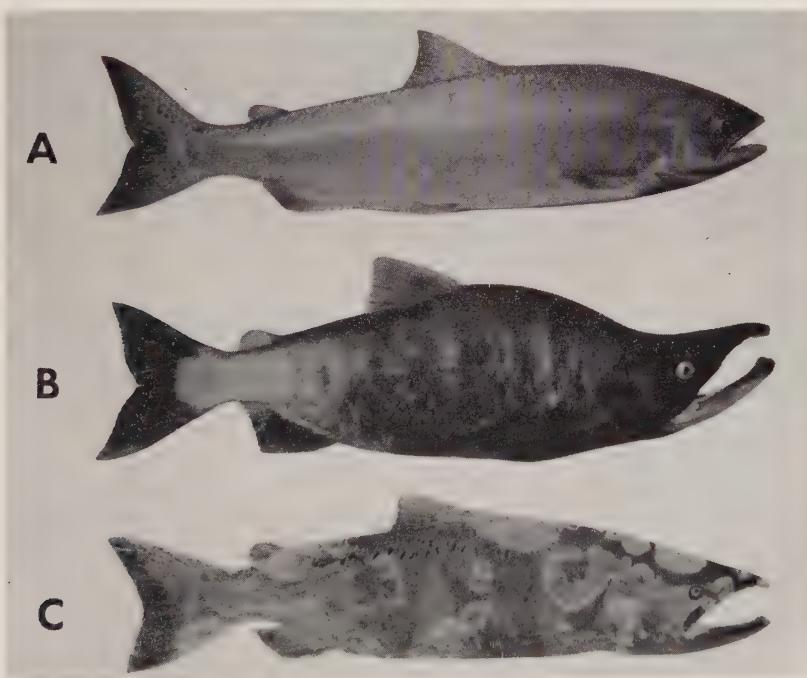


Figure 17-7. Normal (A), spawning (B) and spent (C) salmon. A. Normal, full grown but sexually immature sea salmon (Kokanee) just prior to its upstream migration. Note its normal appearance and contour—it is silvery in color. B. Spawning salmon. This fish is sexually mature. Note the hooked jaw, sunken eyes, humped back and red-colored skin. These fish have diabetes, arteriosclerosis, hyperplastic and hyperactive adrenal glands and the full spectrum of degenerative changes characteristic of Cushing's disease and manifest definite progeric changes. C. Spent salmon, taken on the spawning grounds after a long migration. Note extensive distribution of patches of fungus. These fish have a severely involuted reticulo-endothelial system concomitant with hyperplastic adrenal tissue and have decreased resistance to infection.



Figure 17-8. Slice of head kidney of an immature, sea salmon (King). No adrenocortical tissue is visible grossly. $\times 2\frac{1}{2}$.



Figure 17-9. Slice of head kidney from spawning salmon (King). Adrenal cortical tissue is now quite plentiful and can be detected grossly (white patches in photo). Contrast with specimen shown in Figure 17-8. $\times 2\frac{1}{2}$.



Figure 17-10. Coronary artery of a spawning, fall-run, male salmon. Two atheromata which are lipid-free. The hyperplastic intima projects into the lumen. The media (light grey in photo) has become relatively thin and individual muscle fibers are disrupted. The adventitia shows the characteristic thickening due to progressively increasing deposition of collagen during the upstream migration. Both intima and media contain unusual quantities of acid mucopolysaccharides. H&E, $\times 150$.



Figure 17-11. Myocardial infarct in a spawning salmon. The large, white, punched-out triangular area is a confluent patch of necrotic tissue. H&E, $\times 150$.

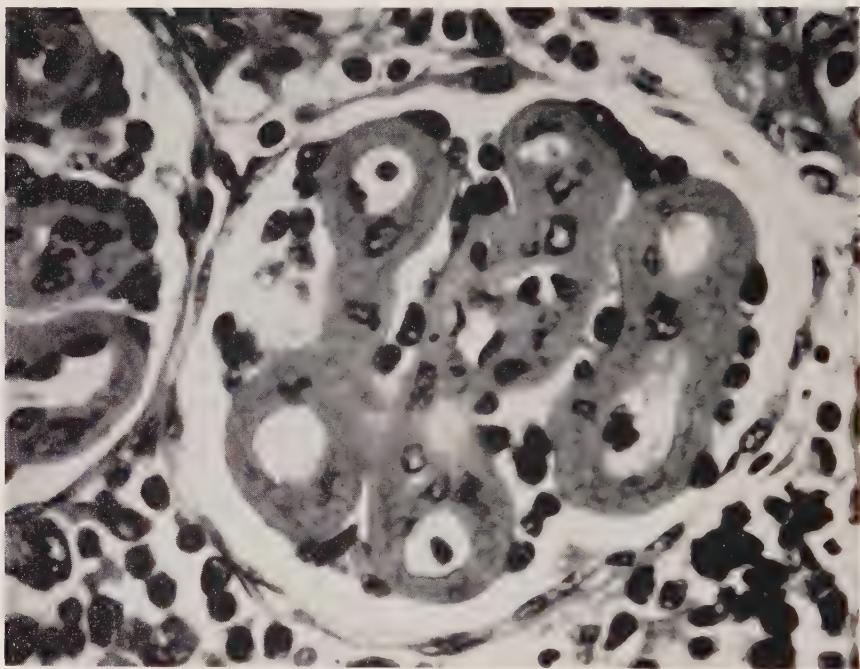


Figure 17-12. Glomerulus of a spent salmon showing extensive capillary thickening, i.e. glomerulosclerosis. H&E, $\times 250$.



Figure 17-13. Rainbow trout given lethal dose of hydrocortisone. Fish is covered with fungus, ichthyophthiriasis. Each white dot in the photo represents a focus of infection. This photo was taken 17 days after implantation of a steroid pellet. This fish expired on the 20th day.

of scar tissue (Robertson and Wexler, 1962a & b). During the active upstream migration the interrenal organ which consisted of glomules of cortical cells undergoes accelerated mitoses and sheets of adrenocortical cells grow radially outward arranged in columns resembling the zona fasciculata of the mammalian adrenal cortex (Figs. 17-8, 17-9). At this time, the salmon produce great quantities of adrenocorticoids (Robertson, *et al.*, 1961; Robertson, *et al.*, 1963), some of them being Compounds B, E and F—the predominant glucocorticoids produced by man. During this period of increased adrenal steroid production (Hane, *et al.*, 1966) fat is mobilized from the liver and peripheral adipose tissue sites and their sera becomes hyperlipemic. The fish has discrete islets of Langerhans sequestered between its pyloric villae. While in the ocean, the islets are barely detectable but during the upstream migration the islets become hyperplastic or giant-sized and the insulin-producing beta cells become degranulated (Robertson and Wexler, 1960). The salmon become hyperglycemic. In fact, the renal glomeruli exhibit wire-loop lesions which resemble the Kimmelstiel-Wilson lesions found in some human diabetics (Fig. 17-12). The skin becomes discolored, thin, and completely covered by patches of fungus disease which cause erosion and loss of vital body fluids (Fig. 17-7). The vertebrae fuse and the fish become kyphotic (Fig. 17-7). Although we have never been able to actually demonstrate the presence of osteoporosis in fish we are strongly suspicious of its presence. The jaw becomes prognathic giving the salmon its classic "hooked-jaw" appearance (Fig. 17-7). Enophthalmos is most pronounced as the eyes sink deep into the head. The muscles, gastrointestinal tract, thymus and other reticuloendothelial organs become greatly involuted which contributes to the fish's loss of resistance to infection. Chalk-like calcific concretions in the kidney are a frequent finding. The female salmon fills its redd with eggs which is inseminated by the male. A week or two later, after drifting weakly and aimlessly, the fish die. During this time, the once compactly arranged cortical cells become completely distorted by flame-shaped hemorrhagic areas so that the adrenal tissue resembles the morphologic pattern of the mammalian zona reticularis, i.e. zone of senescence.[‡] At the time of death, the appearance of the adrenal tissue is strongly reminiscent of the hemorrhagic and thrombosed adrenal glands of patients with the Waterhouse-Friderikson syndrome, i.e. adrenocortical exhaustion (Table 17-I).

[‡] It is believed that the cortical cells of the adrenal originate in the zona glomerulosa which serves as a "stratum germinativum" and that the cortical cells migrate downward between the capillaries of the zona fasciculata where they are altered in size, morphologically, tinctorially, and in their enzymatic ability to convert steroid precursor into definitive steroid. Finally, as the cortical cells mature they pass into the reticulo-endothelial tissue of the zona reticularis where they are destroyed. For this reason, the zona reticularis is called the "zone of senescence."

TABLE 17-I
HISTOLOGICAL CHANGES IN SPAWNING SALMON, CUSHING'S SYNDROME,
EXPERIMENTAL HYPERADRENOCORTICISM AND AGING

	<i>Spawning Salmon</i>	<i>Cushing's Syndrome</i>	<i>Experimental Hyperadrenocorticism</i>	<i>Aging in Mammals and Man</i>
Adrenal	Hyperplasia and degeneration	Hyperplasia and tumors	Hyperplasia	Hyperplasia followed by slight atrophy
Pituitary	Degeneration	Hyaline change in basophils	Minimal degeneration	Degeneration
Spleen	Depletion of lymphocytes, fibrosis	No reported change	Depletion of lymphocytes	Atrophy, depletion of lymphocytes, fibrosis
Thymus	Involution, depletion of thy mocytes	Involution, occasional tumor	Depletion of thymocytes	Involution
Liver	Degeneration	Fatty degeneration	Fatty degeneration	Slight degeneration
Kidney	Degeneration	Degeneration	Degeneration	Atrophy and degeneration
Pancreas	Hypertrophy of islets	Hypertrophy of islets variable	Hypertrophy of islets	No distinctive change
Stomach	Atrophy and degeneration	Occasional ulcers	Atrophy of epithelium and occasional ulcers	No change
Thyroid	Atrophy and degeneration	Atrophy of follicular epithelium	Atrophy of follicular epithelium	Involution
Gonads	Degeneration of testes	Atrophy	Degeneration	Atrophy and degeneration
Muscle	Degeneration of masseter	Atrophy	Atrophy	Atrophy
Cardiovascular system	Degeneration and arteriosclerosis	Arteriosclerosis	Arteriosclerosis	Arteriosclerosis
Skin	Hypertrophy followed by atrophy	Atrophy	Atrophy	Mostly atrophy; in some regions hypertrophy

Young fish, which have "never been to sea" have been implanted with pellets of various adrenocorticoids and they too, promptly develop the hooked jaw, humped back, and other pathophysiologic changes (Fig. 17-13) reminiscent of Cushing's disease in man or hyperadrenocorticism in breeder rats (Robertson, *et al.*, 1963). We have castrated sea salmon prior to their upstream migration, held them in special holding tanks and these castrate specimens double and triple their normal life span (Fig. 17-14) but eventually develop some of the stigmata and senile changes (Table 17-II) characteristic of spawning salmon (Robertson and Wexler, 1962c). It is of interest that as the melting glaciers formed into lakes in prehistoric times, the rainbow trout, which is a land-locked salmon, was not obligated to make long migrations in connection with the spawning effort. Nonetheless, the rainbow trout develops many of the untoward stigmata described in their cousin salmon. However, they do not age as explosively or die after a single spawning as the Pacific salmon. In this same vein, it is also of interest that the Atlantic salmon spawn many times before this same de-

TABLE 17-II

COMPARISON OF HISTOLOGICAL CHANGES IN AGED CASTRATED KOKANEE AND SPAWNING SALMON

	Aged Castrated Kokanee	Spawning Salmon
Pituitary—degeneration	+	+
Adrenal		
Hyperplasia	+	+
Degeneration	±	+
Pancreas—islet hyperplasia	+	+
Spleen		
Depletion of lymphocytes	+	+
Increased fibrosis	+	+
Thymus—involution	+	+
Liver—degeneration	+	+
Kidney		
Capillary glomerulosclerosis	+	+
Tubule degeneration	+	+
Thyroid—atrophy	+	+
Heart muscle		
Vacuolization	+	+
Degeneration	+	+
Blood vessels—beginning arteriosclerosis	+	+
Stomach—atrophy and degeneration	+	+
Skin		
Atrophy	+	0
Hyperplasia	0	+
Scales—absorption	0	+
Muscle degeneration		
Masseter	+	+
Lateral	+	0

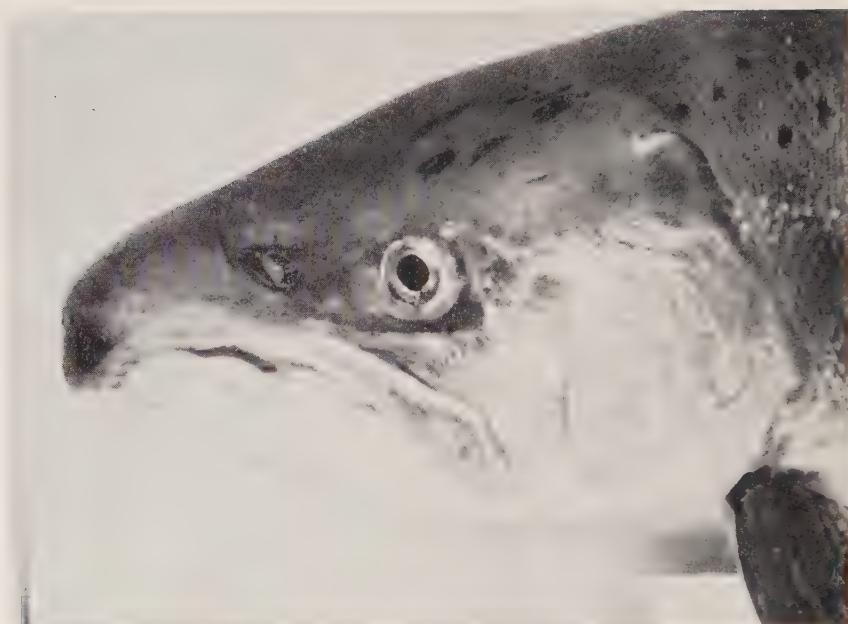


Figure 17-14. Ovariectomized, female salmon (Kokane) which doubled its life-span. Close-up shows a slight rounding of the head and increase in size as well as beginning prognathism. Castrated or ovariectomized salmon which effect regeneration of their gonads lose their "protection" against the development of the progeric stigmata and their life-span is correspondingly shortened.

generative syndrome ultimately causes their demise. Teleologically, the author finds it attractive to think of this difference between the Atlantic and Pacific salmon as some built-in, Darwin-like mechanism, whereby the setting of the "biological time clock" within the hypothalamus of the Atlantic salmon "fires-up" the pituitary-adrenal-gonadal axis in a manner more appropriate to affect completion of migration and the reproductive effort. In the Pacific salmon, it would seem that the hypothalamus, like a thermostat, becomes caught in an open or "full-throttle" position, and this species suffers from the unrelenting driving stimulation of all of its pituitary tropic hormones producing the untoward pathophysiologic changes, the accelerated aging and death after completion of only one reproductive mission.

Finally, one of the most outstanding aspects in these investigations is our finding of coronary arteriosclerosis (Fig. 17-10), myocardial infarction and generalized arteriosclerosis (Fig. 17-11) in spawning Pacific salmon (Robertson, *et al.*, 1961). Again, as in breeder rats, despite severe hyperlipemia and hyperglycemia, the arterial lesions consist of little lipid. Rather, the arterial lesions contain unusual accumulations of acid mucopolysaccharides.

saccharide and collagen or scar tissue (in keeping with other gerontological investigations fish also manifest generalized increased deposition of collagen, e.g. progressively thickening collars of collagen about the entire perimeter of their arteries—all occurring during their brief upstream migration). These findings of vascular disease in fish were confirmed by Van Citters and Watson (1968) in rainbow trout. The finding of arteriosclerosis and myocardial infarction in fish is of fundamental import since it demonstrates that heart and vascular disease is a biological as well as a medical problem. Of further import is the association of hyperadrenocorticism, degenerative changes, explosive aging and death in connection with the reproductive effort in a lower form of life. One cannot help ponder what Malthus or Darwin-like implications this natural phenomenon may have.

CONCLUSIONS

In the spawning fish, repeatedly bred rats and in human pregnancy certain dynamic pathophysiologic events occur which can be linked with the activation of the hypothalamic-pituitary-adrenal-gonadal axis in connection with the reproductive effort. The excess production of adrenal corticoids, in particular, can precipitate certain metabolic and connective tissue alterations which lead to cardiovascular degenerative changes, accelerated aging and even death. Because these untoward changes appear to be conditioned by the intensity or frequency of the reproductive effort this phenomenon has potential applicability to the problems, not only of cardiovascular disease and aging, but also to the growing threat of a population explosion.

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CHAPTER 18

REPRODUCTION AND AGING

A. ÁRVAY

SUMMARY

THE EFFECT OF REPRODUCTIVE processes on the rate of biological aging was studied in the female rat. Biological aging was measured in tail tendon collagen.

In the reproductive period, rats which were pregnant many times were biologically the oldest. Virgin female rats were younger, but the youngest were those which had been castrated early in life. It was shown that the rate of biological aging could be increased by treatment with hormones normally secreted in greater amounts during pregnancy. For example, estradiol, ACTH and adrenocortical steroids increased the biological age.

In the post-reproductive period however, female rats which had littered many times proved to be the youngest biologically.

INTRODUCTION

One of the most important problems of experimental gerontology is: What causes aging? May we emphasize only one change or can several organic, organ-system, morphological or functional changes be brought into causal relationship with the process of aging? Are there physical, chemical, psychological, social or other kinds of factors which could significantly influence the life-span of man?

If it is clear that the average and characteristic life-span of a species is determined by genetic factors, and that the beginning of aging and its course are directed by external and internal environmental influences, it is impossible not to ascribe a role to the neuro-endocrine system. If the above conclusion is accepted, we may raise the question of whether there is any interrelationship between the process of aging and reproductive processes? Does reproduction or the nature of participation in reproductive processes influence the course and rate of biological aging, or to turn the question around: what influence does aging exert on the reproductive processes?

On the basis of the above mentioned hypothesis, an interrelationship between reproduction and the aging of the organism seems highly probable. In the course of life there are no functional and structural changes in the

neuro-endocrine system which are more far-reaching than those connected with reproduction. If we only consider the human species, the female organism is the scene of cyclic changes repeated every 28 days through more than three decades. These changes—though manifested most markedly in the ovaries and endometrium—affect the whole organism. Or if we take into consideration the period of gestation, and gravidity, there are not only the far-reaching structural and functional changes of the endocrine system, but also the development of a new endocrine gland, the placenta. During pregnancy changes occur in the whole endocrine system, in the structural and functional state of most of the organs and organ-systems, in most of the life functions and in the adaptability of the organism.

At my clinic we have been investigating how reproduction influences the rate of biological aging. This is outlined in the first part. In the second part is reviewed the influence of biological aging on the reproductive processes.

THE EFFECT OF REPRODUCTIVE PROCESSES ON BIOLOGICAL AGING IN THE REPRODUCTIVE PERIOD

By means of animal experiments we studied the interrelationship existing between the reproductive processes and biological aging (Árvay, Takács and Verzár, 1963). White rats of our own breeding (*R. norvegicus*) were used in our experiments. Our animals were kept under constant experimental conditions and fed the same diet in all experiments.

Effects of Breeding

Female rats belonging to the first experimental group were divided into 3 groups. The 124 animals of the first group were mated when 10 to 12 weeks old at the beginning of their reproductive period. At the end of their pregnancy they were placed in separate littering cages. After suckling the females were again confined with males. Thus, to the end of the reproductive age they littered 8.1 times on the average and during this time each brought forth an average of 51 pups.

The 119 females of the second group were kept separate from the males to the end of their lives. Hence, they did not participate in breeding. The 108 females of the third group were castrated when 6 weeks of age. In these experiments we endeavoured to obtain an answer to the question of whether the difference in the participation of sexual functions is reflected in any way in the course and rate of biological aging. As regards the difference, we drew conclusions partly from the average life-span of the animals of the different groups, and partly from the result of the thermoisometric tension measurement of the collagen fibers of the tail (Brocas and Verzár, 1961). In some cases we determined the percentage of the so-called

"labile hydroxyproline," that is the quantity of hydroxyproline dissolved in Ringer solution during 10 minutes at 65°C.

Our experiments showed (Table 18-I) that during the reproductive period of life (5.5 to 24 months) the biologically oldest animals were those which were confined with males from the age of fecundity and, consequently, littered several times (group I). Next in order was group II which did not litter even once, being kept isolated from the males. The biologically youngest group consisted of females which were castrated at the age of 6 weeks (group III). However in senescence (age 33 months), after the age of fertility, the character of the biological ages of collagen changed. Now the biologically youngest animals were those which had often been pregnant in their period of fertility, while the castrated females proved to be the oldest. The average life-span also was highest in the group of breeders (Árvay and Takács, 1966).

According to our experiments, therefore, if the investigated properties of the collagen fibers provide us with evidence regarding the actual state of the biological aging of the organism, there must be a demonstrably significant correlation between the character of participation in the reproductive processes and the rate of biological aging. Having ascertained these correlations, two questions may be raised:

1. What factors cause females to be biologically older when they intensively participate in the reproductive processes in the reproductive phase of life?
2. How can we explain the fact that in the period of senescence those females which are biologically the youngest were the oldest in the previous period of life, i.e. those which had participated most intensively in the reproductive processes and had littered the most?

As regards the first question raised we must take into consideration first and foremost the difference between the groups of the biologically oldest and youngest animals determined by the character of the experiments. We had to take into account the difference as regards participation in sexual functions; pregnancy played a frequent role in the first group, but was totally excluded from the second group.

This problem was studied on rats between the ages of 4 and 13 months (Árvay and Takács, 1964-65). The degree of thermal-contraction of the collagen fibers in tail-tendon measured at 62°C, furthermore the magnitude of the weight preventing total contraction (100% inhibition), as well as the weight maintaining 50 percent contraction (50% inhibition), and also the magnitude of the weight causing the collagen fibers to break, were determined during the days directly preceding gravidity, in the first and third week of gravidity, and at different times after littering, but

TABLE 18-I
THE EFFECTS OF BREEDING AND CASTRATION ON THE BIOLOGICAL AGE
OF FEMALE RATS^a

Series	Weight in g Which Results in Breaking of Contracted Fibers				Significance Between Animal Groups Age: 24 Months
	5.5	12	18	24	
I. Multiparous females	3.0	6.5	6.5	8.6	I-II P < 0.01
II. Females separated from males ..	3.5	6.0	5.7	7.5	
III. Castrated females	3.2	5.2	6.3	6.5	II-III P < 0.01

^a Árvay, Takács and Verzár (1963); Árvay and Takács (1966).
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TABLE 18-II
THE EFFECTS OF HORMONES ON THE BIOLOGICAL AGE OF FEMALE RATS*

Treatment per Day For 16-18 Days	Number of Rats	Height of Contraction cm	P
Control	6	9.79 ± 1.09	
Human chorionic gonadotropin (1,500 U)	6	9.46 ± 0.83	> 0.05
Estradiol dipropionate (2 mg)	6	10.45 ± 0.85	= 0.05
Progesterone (10 mg)	6	8.81 ± 1.02	> 0.05
ACTH (6 U)	6	13.81 ± 1.14	< 0.001
Hydrocortisone acetate (2 mg)	6	11.27 ± 0.88	< 0.01

* Árvay and Takács (1966).
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with regard to the control experiments, always on identical days. It may be remarked that the experiments serving as controls were carried out, wherever possible, on sisters, and always on animals of identical age.

On the basis of our experiments we were able to establish that during pregnancy such changes in the thermal contraction of tail collagen fibers and other properties mentioned above indicated an acceleration of the biological aging of the organism. These changes are not significant on the 3rd to 7th day of pregnancy. At the end of pregnancy, on the days preceding the littering, however, significant differences are apparent as compared with the values obtained before pregnancy. The values which provide evidence for the acceleration of biological aging slowly normalize after littering so that the rate of biological aging slows down. We may thus draw the conclusion that the changes ensuing in the properties of the tail collagen fibers examined during pregnancy are reversible, though only to a certain extent, because they do not quite return to the initial values.

Our investigations indicate that pregnancy significantly accelerates the rate of biological aging in our experimental rats. It was questionable however as to what causal significance could be attributed to the many other factors acting during pregnancy. In the course of our experiments the role of the hormones may rightfully have been brought up, because they showed characteristically quantitative, in fact, qualitative changes. Therefore, in further experiments we studied on the one hand the effect of hormones administered parenterally, and on the other hand the effect of the changed endogenous hormone-milieu of the organism on the rat of biological aging (Árvay and Takács, 1965).

Effects of Hormones

In our experiments the following hormones were administered daily to 6-month-old female rats for 16 to 18 days, taking into consideration the duration of pregnancy: human chorionic gonadotropin (HCG) (daily 1500 U), estradiol dipropionate (2 mg), progesterone (10 mg), adrenocorticotropic hormone (6 U), and hydrocortisone acetate (2 mg). After treatment the rats were killed and the tail tendons were tested by the method described earlier. According to our investigations neither HCG nor progesterone influenced the rate of biological aging. On the basis of the values obtained estradiol-dipropionate accelerated biological aging. ACTH ($p < 0.01$) also significantly accelerated the rate of biological aging. After the administration of ACTH the acceleration was more than 80 percent, after hydrocortisone acetate it was nearly 40 percent. See Table 18-II.

Effect of Castration

In order to confirm the effect of the change elicited artificially in the endogenous hormone milieu of the organism in another way, two further se-

ries of experiments were begun. In one of these (using female rats 6 months of age) we investigated the effect of the deficit of the endogenous ovarian hormones, resulting from castration at the age of 6 weeks. It was ascertained that the castrated animals proved to be younger biologically than the control animals of identical age. The difference in our experiments was significant ($p < 0.05$). This result confirms and explains the conclusions of our first experiments. In the previously described breeding experiments (Table 18-I) the castrated ones were biologically the youngest because in these rats estrogen (which causes an acceleration of aging) was lacking.

Effects of Intensive Neural Stimuli

The effect of the parenterally administered ACTH was confirmed in those investigations in which an increased production and mobilization of endogenous ACTH and corticosteroid was experimentally evoked. The animals were exposed to the effect of continuously stressing neural stimuli. As is well known, stressful stimuli lead to increased production of ACTH by the pituitary and consequently of adrenocortical hormones. Seventy-five stressed animals were compared with 75 untreated controls. According to the results obtained, after the traumatization period the thermal contraction of the collagen fibers increased when compared with the normal control. Hence the continuous stress which increases the production of ACTH and adrenocortical hormones, significantly accelerates the rate of biological aging. Effects of similar character were registered in our previous experiments after the parenterally administered ACTH and hydrocortisone respectively (Árvay and Takács, 1965; Árvay, *et al.*, 1967).

According to the results of our experiments, ACTH, the adrenocortical hormones, and also the estrogens increase the rate of biological aging. Thus—in addition to the effects of other incidental factors—the hormones play a role in accelerating the rate of biological aging during pregnancy. It may be due to these hormones that in the reproductive phase of life the female rats that are the oldest are those which participate intensively in the reproductive processes.

Effect of Reproductive Processes on Biological Aging in the Post-Reproductive Period

Our second question: Why does the rate of biological aging change after the reproductive phase of life has run its course? In other words, why do the aforementioned females become younger and have a longer life-span than those which did not participate in the reproductive processes and did not litter even once in the course of their lives?

When answering these questions we may refer to the fact that many

events take place in the female organism during the reproductive phase which mean an increased loading for the organism. Here we must think of the ovarian steroids which exert their influence throughout a very long phase of life, thereby increasing the rate of biological aging. We must also take into account the surplus loading with which the pregnancies burden the whole organism. The organism is freed of all these loadings when the vegetative and germinative functions of the ovaries cease. Perhaps it would not be too daring to assume that the reaction of the organism to being relieved of the effects increasing the rate of biological aging is all the more marked, the greater the loading was during the reproductive stage, and the more pregnancies there were within this period.

Human Mortality and Marital Status

Though the results of animal experiments—especially those concerning the nervous system and the endocrine system—can be applied only with severe criticism in human physiology and pathology, yet we consider it interesting to exhibit the statistical data elaborated in Table 18-III (Árvay and Takács, 1966). The table takes into account the whole female population

TABLE 18-III

TREND OF MORTALITY ACCORDING TO THE MARITAL STATUS AND AGE
GROUPS OF THE FEMALE POPULATION IN HUNGARY. MORTALITY RATE IS
EXPRESSED PER 1000 OF WOMEN OF CORRESPONDING
AGE AND MARITAL STATUS

Year	Marital Status	25-29	30-34	35-39	40-49	50-59	60-69	70-x
1961 . . .	Unmarried	1.6	3.2	4.0	5.5	10.5	21.9	76.3
	Married	0.7	1.0	1.6	2.7	6.6	17.3	58.8
	Widowed	0.5	0.9	1.5	3.3	7.3	20.4	86.3
	Divorced	0.7	1.3	1.9	4.4	7.5	21.2	81.5
	Married +	0.6	1.1	1.6	3.4	7.1	19.6	75.5
	Widowed +							
1962 . . .	Divorced							
	Unmarried	2.5	2.8	4.4	5.2	10.4	25.7	88.7
	Married	0.6	1.0	1.6	2.8	6.9	18.8	63.6
	Widowed	2.5	1.4	1.7	3.8	7.9	21.6	99.8
	Divorced	1.1	1.9	2.4	3.4	8.6	21.6	87.2
	Married +	1.4	1.4	1.9	3.3	7.8	20.6	83.5
1963 . . .	Widowed +							
	Divorced							
	Unmarried	1.7	2.9	3.8	5.7	9.6	23.5	81.9
	Married	0.7	0.9	1.3	2.8	6.3	17.7	55.6
	Widowed	1.1	2.6	2.5	3.6	7.7	20.7	86.0
	Divorced	0.9	1.1	2.4	4.2	6.7	18.9	83.7
	Married +	0.9	1.5	2.1	3.5	6.9	19.7	75.1
	Widowed +							
	Divorced							

(Reproduced with the permission of the Hungarian Central Statistics Bureau.)

TABLE 18-IV

LIFE EXPECTANCY IN HUNGARY OF MARRIED, WIDOWED, DIVORCED WOMEN
ACCORDING TO THE NUMBER OF CHILDREN 1959/60

Age	Total	<i>The life expectancy of married, widowed, divorced women who bore children</i>					
		0	1	2	3	4-5	6-x
50-54	32.92	32.31	32.63	32.75	32.93	33.05	33.42
55-59	28.09	27.54	27.80	27.91	28.09	28.22	28.57
60-64	23.33	22.85	23.07	23.15	23.31	23.44	23.74
65-69	18.66	18.23	18.40	18.48	18.62	18.75	19.03
70-74	14.12	13.82	13.92	13.97	14.06	14.17	14.38
75-79	9.71	9.52	9.56	9.58	9.65	9.75	9.91
80-x	5.40	5.40	5.40	5.40	5.40	5.40	5.40
<i>Life expectancy of married, widowed, divorced women in % of those with no children</i>							
50-54		101	101	102	102	103	
55-59		101	101	102	102	104	
60-64		101	101	102	103	104	
65-69		101	101	102	103	104	
70-74		101	101	102	103	104	
75-79		100	101	101	102	104	
80-x		100	100	100	100	100	

From Árvay and Takács (1966), reproduced with permission of S. Karger, Basel.

of Hungary, every deceased woman within a period of 3 years (1961-1963) figures in it in groups corresponding to her family status and her age. The data represent the mortality rate of 1000 women of similar age and family status.

It is unquestionable that for the purpose of comparison with our rat experiments it would have been better to have evaluated a statistical table showing the number of births and the number of pregnancies respectively, instead of groupings according to family status. Such a classification would show much more definitely whether the number of pregnancies influence the mortality rate of similar age groups. Such a grouping was not available. We know that objections can be raised against our conclusions, for example, the unmarried women figuring in the tables may have had pregnancies, and there are sure to be such married women who never gave birth to a single child. Nonetheless we are of the opinion that our table—which indicates that the mortality rate of the unmarried women is higher in every age group than that of the combined married, divorced, widowed group, shows that there must be some connection between pregnancies, births, and the mortality rate.

Table 18-IV gives a much more definite answer to our question. From the statistical groups referring to 1,330,362 women it undoubtedly comes to light that—*independent of the family status—above 50 years in each*

age group the life expectancy increases in proportion to the number of children.

From our table of statistics which we consider verified by the law of large numbers, it appears that in the human population there is an undoubtedly connection between the reproductive processes, the number of pregnancies and births and the mortality rate or life expectancy. The increased survival of women with large families may have a genetic basis, either dependent or independent of endocrine factors. Women who are biologically strong might be expected to have large families and to be long lived.

Species Differences between Women and Rats

The correlation between reproduction and aging is unquestionable in rat experiments. There is however a species difference as regards the character of the relationship. To explain this we must refer to the fact that the cessation of the vegetative and germinative function of the ovaries in women is different from that occurring in rats (Aschheim, 1965 and 1966). In women the regular follicular maturation processes cease within a relatively short time after the climacteric, while in rats the morphological and functional signs of ovarian function—though to a lesser and lesser extent—can be demonstrated even in late senescence. Thus, the aging effect of estrogens is eliminated later and at a slower rate in rats than in women. We are of the opinion that this difference explains why the effect of participation in reproductive processes on the course of biological aging—though similar as regards its course is manifested differently in women than in rats.

THE SIGNIFICANCE OF AGING IN THE REPRODUCTIVE PROCESSES

The correlation between the biological aging of the organism and the reproductive processes had already been investigated long ago and in many directions. Not only observations relating to human beings, but also the results of animal experiments suggest that in the course of aging the fecundation ability of females declines, the conditions for the development of the intrauterine fetus become less favorable, and the life-prospects of the newborn decrease. These documented alterations raise the question of whether the changes connected with aging in the reproductive organs can be brought into a causal relationship with the above mentioned alterations of the reproductive processes.

Fertility and Age

In recent years numerous investigators have verified that in animal experiments with golden hamsters and mice the fertility of females declines with age (Thorneycroft and Soderwall, 1969a; Soderwall, *et al.*, 1960;

Greenwald, *et al.*, 1967; Greenwald, 1967; Blaha, 1964a). According to Rugh and Wohlfomm (1967) the average litter size of mice 3 to 5 months old is 9.48, and of mice 10 to 12 months old it is 7.63, the percentage of normal offspring 83.03 percent and 73.65 percent respectively, dead fetuses 1.31 and 1.88 percent respectively, percentage resorbed *in utero* 13.18 and 17.89 percent respectively. The total percentage of anomalous mice at birth increases from 2.29 to 3.26 percent with age. According to Finn (1962) there is a period initially in which the size of litter is fairly constant. This is followed by a phase during which successive litters become progressively smaller until breeding ceases.

In the literature we find different explanations of why the litter size decreases with age. An obvious assumption is that the cause of the reduced litter size, lies in the ovaries due to the decreased ovulation rate as a consequence of aging. It may also be assumed that the receptive capacity of the ovaries decreases with age, or perhaps the decidual transformation of the endometrium is not satisfactory. Another assumption would be that the intrauterine development of the fetuses is not adequately ensured, this again being a consequence of aging, and hence they die at the end of pregnancy and are resorbed. The fall in litter size in senescent hamsters appears not to be due to a reduction in the ovulation rate, but to a sevenfold increase in preimplantation deaths and a twofold increase in resorption (Thorneycroft and Soderwall, 1969a). According to other workers serial sections of pregnant uteri revealed no reduction in the implantation rate. The reduction in litter size was the result of fetal resorption in the last two or three days of pregnancy (Soderwall, *et al.*, 1960). Blaha (1964a) reported that in hamsters with increased age, resorption occurred earlier in pregnancy and with implantation failing, even blastocysts were present *in utero*. Blaha (1964b) also reported that ova from senescent hamsters were less viable than those from young females. According to Greenwald (1967) ovaries from senescent hamsters contained fewer nonatretic follicles than those from young animals.

Pituitary-Ovarian Function and Fertility

According to Greenwald (1967) FSH secreted by the pituitary is one requirement for the maintenance of pregnancy in the hamster, since any reduction in its secretion rate or in the ovary's response would most probably result in fewer follicles maturing. In his opinion the changes found in aging hamsters can be related to the functional changes of the pituitary-ovarian axis.

In certain respects Greenwald's conception is supported by the investigations carried out by my co-workers (Borsos, *et al.*, 1971). They confined 44 female rats 18 months of age and 30 female rats 28 months of age with

males twice for periods of 10 days. As not one of the 74 rats became pregnant during this time, transcerebral electrostimulation of the hypophysis of the animals was performed under ether anesthesia on 4 consecutive days. Vaginal cytological examinations were carried out for 7 days before and after treatment. According to their investigations readily observable changes could be seen in the cytological picture of the vagina of the 18-month-old animals under ether anesthesia, as well as with electrostimulation under ether anesthesia. Some presented a picture of regular cyclic changes, providing evidence of ovarian function. Of the 14 control animals not a single one became pregnant. Seven of the treated 30 animals however became pregnant and littered 31 pups. The average litter size was 5.3. Of the 31 pups however 15 died a few hours after birth and only 15 percent of the pups survived. The vaginal cytological picture of the animals 28 months of age was not influenced by electrostimulation. Not a single animal became pregnant after the treatment.

The investigations of my co-workers indicate that the change in the function of the pituitary-ovarian axis may play a role in decreasing the fertility as a consequence of the aging of the rat. Therefore in old animals past their phase of fertility—but not yet in the senium—we successfully stimulated the hypophysial ovarian axis and in consequence some of the treated animals became pregnant and also littered.

Morphological Changes in the Ovary

Of the morphological changes of the ovary during aging we may lay particular stress on sclerosis, an accumulation of the interstitial tissue at the expense of the generative epithelium (Thung, 1961; Pincus, 1961; Harrison, 1962; Milcu, *et al.*, 1965; Árvay, 1968). It is also suggested that the generative epithelium—in common with neurones, the thymus and collagen cells—is amitotic epithelium without turnover (Verzár, 1965).

According to the investigations of Takács (1968), the collagen content of the ovaries of rats as referred to the dry material increases with aging. The collagen content of the ovary is the highest at the age of 14 to 16 months, and then it decreases gradually. Takács has also shown that the percentage of ovarian collagen soluble in Ringer at 65°C rapidly decreases in the course of aging.

Thorneycroft and Soderwall (1969b) described in detail the morphological changes in the ovaries of pregnant hamsters during aging. Ovaries from young and senescent hamsters were examined morphologically on days 8, 12 and 14 of pregnancy. According to their investigations the senescent hamsters had fewer follicles present than the young ones on all days of gestation. In the senescent females the corpora lutea underwent the greatest growth between days 8 to 12, whereas corpora lutea from young

animals grew the most between days 12 and 14. Treatment with PMS revealed that the senescent ovary was refractory. These investigators are of the opinion that the ovaries of young and old hamsters differ only quantitatively during pregnancy with regard to morphology.

Hormone Production by the Aging Ovary

During the process of aging the changes occurring in the hormone production of the ovaries differ according to species. In connection with this I can only refer to the extensive investigations of Aschheim (1965 and 1966). In the human female the steroid hormone production of the ovaries gradually decreases during the menopause despite the fact that the gonadotropic hormone production of the hypothalamo-hypophysial system increases temporarily. The question may be raised of the decrease in the receptive capacity of the ovary, in that it becomes refractory to gonadotropins. The hormone production of the hilus-cells in the phase of aging and in the senium and the role of these cells in physiological and pathological processes is still a much debated question.

Age Changes in the Uterus

When dealing with the effect of aging on the reproductive processes and the reproductive organs, mention must be made finally of the uterus also. According to several concordant investigations the sclerosis of the uterus also increases in parallel with aging, as in the case of the ovaries. Schaub (1964-65) described a significant accumulation of the total collagens of the uterus in aged rats. Woesner (1963) found the collagen-content maximum in the uterus of women between the ages of 30 to 35 years, after which he recorded a decrease.

Several relationships are known to exist between the collagen content of the uterus and the effect of the sexual steroids (Smith and Kaltreider, 1968; Adams and Leathem, 1965; Morgan, 1963). A close relationship has been shown between the hormone production of the ovaries and the collagen content of the uterus. It has been demonstrated that castration decreases the collagen content (Cullen and Harkness, 1964).

The investigations carried out with my co-workers (Árvay, Takács and Ladanyi, 1971) also verified the significance of the ovarian and adrenocortical steroids in bringing about sclerosis of the uterus. Loading with neural stimuli and prolonged stress leads to an accumulation of the total collagen in the uterus and a decrease of the percentage of the labile hydroxyproline.

According to the investigations of Takács and Verzár (1968) tension plays a role in raising the collagen content of the uterus during pregnancy.

The investigations of Murashima (1967) show that the estrogen sensitiv-

ity of the uterus and the vaginal mucous membrane changes with age. In rats the sensitivity is lowest between months 10 to 13, increases gradually till the age of 28 months, and then decreases strongly in the senium. It may be assumed that changes in the enzyme systems and their dynamics play a role in the change of the estrogen sensitivity in the course of aging.

CONCLUSIONS

In the female rat, participation in the reproductive processes of pregnancy and littering accelerates biological aging during the reproductive period of life. It is assumed that the properties of collagen fibers in tail tendon indicate the actual state of biological aging of the organism.

As a reaction to being liberated from the increased loading of many pregnancies, in the post-reproductive period in the senium, the female rats which have littered the most become the youngest biologically.

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CHAPTER 19

AGING IN THE HYPOTHALAMIC-HYPOPHYSEAL OVARIAN AXIS IN THE RAT

PIERRE ASCHHEIM

SUMMARY

NORMAL FUNCTIONING of the hypothalamic-hypophyseal ovarian axis which is seen in the succession of estrous cycles does not cover even the first half of the total life duration in a population of rats. Senile deviations of the cycle, in the form of permanent estrus and the repetitive pseudopregnancies, occupy the major part of the life time. There is no cessation of either ovarian function or its hypothalamo-hypophyseal control, but there is modification of the mode of central regulation. The ovary is not primarily responsible for this senile change; we have no arguments in favor of a primary role for the hypophysis, although we cannot formally dismiss it. On the other hand convergent facts point to a primary responsibility of the hypothalamic areas which control the gonadotropic function of the hypophysis. The senile change manifests itself first in an overall hypersensitivity of these hypothalamic areas to estrogen. It becomes evident, beginning with adulthood in cyclic rats, by a growing aptitude to react by pseudopregnancy to a decreasing external estrogenic stimulation. This fact is used as the basis of a test for aging. Later on, this hypersensitivity leads to a hypofunctioning of the hypothalamic centers which regulate tonic LH secretion. This is followed by the senile deviations of the cycle caused by inadaptation to the environmental modifications previously compensated for and to their neuro-endocrine expression. The senile deviations of the cycle are for the most part reversible. The aging of the hypothalamic-hypophyseal ovarian axis can be advanced by early hemicastration. It is suspended during castration or hypophysectomy, followed by the replacement of the removed organ, that is to say, during the cessation of the steroid information of ovarian origin. It is delayed after chronic treatment with estrogen or estrogen-progestin.

INTRODUCTION

Various dialectic approaches to the study of the senile ovary have been followed during the past hundred years (Waldeyer, 1870). One concerns

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the double ovarian function, the external or reproductive, and the internal or hormonal function. How do they age? Simultaneously or differentially? Independently or not? Another dilemma is presented by comparative physiology: the menopause, the cessation of the double ovarian function, and the structural regressions that occur is a phenomenon which has been progressively recognized as being limited to humans. What is it that replaces the menopause in animals? Is there nevertheless a common process underlying the ovarian aging in humans and animals and, if so, what are its limits? One other problem is that of comparative sexual senescence in the male and the female.

Depending on the period, emphasis has been placed on one or the other of these different aspects.

The first phenomena investigated were the *morphological modifications of the human ovary* after the menopause, as well as the *diminution with age in the reproductive performance* of different species of animals.

At the time of the "explosive" period of sexual endocrinology (since 1920) and up to 1945, the *hormonal aspect* (hypophyseal and gonadal) has been considered most important. The understanding of the human menopause has undeniably benefitted from this, but the therapeutic deductions drawn, with a view to a general "rejuvenation" on a gonadal basis, have partly discredited endocrinology. Its true place was thus restricted at the very moment when the basis of experimental gerontology was being laid (see respectively Korenchevsky, 1961 and Chap. 9 of Verzár, 1963). From this time, date the first morphological studies (Romeis, 1931; Wolfe, 1938, 1943) and experimental data (Zondek and Aschheim, 1927; Hoffman, 1931) on hypophyseal-ovarian relationships in senile rodents.

The *diminution in the number of oocytes with age* has then been the dominant preoccupation for about 15 years. This oocyte "depletion," an incontestable fact in all mammals studied, has become for many authors the central event in the aging of the ovary (Thung, 1961). The excesses of the "hormonal" period have been succeeded by the dogma of the primacy of the egg. This position clashes with the following facts: "The decline in oocyte numbers is best described by an equation which implies that a constant proportion (and not a constant number) of the population is lost per unit time. This finding implies further, that oocytes maintain a constant level of vulnerability. They do not behave, therefore, like a classical aging system which should become increasingly vulnerable with advancing age" (Krohn, 1967a). The greatest loss of oocytes comes about in the stage prior to puberty. The regression line representing the decrease of oocytes in relation to age never reaches zero till after death in most of the species studied (except for some strains of mice). Furthermore, it is known that after irradiation of the ovaries with x-rays or grafting of

ovaries preserved by refrigeration, the endocrine function persists for a certain time, even if it is modified, in the presence of a small number of follicles or even in their absence. The intrasplenic ovaries after tumorous transformation, which produce steroids, are equally devoid of oocytes.

For the last 15 years the new impetus to the study of the senile ovary has come from *neuroendocrinology*. This is why this chapter is entitled "The Aging of the Hypothalamic-Hypophyseal Ovarian Axis in the Rat," and is found in a work devoted to the relation of the hypothalamus and pituitary to senescence. We will in fact study *the aging of a system of regulation*, the one that controls the gonadotropic activity of the hypophysis in the female. In all the classical treatises on sexual endocrinology (Young, 1961; Chester Jones and Ball, 1962; Rowlands and Parkes, 1966) the need for such a study is stressed when the function of senile gonads is discussed. From the experiments of Krohn (since 1957), of Lipschütz, *et al.* (1963, 1965) with the mouse, from observations of Bloch and Flury (1959, 1961) of Mandl and her group (from 1958 to 1961) on the rat, there emerges the notion of an extraovarian, hypophyseal factor "responsible" for ovarian aging.

Since 1961 my own work has implicated the hypothalamus in this process, and thus completes the regulatory circuit by introducing a "post-mitotic" element (the hypothalamic neurons do not divide). New perspectives result therefrom:

1. the hypothalamus is at the center of the regulatory system under study,
2. the aging of its nonrenewable neurosecretory cells is certain,
3. it manifests itself by a modification of the sensitivity of the specialized neuronal areas to the circulating steroids,
4. the hypothalamus occupies a central position in other regulatory functions (hormonal or other),
5. their aging, which is characterized above all by an increasing inadaptability, is said to be differential. If that is the case, how does it work inside the same regulatory organ, the hypothalamus? Are there correlations in the senescence of its different functions of control?

The information furnished by the vaginal smear will act as a leading thread for our study. The *estrous cycle and its senile deviations* reflect, through the ovary, the activity of the follicle-stimulating hormone (FSH), the luteinizing hormone (LH) and the luteotropic hormone (prolactin) of the hypophysis and the hypothalamic control over this activity. The division of our aging animals into cyclic rats, those in permanent estrus (PE) and those in repetitive pseudopregnancy (RPP), with each category corresponding to a different and well defined neuroendocrinological situa-

tion, has created a rational basis for experimental analysis compared with most of the earlier work. We will analyze and summarize these data in the first part of this chapter. The second part will specify the localization, the characteristics and the nature of the senile disturbances of the estrous cycle. The third part will deal with the temporal shifts of the onset of the deviations and the biological measure of the aging of the function under study.

We will not discuss the subject of senile sterility raised in the next chapter. It has been considered in a number of recent publications (*Aging and Reproduction, Journal of Reproduction and Fertility, Suppl 12, 1970*).

THE ESTROUS CYCLE AND ITS VARIATIONS IN AGING RATS

Numerical Data

Fifty percent of our Wistar rats alive at 1 month reach the age of 24 months. The extreme longevity of some animals is 36 to 40 months. These figures are comparable with those of Verzár (1963).

With increasing age the estrous cycles of 4 or 5 days have an increasing tendency to become irregular and are replaced from the age of 12 to 15 months on by two senile deviations of the estrous cycle: the senile permanent estrus (SPE) and the senile repetitive pseudopregnancy (SRPP). Table 19-I gives the percentage distribution of these modalities as a function of age. It combines two different longitudinal studies (nulliparous from 6 to 12 months and multiparous from 15 to 27 months). In cross-sectional studies over a period of 10 years we have repeatedly found a similar distribution.

Table 19-I shows that SPE predominate in the second year and SRPP during the third year. The cyclic females represent between 5 and 10 percent after the age of 18 months. The passage from one state to another cannot be deduced from the Table because it does not take into account mortality, which is noticeable after 12 months. In most cases the cyclic rats undergo only one transformation to a terminal state of either SPE or SRPP. The passage from cycles to SPE and then to SRPP is rare, but unquestionable and interesting for it implies the apparently spontaneous restoration of the ovulatory mechanism suspended during PE. The "cycles" which oc-

TABLE 19-I
PERCENTAGE DISTRIBUTION OF ESTROUS CYCLES, PERSISTENT ESTRUS,
AND REPETITIVE PSEUDO-PREGNANCIES IN RELATION TO AGE

Age in months	6	10	12	15	20	23	27
Estrous cycles	89	72	60	40	8	8	8
Persistent estrus	7	9	20	40	64	52	20
Repetitive pseudo-pregnancies	4	19	20	20	28	40	72

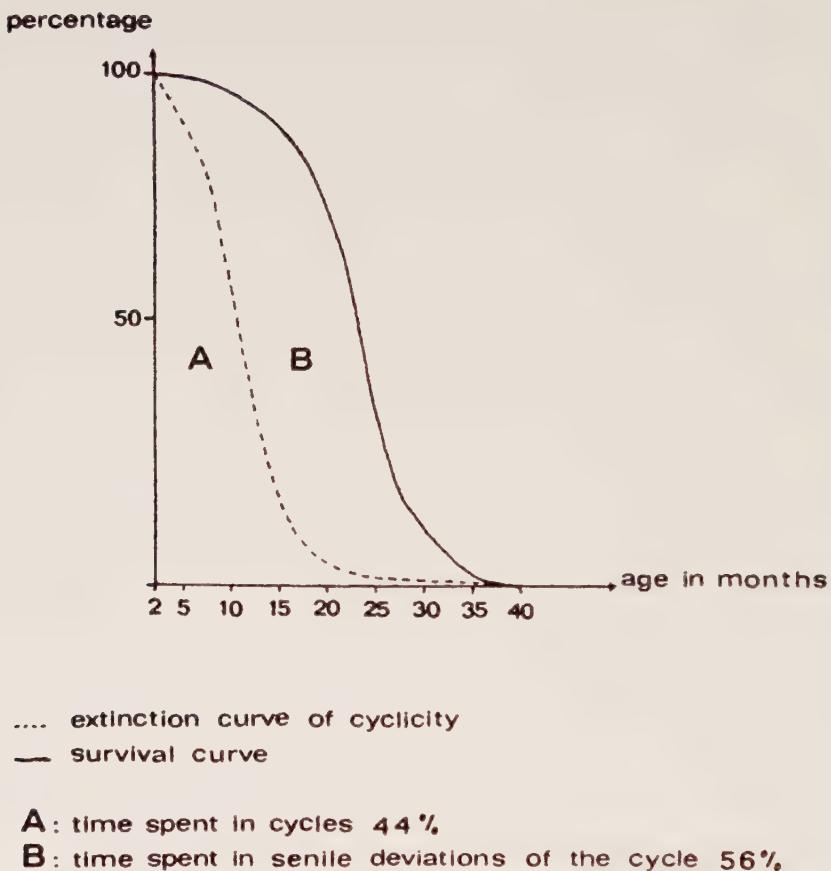


Figure 19-1. Time spent in estrous cycles (A) or in senile deviations of the cycle (B) by a rat population. Curve on left: percentage of cyclic rats. Curve on right: percentage of surviving rats.

casionally follow a PE are mostly anovulatory and aluteal; but again there are a few rare ovulatory cycles. These are more frequent in other strains of Wistar rats, which have a higher rate of PE at 10 to 14 months, but which only stabilize 4 to 6 months later. Everett (1970) had available a strain of rats (DA) almost all of which had PE at 6 to 7 months of age. Our proportions of SPE are comparable to those given by S. Bloch (1959). Mandl (1961) reports a greater number of cyclic rats in the senile multiparous than in the nulliparous group.

Figure 19-1 is a diagram applicable to our nulliparous rats. The coordinate and two curves, on the right the percentage of rats surviving, on the left the percentage of cyclic rats, adjusted for the mortality, define two areas. One can see that after the age of puberty (about 2 months) and up to the disappearance of the population, the total time spent by rats in

estrous cycles (surface A) is less than that spent in senile deviations of the cycle (surface B), 44 percent as opposed to 56 percent. This is a graphic justification of the study of the senile period.

Description

In this histophysiological description of the estrous cycle and its senile deviations, we will mention the *experimental* facts only in as much as they serve to identify the different categories.

SENILE CYCLIC RATS. The estrous cycles of aging rats are often more irregular than those of young adults. Their duration can range from 4 or 5 days to 6, 7 or 8 days, either from a lengthening of the periods of estrus or from a lengthening of the diestrus. The ovary has a mean weight of 30 to 40 mg. The number of eggs ovulated and corpora lutea formed is normal (Aschheim, not published), in spite of the diminution of oocytes which has been thoroughly studied by Mandl and Shelton (1959). Follicles and cyclic corpora lutea are comparable to those of adults. As regards the interstitial tissue which shows morphological and functional signs of senescence, we will study it later for all of the senile categories. The appearance of the genital tract is normal and the mammary glands are neither proliferated nor secreting.

Rats in SPE or in SRPP experimentally reset in estrous cycles show the same histophysiological picture as the spontaneously cyclic senile rats.

SENILE PERMANENT ESTRUS (SPE). This has been known for a long time (see the bibliography in Bloch and Flury, 1959). It is characterized by a persistent cornified smear. One can assume that there is permanent estrus when one recognizes this cytological aspect in 80 to 90 percent of the smears taken on successive days. The polynuclears which appear from time to time among the cornified cells can be due to a uterine inflammation, but sometimes there are also "pseudocyclic sequences" (Everett, 1939) without ovulation.

Permanent estrus is an anovulatory state. The ovaries are quite small (mean weight of 20 to 30 mg per ovary), devoid of corpora lutea but with healthy and atretic follicles of every size. There are always a few ripe follicles, sometimes cystic ones. The interstitial cells are qualitatively similar to those of other senile categories but occupy a relatively more important place, due to the absence of corpora lutea. The biochemical study of the steroids produced has been effected only *in vitro*. According to Weisz and Lloyd (1965) there is in these PE ovaries an increase in the conversion of labeled progesterone into estrogen and an accumulation of testosterone and androstenedione. The genital tract may be the seat of inflammatory phenomena. The uterine horns are stimulated by estrogen, but are not turgescent. The vagina is cornified. The mammary glands are hypertro-

phied and secreting (Aschheim, 1962); the hypophysis shows a hyperactivity and a proliferation of the prolactin cells (Aschheim and Pasteels, 1963), characteristics which SPE share with all the other categories of senile rats, except the cyclic.

SENILE REPETITIVE PSEUDOPREGNANCY (Aschheim, 1961). This is characterized on the vaginal smear by an estrus which intervenes every 12 to 14 days; sometimes the diestrous interval continues for 30 days or more. Estrus can last 1 or 2 days, be transient or be replaced by a proestrus-postestrus sequence. Ovulation is qualitatively and quantitatively normal at the time of estrus; it can be seen in more than half the cases when a postestrus follows a proestrus (Aschheim, not published). The use of a marker, such as Evans blue, indicates that every pseudopregnancy results from the formation of a new generation of functional corpora lutea. Sometimes an estrous cycle of 4 or 5 days occurs between two pseudopregnancies. The SRPP have a tendency to lengthen progressively.

The ovary of such rats weighs between 40 and 50 mg. As the RPP is a later senile deviation than PE, there is here an exception to the rule of ponderal involution of organs with age. The functional corpora lutea measure between 1.3 and 1.4 mm (pseudogestational size). The ovary of the SRPP contains at least 3 generations of corpora lutea at a time, while that of adults in experimental RPP (by renal graft of a supplementary pituitary) has only one generation (Quilligan and Rothchild, 1960). Follicles of all sizes are present. In diestrus the uterine horns are thin and the vagina shows a mucification of the lactational type (a layer of basal cells surmounted by a single layer of mucous cells), indicating a very low or nonexistent estrogen production, plus progesterone. The mammary glands and the pituitary indicate as before a hypersecretion of prolactin.

The pseudopregnant character of prolonged diestrus (or the secretion of progesterone by the corpora lutea) is demonstrated in 2 ways: 1) by the ready development of traumatic deciduoma after the passage of threads in the uterine horns on the 4th or 5th day after estrus (Aschheim, 1961); 2) at any moment of PP, by the fact that the injection of 1 µg of estradiol benzoate daily for 3 days does not result in a vaginal keratinization, as with castrates of the same category, but in a mucification of the gravid type, indicative of a progesterone estrogen synergism (or a progesterone antagonism to keratinization). In this way we have been able to ascertain the functional character of these pseudogestational corpora lutea up to more than 30 days after their formation (Aschheim, not published).

SENILE RATS "ANDROGENIZED" AT BIRTH. We have followed the senescence of rats, which, after injection with 1.25 mg of testosterone propionate at the age of 5 days, enter into permanent estrus at the time of their puberty. They are sterile (androgen-sterilized = AS) because they are anovulatory.

At the time of their senescence the vaginal estrus persists, however in a less regular manner. The ovaries, still deprived of corpora lutea, contain fewer follicles than before, but up to the age of 23 months there is no depletion of the oocyte stock. The mammary glands hypertrophy and become secretory (Aschheim, not published).

SENIQUE RATS IN ANESTRUS. An occasional senile rat does not fit into the preceding categories. The vaginal smear shows a prolonged diestrus of several weeks, interrupted irregularly by an estrus which can last one or several days. The ovary is often very small (10-15 mg), and devoid of corpora lutea, but primary follicles and occasional maturing or mature follicles are always present. The uterus is atrophic, the vagina is unstimulated, without mucification or keratinization. The mammary glands are developed as before.

Anestrus coupled with an atrophic ovary is, according to Bloch and Flury (1959), a terminal state rarely attained by senile rats. One may question however, whether one still deals with a normal senescence, for this atrophy is usually seen only in our apparently sick animals as it is in adults under severe chronic stress (Selye, 1939). In no case is there oocyte exhaustion before death in the rat (Bloch and Flury, 1959; Mandl and Shelton, 1959) as there is with the CBA mouse from the age of 14 months on (Jones and Krohn, 1961a) or in the human female after menopause. Even the Wistar rats of the W₁₂ variety of the CNRZ (kindly placed at my disposal by Mr. Mauleon), which undergo a very rapid oocyte loss in the first weeks of life (Mauleon and Rao, 1963), retain a few ovarian follicles between 21 and 24 months, although the ovarian weights indicate a strong atrophy (Aschheim, not published).

The ovaries of animals in anestrus respond to gonadotropic hormones both exogenous (Bloch and Flury, 1959) and endogenous (intrasplenic ovary, Aschheim, 1968b). The formation of corpora lutea, the repair and then the hypertrophy of the interstitial cells in the intrasplenic ovarian graft indicate: 1) that the ovary *in situ* exerted a restraining action through negative feedback on the hypophysis, and, 2) that the latter is capable, once unrestrained, of functioning as it does in the case of castration. Therefore senile anestrus in the rat is not a spontaneous condition similar to that of the human menopause.

Cytohormonal Data

THE OVARIAN INTERSTITIAL TISSUE, ITS DEFICIENCY CELLS, AND THE CIRCULATING LH. A certain number of morphological signs of senescence are common to the ovaries of all types of old rats. These changes develop progressively after 12 months. There are cells (probably macrophages) filled with senile pigment (lipofuscin). There is always an important proliferation

of the germinal epithelium. Above all, formations appear which resemble "testis-like tubules" (Romeis, 1931) as well as cords of cells of epithelial shape. These elements have been described in detail in the mouse by Thung (1958). It has been shown recently that testis-like tubules derive from atretic follicles as a special consequence of the decrease in circulating gonadotropins (Arias and Aschheim, 1974) and that the cellular cords can be "luteinized" by HCG (Arias-Crumeyrolle, *et al.*, to be published).

Another senile feature is the existence of deficiency cells (DC) or "wheel-cells" in the ovarian interstitial tissue. They have a specific morphological aspect. Their functional meaning is known: the absence or insufficiency of the circulating LH. The DC have been described by Wolfe (1940) and Mandl (1959) in old rats, by Green (1957) and Jones and Krohn (1961a) in old mice. They are repaired for the most part by the daily implantation of a male rat's pituitary (Burack and Wolfe, 1959); 20 IU of PMS are effective in mice (Green, 1957). We have seen the DC in *all* senile categories after 13 months, including those that ovulate periodically, releasing a physiological amount of ovulatory LH. We have shown that the DC have not become insensitive to the gonadotropic hormone, since they resume a normal appearance 1) in a young adult gonadotropic environment (by heterochronic graft of the ovaries) 2) in the senile rat itself, in an intrasplenic graft (they hypertrophy because of the increase in LH due to castration) 3) after injection of HCG (Aschheim, 1968b). The existence of the DC clearly indicates a reduction in the level of basal circulating LH compared with that of adult rats.

There is no vascular sclerosis in the ovary of the aged rats.

THE SENILE PITUITARY AND ITS CONTENT OF PROLACTIN, LH AND FSH. The "chromophobic adenoma" of the hypophysis is frequent in the old rat, especially in the female (Wolfe, *et al.*, 1938). It has been established (Aschheim and Pasteels, 1963) that the prolactin cells are hyperactive in all our senile *noncyclic* Wistar rats including castrates. This explains the mammary secretion, already indicated. On the other hand, these same elements proliferate, ranging from small clusters of giant prolactin cells, to limited adenomas whose cells retain their typical erythrosinophilic granulations, and to the previously mentioned "chromophobic" tumors.

The hyperactivity of the prolactin cells in the senile pituitaries is reflected both by an increase in the circulating hormone (Aschheim, SRPP 1961, SPE 1962, SAS 1965) and by an elevated prolactin content of the pituitary (Clemens and Meites for the SPE, 1971).

The total gonadotropin content is elevated according to Lauson, *et al.* (1939) in 3 rats aged 2½ years, only one of which had corpora lutea.

Matsuyama, *et al.* (1966) confirmed this observation in a pool of 4 hypophyses from SPE aged 12 months.

The same authors report in the same case of SPE a low content of LH measured by Parlow's ovarian ascorbic acid test. Kabak and Sokolova (1963), with a nonquantitative method, find a concentration of LH greater than that of the cyclic rat at the time of estrus, which is minimal.

Table 19-II shows our results (Aschheim, 1968a) of the hypophyseal LH content by the Parlow test in the different categories of senile rats. After ovulation, the cyclic rats have a comparable content at 4 and at 19 months. Similarly in rats androgenized at birth (AS), the LH content is the same at 5 and 19 months. The amount in the SPE is low, being 3 to 4 times lower than that of the SRPP, at the end of pseudopregnancy. Ovulation in the latter is accompanied by a significant reduction in the hypophyseal LH. SPE and SRPP have respectively the amounts of LH corresponding to

TABLE 19-II^a
THE AMOUNT OF HYPOPHYSEAL LH IN DIFFERENT TYPES OF
SENILE FEMALE RATS

Age in Months	Number of Pituitaries Per Group	Content in μg of LH ^b Per Gland	95% Confidence Limits	Functional Type	Time of Autopsy
12-24	5	35.0	11.1-111.0	SRPP ^c	Proestrus
23-25	4	36.5	10.9-122.5	SRPP	Diestrus of 9-11 days
24-25	5	24.6	11.7- 52.0	SRPP	Diestrus of 7-10 days
33	4	17.9	8.2- 39.4	SRPP	Diestrus of 4- 8 days
30	1	26.4	13.5- 51.8	SRPP hemicastrated	Diestrus of 14 days
23	1	22.3	10.3- 48.6	SRPP hemicastrated	Diestrus of 12 days
30	1	22.0	9.9- 49.0	SRPP hemicastrated	Diestrus of 10 days
20	1	16.3	8.4- 31.3	SRPP hemicastrated	Diestrus of 5 days
22	1	8.5	3.5- 20.9	SRPP hemicastrated	Post estrus
20-27	6	5.5	2.3- 13.2	SPE ^d	
21-24	6	8.1	5.6- 11.7	SPE	
24-26	6	6.8	3.1- 14.7	SPE	
22	4	11.9	5.6- 23.5	SPE	
20	3	10.9	4.5- 26.9	SPE	
22	4	13.7	7.0- 26.9	SPE hemicastrated	
19	6	22.8	12.1- 43.0	AS ^e senile	
5	8	19.5	8.3- 45.4	As adult	
19	6	8.0	3.5- 18.3	Cyclic senile	Post estrus
4	5	8.4	5.1- 14.0	Cyclic adult	Post estrus

^a From Aschheim (1968a), simplified.

^b LH-NIH-S_s, gift of Endocrinology Study Section, NIH, Bethesda, Md.

^c SRPP—senile repetitive pseudopregnancy.

^d SPE—senile permanent estrus.

^e AS—androgen-sterilized.

those of adults affected experimentally with the same anomalies of cycle (PE under permanent light or after anterior hypothalamic lesion; PP by cervical stimulation). In summary at the same age, the hypophyseal LH content of senile rats varies with the nature of their estrous and ovarian rhythm. In the same neuroendocrinological situation adult and senile rats have a comparable content of LH.

Labhsetwar (1969) found a raised LH content (Parlow test) in irregularly cyclic rats of age 9 months, estimated from their weight, killed in diestrus and with corpora lutea in their ovaries. It is the same increased amount of LH that we have shown in our pseudopregnant rats and may reflect the same endocrinological condition. The pituitary of these animals is 2 to 3 times richer in FSH (Steelman and Pohley test) than that of younger rats.

As to FSH, Takasugi (1963) indicates that adult mice placed in parabiosis with intact senile ones show PE just as they do with adult castrates, whereas when united with intact adults, they remain cyclic, as do their parabiont partners. This activity of the senile gonadotropic hormones cannot be seen in the ovaries of the senile mice, which do however remain responsive, as shown by the parabiosis with adult castrated mice! These results seem to me difficult to interpret (for example by hypersecretion of FSH), in the absence of data from adult hypophysectomized mice parabiosed with senile mice, with or without ovaries.

THE HYPOTHALAMUS of rats in SPE contains as much prolactin-inhibiting factor (PIF), less luteinizing-hormone-releasing-factor (LRF), but more FSH-releasing-factor (FRF) than those of adults (Clemens and Meites, 1971). We lack data concerning the modifications with age in the production of hypothalamic monoamines and in the vascularization of the region.

There is little certainty as to the diminution in the number of hypothalamic neurons with age, a phenomenon quite evident in other sectors of the central nervous system, such as the cortex, the cerebellum, the thalamus. On the contrary, Andrew (1956) insists on 2 points: 1) there is no cellular destruction in the supraoptic and paraventricular nuclei of the human senile hypothalamus, nor any proof of real degenerative changes, 2) Buttlar-Brentano (1954) describes the following modifications in these nuclei: an increase in the cellular volume, leading to giant neurons, with a surface 8 to 10 times the adult size. These cells are bi- or trimuclear, with an increase in the nuclear "basophilia," and 2 to 6 nucleoli. Andrew interprets this as a reactive or defensive phenomenon. In any case, we know nothing about similar age changes in the rat's neurons responsible for gonadotropin regulation: those of the preoptic area and the arcuate and ventromedial nuclei. In a recent paper, Babichev (1973) states that in old cyclic female

rats, these same neurons become less sensitive to estradiol, although those of the arcuate region remain more sensitive than those of the preoptic region.

Summary and Neuroendocrinological Significance

Table 19-III attempts to summarize the hormonal status in the different senile conditions, either by means of biological or biochemical measurements or by the histophysiological study of the appropriate target organs.

TABLE 19-III
HORMONAL STATUS IN THE SENILE FEMALE RAT

	Cyclic adults	Cyclic seniles	SPE	SRPP	SAS	Means of detection
Estrogen (ovarian in vitro) (circulating)	N		↗			biochemistry
	N	N	→ ^a	↘	→ ^a	histological aspect of target organ
Testosterone (ovarian in vitro)	N		↗			biochemistry
Progesterone (circulating)				↗		histological aspect of target organ
Total gonadotropins (hypophyseal)	N		↗			bioassay
FSH (hypophyseal)	N		↗	↗ ^b		bioassay
LH (hypophyseal) (circulating)	N	N	↘ ^c	↗ ^c	N ^d	bioassay
	N	↗	↘	↘	↘	histological aspect of target organ
Prolactin (hypophyseal) (circulating)	N		↗			bioassay
	N	N	↗	↗	↗	histological aspect of target organ
FRF (hypothalamic)	N		↗			bioassay
LRF (hypothalamic)	N		↘			bioassay
PIF (hypothalamic)	N		N or ↘			bioassay

SPE senile permanent estrus

SRPP senile repetitive pseudopregnancy

SAS senile androgen-sterilized rats

N normal value or situation for adults within the limits of variation during the cycle.

↗ increase ↘ decrease in senile rats compared with cyclic adults

a persistent secretion of estrogen; amount ignored.

b if the information from Labhetswar (1969) concerns the SRPP

c but N in comparison with adults, in persistent estrus by continuous illumination or hypothalamic lesion, or pseudopregnant after cervical stimulation.

d N by comparison with androgen-sterilized adults.

What seems to emerge from the table is not so much the need to fill the empty spaces, but the overall significance that leads to an experimental approach. Two facts are dominant:

ALL THE SENILE GROUPS HAVE A DIMINISHED BASAL CIRCULATING LH while the hypophyseal content of the hormone varies significantly from one group to another. Synthesis and release are diminished or slowed down in the SPE. In the SRPP, the lack of release during pseudopregnancy is accompanied by a progressive accumulation of LH in the pituitary which does not indicate the intensity or speed of synthesis. After ovulation, the reduction in the hypophyseal LH content is greater in absolute value than in the cyclic rat.

ALL THE SENILE NONCYCLIC GROUPS SHOW A HYPERSECRETION OF PROLACTIN. The hypothalamic control of this secretion being inhibitory, this means that there is a hypothalamo-hypophyseal "disconnection" for the function studied. It develops *in situ*. It concerns only prolactin, since the ovulatory LH "passes" at the beginning of each pseudopregnancy and since the production of estrogen in the SPE necessitates a basal secretion of FSH and LH. It will be seen later that it is for a great part reversible. At any rate it serves to draw attention to the senile hypothalamus.

EXPERIMENTAL STUDY OF THE SENILE MODULATIONS OF ESTROUS REGULATION

This section deals with the localization of the senile disorder in the regulation of the estrous cycle, its characteristics and its nature. This *experimental* study and the methods used are relevant both to gerontology and to neuroendocrinology.

Primary Responsibility of the Hypothalamus

The hypothalamus of the noncyclic senile rat thus functions in a different manner from that of the cyclic adult female. Does the primary cause of this difference reside in the hypothalamus itself? Or do the hypothalamic regulating centers merely reflect a disturbance of peripheral origin? The part played in this respect by the ovary and the hypophysis can be specified by their "heterochronic graft."

HETEROCHRONIC GRAFT OF THE OVARY. The experiment (Aschheim, 1964-65) consists of grafting, to a female Wistar rat deprived of its own ovaries, the ovaries from a rat of a different age. These homografts are inserted under the renal capsule or in the anterior chamber of the eye.

Whether the replacement of the ovary is immediate or is done one month after castration, the results are identical and can be systematized in this way:

Pre-puberal ovaries (28 to 32 days) grafted into senile female rats pre-

viously in permanent estrus, cause renewal of the permanent estrus. Likewise, pre-puberal ovaries implanted into senile females previously in repetitive pseudopregnancy restore this type of function.

Conversely, senile ovaries, originating from rats in permanent estrus or in repetitive pseudopregnancies assume normal cycles when they are grafted into a young adult rat. We have already noted that under these conditions, the deficiency cells of the senile ovary resume a normal appearance.

It is not the age of the ovary that is responsible for the manner of its endocrine functioning, but the age of the hypothalamo-hypophyseal control system to which the ovary is subjected.

Moreover, if a cyclic rat is castrated at between 8 and 12 months and receives at 24 to 27 months an either immature or senile ovarian graft, it resumes estrous cycles, a very exceptional occurrence in intact controls of the same age. After this long period of castration during which the higher centers are deprived of all steroid information of ovarian origin, they show the same sensitivity as before.

Zeilmaker (1969) has confirmed my results by exchanging ovaries between adults and SPE. A more complete confirmation comes from Peng and Huang (1972) who extend the experiment to senile anestrus rats.

In the mouse, the previous experiments of Krohn seem more difficult to interpret. Jones and Krohn (1961b) transplant ovaries from CBA mice hypophysectomized when young and allowed to become old into some adult CBA's and into a senile CBA. In the adult, the ovary grafted into an orthotopic position assumes cycling and gravidity. However in the senile mouse, both before and after the graft, the estrogen stimulation is weak and irregular. The ovary develops some Graafian follicles but no corpora lutea. The interstitial tissue is deficient. We shall return later to the role of hypophysectomy in conserving the stock of oocytes. This experiment clearly indicates that the functioning of this ovary which is chronologically old, but has been for a long time inactive, depends on the gonadotropic environment, which is normal in the adult recipient but is deficient in the senile recipient.

An orthotopic heterochronic graft of an ordinary senile CBA ovary into the adult assumes only a very irregular estrogen stimulation (Krohn, 1962). This is due to the fact that such ovaries no longer possess follicles and have become refractory to the adult gonadotropins. Senile CBA \times A ovaries, still possessing oocytes, function in a cyclic manner in the adult. It is the inverse experiment that is disturbing. In the senile CBA mice that have been grafted with young ovaries, there is a resumption of estrous cycles where previously they had stopped or a normalization of the rhythm in the mice previously in prolonged estrus or with irregular cycles. For Krohn (1967b), these transplanted ovaries have a normal appearance. The

author concludes (Krohn, 1962) that the gonadotropic function is normal, whereas he speaks of an LH deficiency in the very same senile CBA recipient mouse mentioned in the previous paragraph (see also the discussion of Ber (1968), p. 63).

In my opinion, the following hypothesis remains: if certain CBA mice stop secreting ovarian steroids at about 10 to 12 months and others do not, then the first group could be, until the grafting of young ovaries, in the situation of my rats, castrated young and grafted when senile, or in the situation of the rats of Smith (1963), that were hypophysectomized young and grafted with hypophyses 12 months later (see further on). In this first group the hypothalamic sensitivity to the ovarian steroids would be maintained unchanged in their absence and allows the resumption of cycles after the ovarian graft. The mouse of Jones and Krohn (1961b) would be, on the other hand, an example of the second group.

In the case of the rat there is no primary ovarian deficiency with age. However, this does not signify that ovarian aging is nonexistent, but means that it is not responsible for the reorganization of the hypothalamo-hypophyseal ovarian regulation appearing with age.

HETEROCHRONIC GRAFT OF THE PITUITARY. Smith (1963) hypophysectomizes Long-Evans female rats 40 days old and grafts into their sella turcica 60, 200 or 375 days after hypophysectomy, the hypophysis of a pre-puberal male. The experiments are well controlled and great care is taken to ensure vascular reconnection. Two thirds of the females start their normal vaginal cycles again, the ovaries resume a normal weight and appearance, the interstitial cells become normal, even those in the group of rats grafted at the age of 415 days and autopsied at 16 months. The only anomaly is an absence or a deficiency in the lactation of the females after parturition. In the case of female rats that remain acyclic, there is a gain of weight, stimulation of bone growth and of the thyroid as in the preceding groups, but the adrenal cortex and the ovary remain atrophic as with the hypophysectomized rat.

It is important to note that in the oldest group, the graft still allows at the age of 16 months a normal functioning of the estrous cycle with a normal ovarian interstitial tissue, when at this age intact rats usually have developed deficiency cells. During 375 days, the absence of any ovarian secretion preserves the hypothalamic sensitivity to steroids.

Pecile, *et al.* (1966) hypophysectomize females of 40 days. One month later they transplant into the sella turcica 3 mg of hypophyseal tissue from rats aged respectively 30 days, 8 months and 2 years. The grafts of all ages exhibit good functional capacity with regard to growth hormone but not for the corticotropin and gonadotropic hormones. The gonadotropic function remains nil, except with the pituitaries of 30 days. The transfer of

3 mg of hypophyseal tissue leads one to think that only glandular fragments from the groups of 8 and 24 months have been grafted. Under these conditions, it is as easy for a hypothalamic-hypophyseal reconnection to succeed as with an entire gland? It is difficult to understand that the pituitaries of 8- and 24-month-old rats are equally incapable of being stimulated by a young hypothalamus.

Peng and Huang (1972) replace pituitaries of young females by those from old ones, grafted under the median eminence. In 10 out of 30 cases, there is a resumption of vaginal cycles, 3 of them being also luteal, and one case of fertility. Thus, old pituitaries can function normally in young females, but they seem to do so much less frequently than young pituitaries grafted under the median eminence of young recipients. The graft of young pituitaries into senile recipients has not yet been done.

Some Characteristics of Senile Deviations of the Estrous Cycle

THESE DEVIATIONS ARE NOT FIXED. Bloch and Flury (1959) have described ovulatory cycles which spontaneously follow PE. We have observed (Aschheim, not published) that this is so in certain Wistar strains, although not in the Wistar strain that we have mostly studied. But in the latter the SPE can "spontaneously" pass to SRPP. We cannot indicate the percentage of SPE which change the rhythm in this way, but we do know that about 20 percent of the SRPP come from SPE (Aschheim, 1961).

SRPP exposed to permanent light enter into PE and when put back into alternating light they return to SRPP (Aschheim, 1961).

So these animals, while remaining under a senile neurohumoral command, are led to produce the one ovarian hormone, progesterone or estrogen, which was previously lacking.

THESE DEVIATIONS ARE REVERSIBLE. Unlike the preceding situation here an experimental procedure restores ovulatory cycles which appear self-sustained after the initial, inducing modification.

In 1927, Zondek and Aschheim described the morphological and functional reactivation of the ovary of the mouse in anestrus for 5 months, by the implantation of the cow's pituitary. Hoffman (1931) in the senile mouse, and Romeis (1931) in the senile rat, likewise both obtained the resumption of estrous cycles.

We have reactivated the SPE rats by intravenous injection of LH (1 to 3 µg of LH-NIH), by subcutaneous injection of 10 IU of HCG or by subcutaneous implantation of the pituitary of a rat in SRPP (Aschheim, 1965).

In almost 70 percent of the cases the gonadotropin induces corpora lutea which become functional through the prolactin secreted by the SPE. Pseudopregnancy is followed by ovulatory cycles, due this time

to the ovulatory release of *endogenous LH*. At the same time the secretion of prolactin is inhibited, the mammary glands which previously were secreting, regress, the new corpora lutea are small. It would be interesting to study the hypophyseal prolactin cells previously hyperactive and often tumoral. On the average, these rats have 11 autonomous cycles (5 to 24) of which 75 percent have a duration of 4 or 5 days. The final evolution is towards repetitive pseudopregnancy; only exceptionally is there a return to PE.

The injection, 2 days after the gonadotropic hormone, of ergocornine, a drug which interrupts the secretion of prolactin, and hence the secretion of progesterone by the new formed corpora lutea, leads to a resumption of PE. When exposed to continuous light, the reactivated rats enter permanent estrus, but when returned to alternating light they resume cycles, again preceded in most cases by a pseudopregnancy. Senile androgen-sterilized rats react to the injection of LH by a pseudopregnancy, but this is not followed by a release of endogenous ovulatory LH and the rats return to PE.

The reactivation of the SPE is also possible by provoking a release of endogenous LH, which is achieved by placing in darkness. Persistent vaginal estrus is now replaced by vaginal cycles and in half of the cases this is accompanied by the formation of cyclic corpora lutea (Aschheim, 1965; see also Everett, 1943 and 1970). The daily injection of 0.25 mg of epinephrine for 10 days leads in half the cases to the restoration of numerous normal cycles (Clemens, *et al.*, 1969). The aptitude for the ovulatory release of LH in SPE rats with their small amount of hypophyseal LH is demonstrated by electrical stimulation of the preoptic area of the hypothalamus (Clemens, *et al.*, 1969; Everett, *et al.*, 1970) and through the administration of the LH releasing factor (Aschheim, 1963, not published).

The SRPP rats can also be rendered cyclic again, but the efficiency of the treatment is clearly less. The housing of adult mice in groups leads to pseudopregnancy (Van der Lee and Boot, 1955). It does not in adult rats (Heinecke, *et al.*, 1960). But in senile rats, the opposite operation, the isolation of grouped SRPP rats, renders them cyclic in 5 times out of 7 at 16 to 19 months of age and once in 9 rats aged 20 to 26 months (Aschheim, 1966).

To conclude, the "reactivation" of senile rhythms is more or less easy to realize and generally of limited duration. Its mechanism raises several neuroendocrinological problems, in particular the cessation of the secretion of prolactin. But the correction of senile deviations of the estrous cycle is a fact which implies 3 consequences:

1. Reactivation produces a large number of senile cyclic rats and so per-

mits the comparative study with the few senile rats which remain spontaneously cyclic and with adult cyclic animals.

2. Among the efficient means of reactivation the external environmental factors are interesting. Isolation from light for the SPE, social (olfactory) isolation for the SRPP could create for the animal the "basic" conditions facilitatory for the self maintenance of the cycles, thus masking the increasing inability of the aging organism to adapt to changing conditions of the environment.

3. The reversibility of the senile deviations helps us to understand the cause of aging in the hypothalamo-hypophyseal-ovarian axis. A reversible state seems to be incompatible with the idea of *primary* faults in hormonal production or release of whatever origin (ovarian, hypophyseal, hypothalamic). It points more to a *primary* trouble in the reception or transmission of information, to changes in the sensitivity of the regulatory structures which have already been localized in the hypothalamus. Anyhow, it is this hypothesis of a modification with age of the hypothalamic sensitivity to ovarian steroids and especially to estrogen which we have tried to verify.

The Nature of Aging in the Central Regulatory Mechanism of the Gonadotropic Function

A recent report (Aschheim, 1970) deals with the feed-back of ovarian steroids on the regulation of the LH function in senile rats. The effects of castration and of injection of estrogen in the castrate on the hypophyseal and on the circulating LH and the effect of hemicastration or the administration of steroids on other parameters in the noncastrated female are studied.

FEED-BACK DUE TO CASTRATION OR ADMINISTRATION OF ESTROGEN IN CASTRATES. Senile rats are divided according to their estrous rhythm, whether cyclic or in senile deviation. The duration of castration is one month. The treated female castrates receive during this month 5 μ g of estradiol benzoate every 3 days. Other groups consist of rats castrated when young and allowed to become old, some remain without treatment and others receive 5 μ g of estradiol benzoate every 3 days during the last month of their life; groups at autopsy are spread out up to 28 months of age.

The hypophyseal LH is determined according to the Parlow method, circulating LH is evaluated by the histophysiological appearance of the ovary grafted into the spleen, the formation of corpora lutea and the aspect of the ovarian interstitial tissue.

Results

- a. Castration results in the increase of circulating LH in all senile categories.

- b. Castration results in the increase of hypophyseal LH in all senile categories (Table 19-IV).
- c. The magnitude of feed-back differs according to the categories studied (Table 19-V).
- d. Prolactin restrains the increase of hypophyseal LH after castration.
- e. The restraint put on the LH of castration by estrogen is more efficient in the senile previously cyclic rats than in the adult previously cyclic rats (Table 19-VI).
- f. The animals which are castrated young retain their hypophyseal sensitivity to the withdrawal and administration of estrogen throughout their senescence.

Comments

a. This proposition is derived from the study of more than 80 ovarian intrasplenic grafts. The categories (SPE, SAS) whose ovaries *in situ* are devoid of corpora lutea form these in the intrasplenic grafts one month after the operation. The interstitial tissue, which is deficient in the ovary *in situ* in all categories, is restored and then undergoes hypertrophy in the intrasplenic position. The administration of estrogen during castration causes the deficiency cells to reappear in the splenic ovary.

b. In all the castrated groups, the increase of hypophyseal LH is significant by comparison with the corresponding intact females. Table 19-IV shows that the hypophyseal content is about 80 µg of LH per gland in the castrated adult or senile rats which were previously cyclic (the same in the adult castrates previously androgen-sterilized and not shown in the Table),

TABLE 19-IV^a
HYPOPHYSEAL CONTENT OF LH IN µg OF LH-NIH^b PER GLAND
MEASURED BY THE OVARIAN ASCORBIC ACID DEPLETION TEST

	<i>Intact Female</i>	<i>Female Castrated for 1 Month</i>	<i>Female Castrated and Estrogenized for 1 Month^c</i>
Cyclic rats, 3-5 months old . . .	10.9 ± ^d 2.5 (2) ^e	74.5 ± 6.6 (8)	37.3 ± 3.5 (3)
Cyclic rats, 12-14 months old . . .	12.4 ± 4.4 (2)	80.0 ± 6.9 (6)	20.2 ± 6.8 (3)
Rats in senile permanent estrus . . .	9.4 ± 1.3 (6)	43.9 ± 3.6 (5)	20.9 ± 3.5 (2)
Rats in senile repetitive pseudopregnancy	28.5 ± 4.4 (4)	57.8 ± 3.0 (2)	46.3 ± 13.4 (2)
Senile androgen-sterilized rats	28.8 ^f (1)	47.3 ^f ± 6.7 (3)	28.2 ^f (1)

^a From Aschheim (1970), modified.

^b LH-NIH-S₃ and S₁₂, gift of Endocrinology Study Section, NIH, Bethesda, Md.

^c 5 µg of estradiol benzoate every third day.

^d ± standard error.

^e () number of assays.

^f Without an aberrant value.

and the LH content is about 50 µg for castrated senile rats previously in RPP, PE or AS.

c. Table 19-V illustrates this dichotomy by the relative potencies of LH. They are respectively of 6-7 for the first 3 groups and of 2-3 for the last 3 groups. The column of castrated adults indicates an identical behaviour between cyclic and AS rats. In the column of the senile castrates, the cyclic rats are different from all the other senile categories which behave identically. The line of cyclic rats does not show a change with age, whereas the AS line shows a difference in relative potency according to age.

d. It is prolactin which is responsible for the smaller increase in LH after castration of senile, noncyclic rats.

The suppression of the secretion of prolactin by "reactivation" of cycles previously to castration increases the hypophyseal content of LH from 50 µg to 80 µg one month after ovariectomy. Thus the magnitude of the increase in LH after castration which is clearly less in the most representative senile groups, SPE and SRPP (and also in the SAS), is not a *direct* effect of aging, but a consequence of the *preliminary* modification of the hypothalamic regulation of the estrous cycle, a direct consequence of hypersecretion of prolactin.

e. On the other hand the cyclic senile rats behave like adults with respect to castration. Thus, although very rare, they are of fundamental importance. The easy experimental transformation of SPE into senile cyclic rats similar to the former allows one to increase at will the number of animals in this group. It is indeed this category which, from a gerontological point of view is directly comparable with adults. The restraint put on the increase of LH after castration by estrogen is clearly more efficient in old cyclic rats than in adults; in the former the hypophyseal LH is no more

TABLE 19-V^a
RELATIVE POTENCY OF HYPOPHYSEAL LH IN FEMALE RATS
AFTER ONE MONTH OF CASTRATION^b

	Adult	Senile
Cyclic	6.0 { 6.2 (3.7-10.5) ^c 5.8 (3.5-10.0)	6.3 { 8.0 (4.0-16.0) 4.6 (2.0-10.8)
Senile repetitive pseudopregnancy		3.1 { 3.4 (1.2- 9.6) 2.8 (0.8- 9.7)
Senile permanent estrus		2.7 { 3.4 (1.7- 6.8) 1.9 (0.8- 4.5)
Androgen-sterilized	7.0 (3.2-15.6)	2.0 { 2.6 (1.5- 4.8) 1.3 (0.6- 3.2)

^a From Aschheim (1970).

^b Compared to intact female rats of same age and category.

^c () 95% confidence limits.

TABLE 19-VI^a

RELATIVE POTENCY OF HYPOPHYSEAL LH IN FEMALE RATS CASTRATED AND ESTROGENIZED^b FOR ONE MONTH^c

	3-5 Months of Age	12-14 Months of Age
Cyclic before castration	4.9 (2.8-8.5) ^d	1.2 { 1.7 (0.4-6.7) 0.7 (0.3-2.1)

^a From Aschheim (1970), modified.

^b 5 µg of estradiol benzoate every third day.

^c Compared to intact female rats of same age.

^d () 95% confidence limits.

significantly different from that of intact controls; whereas in the latter, it is about 5 times more potent (Table 19-VI). Thus, contrary to what appears in 90 percent of senile rats (those in senile deviations of the cycle), there is an *increase* with age in hypothalamic sensitivity to estrogen when comparison is made between cyclic rats differing only by age.

f. The results of prolonged maximal working of the hypothalamo-hypophyseal complex are studied in female rats castrated early in life and left to age. Castration is performed between 3 and 6 months of age. The hypophyseal LH content estimated at varying times between 2 and 25 months after the operation is approximately 80 µg per gland, which is similar to that obtained one month after castration. The injection of 5 µg of estradiol benzoate every 3 days for a month reduces this amount to about 20 µg per gland. The persistence of the sensitivity of the system to the withdrawal or administration of estrogen should be compared to another fact already mentioned: the reestablishment of estrous cycles in rats, castrated or hypophysectomized when young, to which the missing organ is grafted at an old age. A prolonged period of ovarian inactivity preserves the central regulatory potentialities.

Griesbach and Purves (1960) have shown in their rats of both sexes, castrated when young and allowed to grow old, a high proportion of hypophyseal tumors of gonadotropic cells. Castration is believed to be solely responsible for this change. The adrenal has been excluded. Houssay, *et al.* (1951) have described in the rat castrated for a long time, adrenal tumors which do not seem to secrete sex hormones active on the genital tract (Houssay, *et al.*, 1952). Estrogen stimulation in these castrated rats which is frequently mentioned in the literature is attributed by these authors (1952) to an incomplete castration.

But what happens when the hypothalamo-hypophyseal system is already aged at the time of castration? We have seen that one month after castration, the hypophyseal LH content is significantly increased in all types of

senile rats by comparison with corresponding intact rats. However it is not known if this value will be maintained for the rest of life. Does prolactin play again a discriminatory role between the different groups? The mediocre development (at the end of 6 months) of intrasplenic ovaries of SRPP grafted at more than 24 months of age, coupled with a low LH content of their pituitaries will lead us to the study of continuous maximal functioning imposed on a system already aged and therefore perhaps no longer homogeneous.

Bøe, *et al.* (1954) found that the splenic autograft of the ovary in rats aged from 18 to 24 months was only weakly developed at the end of 4 to 5 months. In the mouse the splenic homograft leads to tumors both of the young ovary grafted to a senile mouse and of the senile ovary grafted to a young animal (Klein, 1953). According to Ber (1968) the ovary possesses an intrinsic property which controls its capacities for growth, diminishes with age and is independent of the hypophyseal environment.

THE FEEDBACK OF STEROIDS ADMINISTERED TO INTACT SENILE RATS. The existence of deficiency cells in the ovarian interstitial tissue of all groups of senile rats indicates a hyposecretion of LH. Their presence in the anovulatory females (SPE, SAS) shows that this hypofunctioning affects the basal or tonic secretion of LH, regulated by the arcuate and ventro-medial nuclei of the hypothalamus. One of the causes of the hyposecretion of basal LH could well be the increase with age in the sensitivity of these nuclei to estrogen which we have just seen in castrated rats which were previously cyclic. In the intact animal, the situation is the following:

The aptitude for pseudopregnancy triggered by the injection, at estrus, of minute quantities of estrogen increases with age.

Alloiteau (1957) has shown that, in the adult rat, the single injection of 50 or 100 µg of "nonesterified" estradiol at estrus, induces pseudopregnancy in 50 percent of the cases. Gilmore and McDonald (1969) successfully use 5 µg of estradiol benzoate. Acker and Chabardès (1970) with 5 µg obtain 70 to 90 percent of pseudopregnancies by injecting at proestrus, 55 percent at estrus. All rats were "adult" without indication of age.

We have studied this aptitude for pseudopregnancy in relation to age (Aschheim, 1972). Table 19-VII shows in Wistar rats, that for each dose used the percentage of pseudopregnancies triggered by estradiol benzoate increases with age.

Two remarks: (1) In the oldest rats, and only in these, another type of response to the injection of estrogen is added: not pseudopregnancy, but persistent estrus with disappearance of corpora lutea, lasting mostly 10 to 30 days. (2) The pre-puberal rat, in which corpora lutea are induced by human chorionic gonadotropin, requires considerable doses of estradiol benzoate to make the corpora lutea functional.

TABLE 19-VII
THE INCREASING APTITUDE WITH AGE TO REACT BY PSEUDOPREGNANCY
TO ESTRADIOL BENZOATE INJECTED AT ESTRUS IN WISTAR RATS

<i>Estradiol Benzzoate in μg</i>	<i>30 Days^a</i>	<i>2 Months</i>	<i>3-6 Months</i>	<i>7-8 Months</i>	<i>9 Months</i>	<i>11-15 Months</i>
50	10/16 ^b 62.5%	15/24 62.5%	12/15 80.0%	13/15 86.7%	9/11 81.8%	11/12 91.7%
10	5/15 33.3%	6/17 35.5%	9/15 60.0%			
5		4/15 26.7%	12/34 35.3%	45/80 56.3%	11/15 73.3%	18/23 78.3%
3		0/15	5/35 14.3%	10/38 26.3%	17/42 40.5%	18/29 62.0%
1				0/6		
0.5					7/18 38.9%	

^a Corpora lutea induced by HCG.

^b Numerator: number of pseudopregnancies; denominator: number of injected rats.

We will see later on that, derived from data like those in Table 19-VII, a biological aging test for the central regulation of the estrous cycle can be set up.

Steroids injected into intact rats with a senile deviation of the cycle (SPE and SRPP) have the same effects as in adults in spontaneous persistent estrus or in experimental pseudopregnancy.

Thus progesterone (4 mg daily for 3 days) induces ovulation in 60 percent of the SPE (Clemens, *et al.*, 1969) as it does in adult or senile PE of the DA strain (Everett, 1940). Likewise estrogen (10 µg of estradiol benzoate during 1, 2 or 3 days) injected in the SRPP, produces ovulation in 50 percent (Aschheim, 1970), a result which agrees with that obtained by Everett (1947) in pseudopregnant or pregnant adult rats.

One can then admit that the preoptic area, responsible for the ovulatory release of LH can be reactivated in those senile rats in which it is spontaneously out of use or functions only at long intervals. However there is no quantitative data on its sensitivity to steroids.

THE CONSEQUENCES OF HEMICASTRATION IN VARIOUS TYPES OF SENILE RATS. Ingram (1959) indicates that the hemicastration of 7 senile rats at the age of 20 to 23 months leads 6 weeks later to a 1.5 fold increase in the weight of the remaining ovary. Labhsetwar (1967) reports no compensatory increase in the weight of the ovary (10 days after hemicastration) in rats estimated to be 9 months old according to their weight.

We have examined the consequences of hemicastration all together on the maintenance of the pre-existing vaginal rhythm and on ovulatory, luteal and ponderal compensation of the remaining ovary (Aschheim, 1970). The period of observation has been of one month. As usual the cyclic rats and those in permanent estrus and in repetitive pseudopregnancies have been considered separately.

The weight gain of the remaining ovary is statistically nonsignificant in rats in PE or in RPP at 24 months and similarly in cyclic rats of 17 or 20 months. However, there are functional changes.

In the senile cyclic rats, only about 40 percent maintain the cyclic rhythm after hemicastration at 13 to 17 months (20% between 20 and 24 months). Nearly all of these show then an ovulatory, luteal and weight compensation in the remaining ovary one month after operation (for example at 13 months, 11 rats out of 12 show weight and luteal compensation and 9 out of 12 ovulatory compensation). The other senile cyclic rats change rhythm shortly after hemicastration. Thirty percent of the initial animals pass into a persistent estrus which leads to the absence of ovulation and corpora lutea and to a regression of ovarian weight. This explains the absence of weight gain in the remaining ovaries of the whole group of senile cyclic rats. Thirty percent pass into RPP or into alternating cyclic

and pseudopregnant sequences. This leads in the 13-month-old rats to 8 out of 8 weight and luteal compensations and to 4 out of 8 ovulatory compensations. In older animals, the change to pseudopregnancy less often involves compensations.

In rats in SPE, ovulation and luteinization of the ovary remaining after hemicastration are exceptional. The majority of them take up persistent vaginal estrus after a more or less long diestrus. The remaining ovary does not compensate in the number of large follicles nor in weight. The number of eggs ovulated and corpora lutea formed immediately after the injection of LH in these hemicastrated SPE rats is from about 5 to 6 (that is, no compensation). But as this same injection triggers the re-establishment of *autogenous* cycles, in most cases, an ovulatory, luteal and weight compensation is then found (Aschheim, not published).

The rats in SRPP generally continue their rhythm after hemicastration. At 13 months 7 out of 9 compensate for the ovarian weight and the number of corpora lutea and 6 out of 9 for the number of eggs. From 20 to 29 months compensation rarely occurs.

In summary the senile rats in which the inhibitory control of the hypothalamus on the secretion of hypophyseal prolactin is abolished are barely able to compensate functionally for hemicastration. When the inhibitory control is re-established (by the reactivation of the SPE), the compensation reappears. In senile cyclic rats hemicastration forms a test of adaptation, from which a majority, increasing with age, escapes through a change into one of the senile deviations of the estrous rhythm. The operation selects, therefore, among a population of given age those individuals which are still adaptable and those that are no longer. Our aging test, performed before hemicastration, allows one to forecast this potential difference in an apparently homogenous group of senescent cyclic rats (Aschheim, 1971).

CONCLUSIONS ON THE NATURE OF AGING OF THE REGULATION OF THE ESTROUS CYCLE. The ovary being excluded from the primary responsibility for aging of the hypothalamo-hypophyseal-ovarian axis, a modification of sensitivity to ovarian steroids has been proposed as the expression of aging of the central regulation, and its direction and its magnitude have been investigated. In really comparable situations (cyclic rats differing only by age) there appears to be an overall hypothalamic hypersensitivity to estrogen in old age.

However, this notion must be understood "per animal," "per hypothalamus," not "per neuron." It is the result of the action of at least 2 unknown contributing factors which may counteract each other, the number of active neurons and their individual sensitivity. Does the senile decline in neurons of 30 to 40 percent, known for other sectors of the central

nervous system, also apply to the hypothalamus? If it does, the increased inhibition of LRF and PIF manifested by the hyposecretion of LH and the hypersecretion of prolactin, is compatible with all the hypotheses on individual neuronal sensitivity to estrogen, increased like the overall response, maintained or diminished. Even in this last case, an unchanged quantity of estrogen facing a reduced number of only slightly sensitive neurons could bring about a response of increased inhibition. It is known that the hypothalamic contribution to the onset of puberty consists of an abrupt decrease in hypothalamic sensitivity to estrogen. There is no need then to postulate an inverse process for old age in each of the still existing neurons.

Other questions remain equally open. Firstly that of the feedback of other ovarian steroids. Above all that of the hypothalamic areas affected by the overall increase of sensitivity to estrogen. The region of the arcuate and ventro-medial nuclei, regulating the tonic LH, is certainly involved. The tuberal region whose stimulation induces pseudopregnancy (Everett and Quinn, 1966) could also be involved considering what we have said of the increasing aptitude with age to react by pseudopregnancy to estrogen. Now this region includes the dorso and ventromedial nuclei. Nothing is known about the possible implication of the preoptic area which controls the release of ovulatory LH. However, as already mentioned, the administration of estrogen as well as its deprivation (by hemicastration) involves, as age increases, besides the pseudopregnant response, another response of the persistent estrus type. This evokes the results of Pasteels and Ectors (1968) and Ectors and Pasteels (1969). These authors obtain pseudopregnancy or persistent estrus by the implantation in the anterior hypothalamus of progesterone, medroxyprogesterone or estradiol benzoate. Thus none of these areas can be at present excluded from possible participation in the process of aging under study. This is an important fact because it is consistent with the hypothesis of a similar or even simultaneous aging of all neurons sensitive to estrogen.

These changes in the sensitivity of the central regulation system are also responsible for the transition to senile deviations of the estrous cycle. This is obvious for the injection of minute quantities of estrogen. The deprivation of estrogen by hemicastration which leads to similar results could act in the same way. In fact the change towards permanent estrus or pseudopregnancy rarely occurs suddenly after the operation, more often after one or sometimes two vaginal cycles. One could imagine that by an increased feedback on the hypothalamus, hemicastration could result in the supplementary secretion of estrogen before the estrus of such an intermediary cycle and that it is this supplement which starts the senile deviation of the cycle.

When the senile deviation of the cycle is established, then the central sensitivity to castration or to estrogen appears reduced. We have seen that this is related to the hypersecretion of prolactin. These senile animals behave like the castrated rats of Halasz and Gorski (1967) which have undergone an interruption of neural afferents to the medial basal hypothalamus, leaving inside the feedback loop only the arcuate and ventro-medial nuclei.

TEMPORAL ASPECTS AND MEASUREMENT OF AGING OF THE HYPOTHALAMO-HYPOPHYSEAL OVARIAN AXIS

The orientation of this section is gerontological. Because the known facts are few it is in part prospective.

Without a test of aging, it is difficult to assess any procedure intended to retard or advance the onset of senescence, to slow down or accelerate its progression. In the case of thermoregulation for example, the decline in adaptation with age can be measured, because the framework to which the biological test refers remains unaltered, a difference of temperatures or a time needed for restoration of temperature after cooling. The regulation of the estrous cycle is, however, more complex. During cyclicity, it is possible to measure a change with age in a process of adaptation (see page 406). But at a certain point this quantitative change produces qualitative changes, the senile deviations. And then, the system of reference is no longer the same. It is then impossible to measure any further progression in an aging process which is now masked.

Masked Aging

The inverse situation shall also be considered. The senile deviations are reversible. For a variable time and with a variable efficiency, rats in SRPP and SPE are transformed into cyclic rats similar to the spontaneously cyclic ones of the same age. Does that mean "rejuvenation" at least for the central regulation? These animals retain a deficient ovarian interstitial tissue, evidence for their hyposecretion of tonic LH which differentiates them from young cyclic rats. It is conceivable that these rats, when cyclic again, utilize more steroid sensitive neurons, due to restoration of activity in the preoptic area, than at the time of their permanent estrus. This could also explain the restoration of the inhibitory control on the secretion of prolactin. Thus it would be interesting to apply the aging test to "reactivated" rats, especially at an age when no more cyclic controls exist. Meanwhile, the situation is that of a masked aging.

Suspended Aging

This is what is obtained by castration or hypophysectomy carried out in the young adult. The prolonged absence of steroid information apparent-

ly conserves the hypothalamic sensitivity at a level permitting the resumption of cycles when the missing organ is substituted for. For castration (Aschheim, 1964-65) this delay has been such that there no longer exists an intact cyclic rat of the same age. During 25 months the amount of hypophyseal LH in the castrated animal remains constant, just as the LH content remains constant (4 times less) in the castrated animal, estrogenized during the last month before autopsy. It is quite clear that here is simply a suspension of the regulatory *function* under study, the senescence of this function evidently cannot be expressed until after its resumption.

It seems likely that this is what happens in the spectacular experiments which prolong life by caloric restriction as recorded by McCay (McCay, *et al.*, 1935) and confirmed by later workers (see Everitt and Porter, Chap. 30 of this book). Whatever the mode of action of the food restriction, whether or not it acts on the hypothalamus to achieve what is sometimes called a pseudo-hypophysectomy, there is a suspension of the estrous cycle. The latter can be reactivated simply by the reestablishment of the caloric allowance (Asdell and Crowell, 1935).

A report by Zeilmaker (1969) has to be mentioned even if it is not evident that it deals with "suspended aging." Rats receive Lyndiol (mixture of 30:1 of lynestrenol and mestranol), orally at 9 weeks, and for varying lengths of time. The ovaries atrophy while conserving their potentiality. The cessation of the treatment after 6 months leads in 7 out of 9 cases to a resumption of the estrous cycles which continue for 14 ± 1 months; 2 rats enter PE. The cessation of treatment after 14 months leads in 7 out of 9 cases to a resumption of ovarian activity as persistent estrus; 2 out of 9 cases resume cycles for 3 to 4 months. The pituitaries of rats treated for 14 months and then fed normally are, at 20 to 22 months of age, smaller than those of the controls and do not show hemorrhagic foci. The author concludes that the treatment may slightly retard the process of hypothalamic aging.

If there is a suspension of ovarian activity during the treatment, the latter on the other hand continuously supplies steroid information to the hypothalamus. The effect on its biological age of the antifertility steroids may differ according to their hormonal composition and should benefit from the set up of the test of aging. This experiment has now been done. Cycles resume after 4 or 8 months of steroid treatment and their biological age is then considerably younger than in control rats of the same age. This applies to estrogen as well as to estrogen-progestin treatment, but not to progestin alone (Aschheim, 1974). Does long term oral administration of estrogen (about 5 μ g per day) act as a hypothalamic-hypophyseal desensitizer counteracting the natural increase with age in the central sensitivity to the hormone?

Retarded Aging

I am not aware of any situation in which there is a true retardation of the aging of the estrous regulation once the system is functioning in a cyclic manner. Besides the aging test with estrogen, the study of deficiency cells in the ovarian interstitial tissue (present in almost all cases after 13 months of age) could be useful here. An important delay in the appearance of these cells would have gerontological significance.

Advanced Aging

Early hemicastration produces such a situation (Aschheim, 1970; Aschheim, 1971).

The long term effects of early hemicastration have been studied on reproductive performance; among others by Biggers, *et al.* (1962a, b) and Finn (1963) in the mouse. The result is a reduction to half of this performance, affecting the total number of offspring as well as the length of the period of reproduction. In other words during the first half of the period of reproduction of intact animals, the castrated mice show compensation; but during the second half they are sterile. This is due probably to the premature aging of the overburdened functional uterine horn. Adams (1970) reports results in the rat and the rabbit. In the rat the total number of young produced after early hemicastration is 50 percent less than that of intact rats, but this time the reduction results from both a reduction in the number of young born per litter (especially after the sixth) and the reduction in the number of litters, the period of reproduction lasting as long as in the controls.

To my knowledge, only 2 authors discuss the long term physiological compensation of the single ovary from the endocrine point of view. King (1911), cited by Biggers (1962a) states that the ovary of hemicastrated rats shows only little or no compensatory hypertrophy "several months after the operation." Thung (1961) notes that in mice hemicastrated at 13 months, there is, 8 months later, in comparison with intact mice, twice as many (12% compared with 6%) animals whose vaginal smears show an irregular estrogenic activity, including a more or less continuous estrogenic stimulation.

According to the criteria already mentioned (type of the vaginal smears, ovulatory, luteal and weight compensation of the remaining ovary) we have compared (Aschheim, 1970), in the course of their aging, rats hemicastrated at 21 days and their intact controls. The number of animals with disturbed cycles remains equally small in the 2 groups up to the age of 10 months. At 11 months the situation changes abruptly: 9 out of 45 intact rats are in permanent estrus at 11 and at 12 months; in the hemicastrated

rats this number increases to 19 out of 46 at 11 months, 21 out of 45 at 12 months (46.7%). This percentage is not achieved by the intact rats at 17 months of age. Hemicastration has then notably advanced the onset of one of the senile deviations of the estrous cycle. The single ovaries of these PE rats do not compensate.

Moreover, the 24 other hemicastrated females which did not enter PE do not show further functional compensation. Six have vaginal cycles, but are in fact anovulatory rats, with a small ovary devoid of corpora lutea, comparable to the SPE ones. Five others are pseudopregnant rats, either already repetitive or still alternating with cycles; the remaining ovaries containing numerous corpora lutea are very large: 75.8 mg (as against 40.9 mg for one ovary of the intact SRPP rats of the same age). A single female ovulates 11 eggs; the others a mean of 6.3. Thirteen hemicastrated rats remain cyclic with an average weight of 50.8 mg per remaining ovary and 5.9 eggs (as against 31.1 mg for one ovary in 17 intact cyclic rats of the same age and 5.3 eggs for the corresponding oviduct). Histological examination of the serially sectioned ovaries reveals that the number of fresh corpora lutea is only slightly in excess of the number of ovulated eggs. There is no increase in the number of corpora lutea with retained ova. The weight increase of the ovary is due to a deficient luteolysis, maintaining numerous old corpora lutea.

Two recent papers confirm that ovulatory compensation is not maintained for a long time after early hemicastration. It is stopped 6 months after the operation in the 8-month-old rats of Peppler (1971) and after ten cycles in the rats of Chatterjee and Greenwald (1972) operated at about 2 months of age.

In a second experiment (Aschheim, to be published), we have followed the rats hemicastrated at 23 days during the entire period that they maintain estrous cycles. Ovulatory compensation (which means 10-12 eggs versus 5-6 eggs in case of noncompensation) is continuously diminishing. It is maintained in 11 out of 14 rats at 3 months of age, in 17 out of 30 at 7 months, in 3 out of 8 at 10 months, in 0 out of 13 at 12 months in the group described above. Ovarian "hypertrophy" persists in all animals, but its significance changes. Whereas in rats with ovulatory compensation, the hypertrophy due to the increase in fresh corpora lutea is truly compensatory in the others, the weight gain results from maintained old corpora lutea.

In a third experiment (Aschheim, 1971), rats hemicastrated at 23 days, undergo a test of aging (3 µg of estradiol benzoate) at 7 months, which triggers 57 percent of pseudopregnancies, 43 percent of the animals remaining cyclic. After that, 2 cycles are allowed to pass, then the eggs in the remaining oviduct are counted. All the rats which gave a response of the

"young" type to the test (that is, no pseudopregnancy) show ovulatory compensation (10 to 12 eggs); all those that do not compensate (5 to 8 eggs) have given an "old" type response to the test (induced pseudopregnancy).

Early hemicastration then advances aging of the estrous regulation.

Other Situations

Are there changes in the rapidity of aging of the estrous function (acceleration, slowing)? If so are they correlated or not with the onset of senescence, normal, advanced, retarded? Nothing is known about this.

Biological Measure of Aging of the Hypothalamic Regulation of the Estrous Cycle

Cyclic rats show an increasing aptitude with age to react by pseudopregnancy to estradiol benzoate injected at estrus (Table 19-VII) (Aschheim, 1970, 1972). That means that there is an increasing facility with age to release prolactin in response to an estrogenic stimulation at estrus, which in turn results probably from the increasing facility for estrogen to inhibit the hypothalamic prolactin-inhibiting-factor (PIF). This applies to a twentyfold range of doses of estrogen. For a given dose of the hormone, the curve showing the percentage of induced pseudopregnancies in relation to age is S-shaped. The curve representing the quantities of estrogen efficient at the 50 percent level in different age groups is hyperbolic.

At least two different biological criteria must be used to assess the diagnosis of pseudopregnancy, persistent estrus or a maintained cycle, the 3 types of response which can occur. The "old" response is pseudopregnancy or occasionally persistent estrus, the "young" response is a maintained or slightly lengthened cycle.

As for specificity, the frequency of pseudopregnant responses, not to estrogen, but to the stress of injection at the day of estrus is 7 percent, but is not age-dependent. The nonreproducibility of the aging test is also about 7 percent. It does not change with age. Repeated trials, until 4 tests at 3 to 4 week intervals, can be done without altering the response.

The aging test is then a functional test for adaptability, dealing only with physiological stimulation and responses and leaving the rat intact. It can be carried out during the whole period of cyclicity, but must be used with caution at the end of this period, when cycles are "spontaneously" replaced by their senile deviations (12-14 months). It reveals the latent heterogeneity of an apparently homogenous, cyclic population. This heterogeneity is age-dependent.

We do not yet know if this aging test expresses *the* aging of the central

regulation of the cycle in female rats or only one of its aspects. But it is a striking fact that the mechanism underlying the spontaneous senile deviations and the senile responses to the test in cyclic rats is the same.

It may be possible that the rats which remain cyclic when aging represent a population selected for the stability of the central regulation and that this would account for the flattening of the last part of the S-shaped curve. The problem of selection exists in all aging phenomena, but in this particular case, it could be studied experimentally. Indeed, the possibility of reactivating, of "recycling" rats in senile persistent estrus, should permit us to test these animals, which have not been selected for stability (or negatively) and to compare them with spontaneously cyclic rats of the same age.

FINAL CONSIDERATIONS AND PROSPECTS

The Senile Ovary

In the rat it is clear that the ovary is not responsible for the senile sterility which takes place before the oocyte stock is exhausted (Jones, 1970). The ovary is not primarily responsible for senile deviations of the estrous cycle (Aschheim, 1964-65). We are now far from the earlier view that oocyte depletion is the central event of ovarian aging which moreover would lead to a loss of sensitivity to gonadotrophic hormones. The question of the possible relation between ovarian senescence, the number of oocytes, the production of ovarian steroids, and their hypothalamo-hypophyseal feed-back in my opinion is as follows.

Can we reveal or create in the rodent a situation like that found in women after the menopause, that is, a "biological" castration with disappearance of follicles and gonadotrophic hyperfunction of the pituitary? One thinks of CBA mice whose ovaries are deprived of oocytes in the second half of their life. The gonadotrophic activity of their pituitary has not yet been studied; but the presence of deficiency cells in the interstitial tissue of their ovaries makes a gonadotrophic hypersecretion unlikely. After the disappearance of the oocytes, ovarian tumors form (Thung, 1961), which do not appear to secrete biologically active steroids. The C57-BL/6-W^v-W^v mice whose ovaries, through mutation, are practically deprived of oocytes from the very beginning, rapidly develop ovarian tumors which are hormonally inactive: tubular adenomas derived from the germinal epithelium (Russell and Fekete, 1958). The experimental destruction of oocytes and follicles after local irradiation by X-rays (Mandl and Zuckerman, 1956) also leads to the formation of tumors in the ovary of the rat. But here estrogen is secreted for several months in the absence of oocytes and follicles. In the terminal stage of hormonal inactivity of the ovary, the

pituitary reacts as in castration by an increase of its FSH content (Mandl and Zuckerman, 1956) and of its total gonadotropin secretion (Westman, 1958).

Do such sequences result from ovarian aging or simply from destruction of oocytes? The aging test could determine this in certain circumstances.

The Senile Pituitary

Throughout this study the gonadotropic function of the pituitary during senescence has appeared as a passive vehicle of a hypothalamic command altered in its sensitivity to estrogen. Now, this hormone can exert a direct influence on the pituitary, inhibiting the gonadotropic cells (Bogdanove, 1963; Ramirez, *et al.*, 1964), stimulating the prolactin cells (Nicoll and Meites, 1962). (However, we do not know if this action plays a physiological role in the regulation of the estrous cycle.) Moreover the pituitary undergoes structural alterations with age.

AUTONOMOUS AGING OF THE GONADOTROPIC FUNCTION OF THE PITUITARY. Only the result of a heterochronic graft of the pituitary in contact with the median eminence can attribute the respective responsibilities to the hypothalamus and to the pituitary in the aging of the estrous function. In the experiment of Peng and Huang (1972), already mentioned, this responsibility seems to be shared. In any case the reversibility of the senile deviations of the cycle leads us to impute the failure of the organ to a primary defect of information, of sensitivity, not to a primary disturbance of hormonal production or excretion. And in fact the senile pituitary retains all its capacities for the elaboration of the gonadotropic hormones. At the same age, its LH content differs significantly according to the category of estrous regulation; in the same category, the content does not differ with age. The decrease in hypophyseal LH after ovulation of the SRPP is clear and significant. Castration leads, after one month, to a similar increase of LH in the senile and adult cyclic rats. The pituitaries freed of inhibition by castration at a young age maintain their maximal amount of LH throughout their senescence. For Labhsetwar (1970) castration, like hemicastration, of an "aged" rat leads to the same important amount of hypophyseal FSH, similar to that of a castrated adult. There is only the long term restraint from inhibition, caused by castration of the already aged rat, which might perhaps demonstrate an ultimate fading of the feedback (but at what level?).

Like the ovary, the pituitary ages, but there is no evidence for its primary responsibility for the senile changes in gonadotropin regulation. While the ovary can be formally eliminated owing to the results of its heterochronic graft, a kind of hypophyseal collusion remains possible.

HYPOPHYSECTOMY AND AGING. Jones and Krohn (1961b) studied the evo-

lution of the ovary of mice hypophysectomized at the age of 40 and 50 days. The operation clearly retards but does not abolish the oocyte loss with time. The intact CBA mice lose 28 percent of their oocytes in periods of 20 days, the hypophysectomized ones 11 percent. The ovaries of mice hypophysectomized 300 days previously, ovulate after orthotopic grafting into a young recipient, and lead to pregnancies as successfully as young normal ovaries grafted in the same way. Senile ovaries of mice hypophysectomized when young have less lipofuscin pigment and anovulatory follicles than those of intact mice of the same age.

But, as mentioned before, oocyte loss throughout life is not an "aging" process which should be accentuated with age and give an S curve like for example the disappearance of estrous cycles (Fig. 19-1) or the aptitude for pseudopregnancy.

The aging of tail tendon collagen continues, but in a clearly retarded manner, in the hypophysectomized rat, both male (Olsen and Everitt, 1965) and female (Verzár and Spichtin, 1966) whilst longevity, another criterion of aging, diminishes (Everitt and Cavanagh, 1965; Verzár and Spichtin, 1966).

The effects of caloric restriction leading to a "pseudohypophysectomy" are manifest by a retardation in the aging of collagen (Chvapil and Hrúza, 1959) and an increase in longevity (see Chap. 30). From the sexual point of view, there is a cessation or nonappearance of the estrous cycles and an ovarian atrophy with preservation of numerous young follicles (Huseby, *et al.*, 1945; mice), but without corpora lutea. These ovaries, evidence of the lack of circulating gonadotropic hormones, remain sensitive to the injection of these hormones (Mulinos and Pomerantz, 1941). It is interesting to note that the pituitary accumulates, at least for a short term, the hormones which it is unable to release (Rinaldini, 1949; Meites and Reed, 1949; Maddock and Heller, 1947; Srebnik, *et al.*, 1961). After a longer time the gland is deficient in gonadotropic substances (Werner, 1939).

If one adds that early castration does not influence longevity (Asdell, *et al.*, 1967), nor the biological age of collagen (Árvay, *et al.*, 1963; Árvay and Takács, 1966) in comparison with the nulliparous intact rat, one can set up Table 19-VIII. It can be seen that the 3 conditions which "suspend" hypothalamo-hypophyseal-ovarian aging have divergent effects on longevity and on the biological age of collagen. Thus it is not through the gonadotropic function of the pituitary that these parameters are acted upon.

HYPOPHYSECTOMY AND AGING OF THE OVARIAN TARGET CELLS. The gonadotropic hormones induce in the ovary of the rat temporary morphogenetic and steroidogenetic phenomena. Hypophysectomy and replacement therapy allow the study of ovarian target cells in different, stable and continuous conditions of time. Thus, artificial types of cellular aging may be realized.

TABLE 19-VIII

RELATIONSHIP BETWEEN GONADOTROPIN LEVELS AND
TWO CRITERIA OF SENESCENCE

	Gonadotropins pituitary	Gonadotropins circulating	Aging of collagen	Survival
Castrated rat	↗	↗	N	N
Hypophysectomized rat	O	O	↘	↘
Caloric restricted rat	at first later ↗	↘	↘	↗

Value in relation to intact nulliparous rat

N normal

↗ increase

↘ decrease

Hypophysectomy changes the interstitial cells of the ovary into deficiency cells or inactive "wheel"-cells (Selye, *et al.*, 1933). Moreover, the operation stops the formation of new interstitial cells which originate from the thecal cells of atretic follicles. The deficiency cells persist in the hypophysectomized rat. Their morphology and function can be repaired by gonadotrophic hormones with "ICSH" activity (LH, HCG, PMS) up to 30 to 60 days after the operation (Rennels, 1951; Marti and Green, 1965). Four months after hypophysectomy of an immature rat, 50 IU of HCG, previously sufficient for repair, have lost much of their efficiency (Arias and Aschheim, to be published). Does this indicate an "aging" of these dormant cells, or does it result from the general metabolic consequences of hypophysectomy at that time?

The corpora lutea present in adult rats (or in pre-puberal rats artificially luteinized) at hypophysectomy are maintained in an inactive state for a very long time. When stimulated by HCG or LH, these luteal cells are able to produce estrogen, up to 9 months after hypophysectomy (Parlow, 1961). But for Chamorro (1945) corpora lutea of rats hypophysectomized

at 18 months are unable to do so despite a strong, prolonged treatment with HCG, started 3 weeks after the operation.

The luteal cell in the hypophysectomized rat whose gland has been re-grafted in an ectopic position where it becomes a source of prolactin, is active and maintains its production of progesterone for at least 104 days (Everett, 1956).

Thus, hypophysectomy leads to simplified experimental situations which deserve time-related investigations by modern methods of subcellular exploration.

The Senile Hypothalamus

THE STEROID-SENSITIVE HYPOTHALAMUS. With age the hypothalamic target structures for steroids modify their sensitivity to estrogen in the normal cyclic rat. The other sex steroids have not yet been examined. This modification appears as an overall increase in sensitivity. That of the individual neurons concerned remains unknown in the absence of qualitative and quantitative morphological data. What are their cyto-physiological and ultrastructural aspects? What is their number? It is only on these bases that future results of dynamic studies on the uptake of labelled steroids, the hormone-dependent electrophysiological activity or the neuropharmacological reactions could be interpreted.

CORRELATIONS BETWEEN VARIOUS REGULATORY FUNCTIONS OF THE HYPOTHALAMUS. Kennedy and Mitra (1963) have considered body weight and food intake as initiating factors for puberty in the rat. In their opinion the decrease in sensitivity to estrogen which characterizes puberty at the hypothalamic level, may originate from a decrease in the metabolic rate. The latter is a consequence of the decrease in energy balance which is required for the adaptation of the growing organism to its environment, especially as regards thermoregulation.

Nutrition, light, and temperature are well known as initiating factors of sexual activity in seasonal animals.

What can be said about their correlations in the senile hypothalamus? Rothchild (1967), in a brilliant speculation has formed a scheme for it. He suggests that the "syndrome of old age" could be due to the decrease of inhibitory activity of the ventro-medial nucleus on the far-lateral area. Activation of this area increases the appetite (Kennedy, 1966). According to a second hypothesis activation of the far-lateral area leads also to a series of inhibitions, resulting in the decrease of caloric loss (and hence the increase in temperature), of sexual receptivity, locomotor activity, maximal secretion of LH and in the increase of the secretion of prolactin (by inhibition of PIF). One can discuss the second hypothesis, the need to consider the far-lateral area as the starting point of the inhibitory influence

acting upon the functions enumerated which effectively diminish with age. But the initiating role attributed to food intake (Aschheim, 1964-65; Rothchild, 1967), as in puberty, requires consideration.

The decreased activity (or hypofunction) of the ventro-medial and of the arcuate nuclei in the gonadotropic LH regulation of the senile rat is an established fact (Aschheim, 1970). Furthermore, this hypofunctioning is secondary; it results from an altered perception of the steroid environment. Is the inhibitory control upon the far-lateral area and the stimulatory control upon the gonadotropic activity of the pituitary exerted by the same neurons of the ventro-medial nucleus? If so, is the senescence of the two functions necessarily simultaneous? Does a caloric restriction applied to rats in senile deviations of the cycle restore estrous cycles? Thus, once more, but now via the hypothalamus, we face the relationship between food intake, pituitary, ovary and aging.

There is less data (see Chap. 5) available on the correlations of the different hypophysiotropic functions of the senile hypothalamus than there is on the relations between gonadotropic regulation in the senile female and appetite (Kennedy, 1953) or locomotor activity (Farris, 1945). Even more astonishing, scarcely anything is known about aging of the hypothalamo-hypophyseal-testicular hormonal axis (see Bishop, 1970). In a single assay of hypophyseal LH in 4 groups of intact males and castrates aged 6 and 21 months, we found a significant increase in LH after 1 month of castration (at 6 months 3.6 times; at 21 months 2.7 times). The amount of LH in intact males like that of castrates did not change with age (Aschheim, not published). Some interesting studies (Larsson and Essberg, 1962; Jakubczak, 1967) have been made of the sexual behavior of the male rat. There is not an overall decrease in sexual activity with age but a different temporal distribution. These neurological changes occur before any detectable testicular hormonal deficiency develops.

CONCLUSION

We have studied the aging of a specific regulatory system. The primary failure resides in its central link, the senile hypothalamus. But, at present, the latter appears as a "black box." The different tests of adaptation which demonstrate the modification of its sensitivity to estrogen are triggered upside and are recorded downside. This applies also to the described aging-test of the estrous cycle. However, with its use, one can hope for new information on the control and understanding of experimental situations susceptible to modify the temporal course of aging. But the direct study of the neuroendocrine regulatory cell in relation to its age remains to be done.

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CHAPTER 20

THE FEMALE CLIMACTERIC

ARTHUR V. EVERITT

SUMMARY

OVARIAN FAILURE OCCURS in the human female at age 50 years. The secretion of estrogen and progesterone by the ovary falls to very low levels and the secretion of gonadotropins by the pituitary increases markedly. The climacteric appears to be due to intrinsic aging of the ovary, which may be accelerated by changes in hypothalamic function and hypersecretion of pituitary gonadotropins. The loss of estrogen causes atrophy of the uterus, vagina and breasts and is associated with the increased incidence of coronary atherosclerosis, osteoporosis and cancer of the breast and genital tract in postmenopausal women. Some of these changes may be prevented or delayed by estrogen replacement therapy.

INTRODUCTION

Loss of ovarian function in middle age is probably the most outstanding characteristic of aging in women. The sudden decline in secretion of ovarian hormones at the climacteric is often followed by the onset of age-related pathology. Thus ovarian failure is believed by some workers to accelerate aging in other organs.

CHANGES AT THE CLIMACTERIC

The female climacteric is the period between ages 45 and 55 years, often known as "the change of life." The outstanding event is the cessation of menstruation, the menopause. The average age of the menopause is 50 years, and has increased slightly in most European countries during the last century (Frommer, 1964; Vara, 1970).

A male climacteric has been described by Werner (1939) but affects only a small proportion of men.

The Ovary

The size of the ovary begins to decrease after 30 years (Rössle and Roulet, 1932), but the decrease is minimal until about the time of the climacteric. Since birth there has been a continuous loss of oocytes, which appear to be exhausted soon after the menopause (Jones, 1970). The number of graafian follicles decreases considerably after the age of 40 years (Block,

1952). At this time anovulatory cycles appear and the reproductive performance declines (Francis, 1970).

Ovarian Hormones

Associated with the loss of follicles is the decline in the secretion of estrogens. The total urinary estrogen excretion (Fig. 20-1) falls progressively between ages of 40 and 60 years (Pincus, *et al.*, 1954). There is now an extensive literature on urinary estrogen output in postmenopausal women. All three classical estrogens (estrone, estradiol and estriol) are excreted although the most potent estrogen, estradiol, is excreted in the least amount (McBride, 1957; Procope, 1969). It is now obvious that the ovarian stroma produces steroid hormones in the absence of developing follicles and corpus luteum formation (Mattingly and Huang, 1969). The pathway of steroid biosynthesis appears to change at the time of the men-

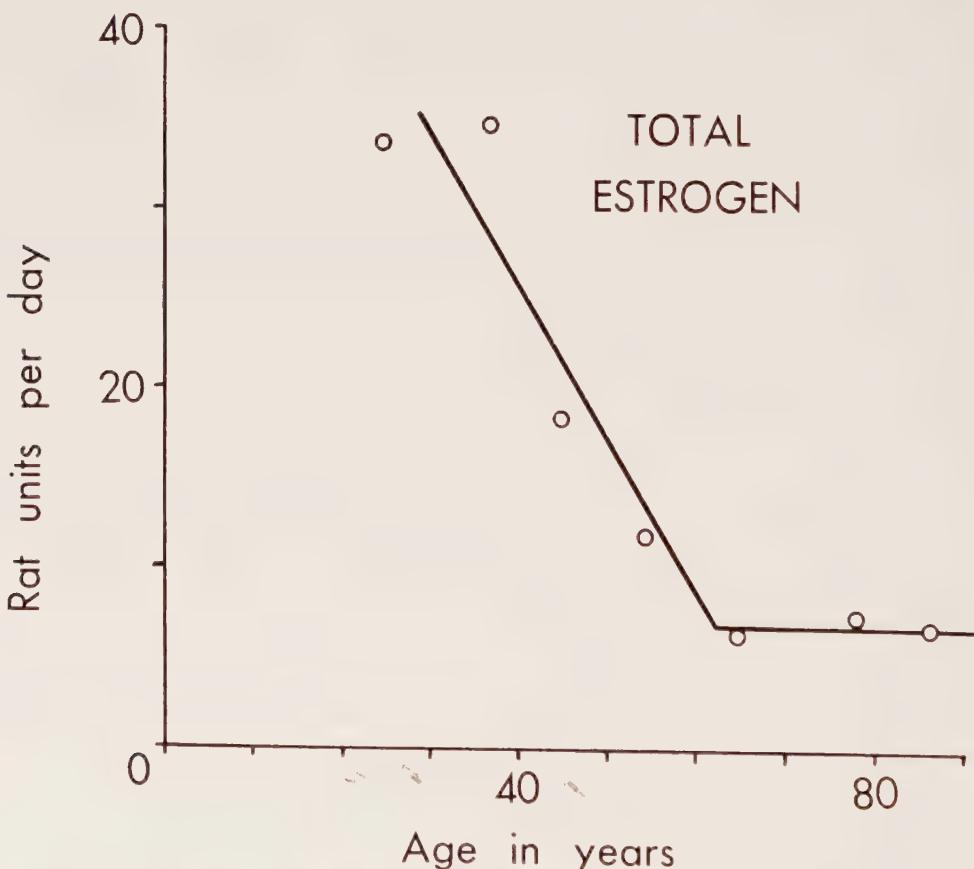


Figure 20-1. The fall in estrogen secretion with advancing age in women (from Pincus, *et al.*, 1954. Reproduced by permission of Gerontological Society Inc.).

opause leading to increased production of androgens (Mattingly and Huang, 1969) and nonclassical phenolsteroids (Dilman, Chap. 32). The production of estrogens by the post-menopausal ovary is very low (Mattingly and Huang, 1969), and may even fall to zero. The main source of estrogens in the circulating blood at this time is from the conversion of plasma androstenedione to estrone (Grodin, *et al.*, 1973). There is no evidence for the secretion of estrone or estradiol by the ovary or adrenal in postmenopausal women (Grodin, *et al.*, 1973).

The excretion of pregnanediol is decreased in women approaching the menopause (Adamopoulos, *et al.*, 1971). This indicates that the secretion of progesterone also declines. The cessation of ovulation reduces the number of theca lutein cells and hence there is a marked reduction of progesterone secretion by the ovary.

Pituitary Gonadotropins

The decreased secretion of ovarian hormones is associated with an increased production of pituitary gonadotropins. The excretion of gonadotropins in urine is very high in postmenopausal women (Heller and Heller, 1939; Heller and Shipley, 1951; Albert, *et al.*, 1956). With the development of specific radioimmunoassays for follicle stimulating hormone (FSH) and luteinizing hormone (LH), both gonadotropins were found to be excreted in greater amounts in women approaching the menopause (Papanicolaou, *et al.*, 1969a; Adamopoulos, *et al.*, 1971) as well as after (Papanicolaou, *et al.*, 1969b), with FSH increased more than LH. The high level of gonadotropin excretion begins to decline 20 years after the menopause, probably due to pituitary aging (Heller and Shipley, 1951; Albert, *et al.*, 1956).

The serum levels of LH and FSH are also elevated in postmenopausal women (Kohler, *et al.*, 1968; Abraham, *et al.*, 1968; Coble, *et al.*, 1969; Nillius and Wide, 1971; Wise, *et al.*, 1973). The production rate for FSH increases 15 fold (Coble, *et al.*, 1969), while that for LH increases only 5 fold (Kohler, *et al.*, 1968), but the metabolic clearance rates are not changed. Thus the high serum gonadotropin levels are due to increased secretion rates by the pituitary, especially of FSH.

Symptoms and Signs of the Climacteric

Climacteric symptoms are numerous and variable. However, there is some argument as to which symptoms and signs can be attributed specifically to the menopause. For example, Rybo and Westberg (1971) concluded from a statistical study of symptoms that only the hot flushes could be specifically associated with the menopause. On the other hand, Vara (1970) accepts a large number of symptoms and classifies these according to the

phase of the climacteric, as follows: In the premenopausal and menopausal phase (age 40 to 50 years) Vara lists the common symptoms as hot flushes, sweats, palpitation, nervousness, insomnia, frigidity, depression, fatigue and weight increase. In the postmenopausal phase (age 50 to 60 years) additional symptoms include psychological disturbances, gastrointestinal spasms, headaches, facial hair, flabby and atrophic breasts, arthritis, low back pain, dyspareunia, pruritis vulvae, incontinence and dryness and loss of elasticity in skin. In the late postmenopause (age more than 60 years) additional signs are pathological changes such as arteriosclerosis, hypertension, osteoarthritis, osteoporosis, Dowager's hump, Heberden nodes, raised serum cholesterol, decrease in height, and advanced skin and mucous membrane changes.

The additional signs of the late postmenopause are probably more associated with old age than with the menopause. The only true symptoms are the hot flushes and sweats. Estrogen replacement therapy is remarkably effective in abolishing autonomic vascular reactions such as hot flushes, sweating and dizziness. Of course loss of estrogen and progesterone leads to degeneration and atrophy of the accessory reproductive organs (uterine endometrium, vagina, vulva).

MECHANISM OF THE CLIMACTERIC

Although it is agreed that ovarian failure occurs at the climacteric, it is not yet settled whether the primary age change is hypothalamic, pituitary or ovarian.

Primary Aging of the Ovary

The classical view is that the menopause is due to the decline in ovarian function (Zacherl, 1928; Zondek, 1930; Wagner, 1955). The aging ovary secretes progressively less estradiol in response to pituitary gonadotropin stimulation (Fig. 20-2). Consequently, there is less estrogen to inhibit the hypothalamus (by negative feedback) and therefore the secretion of pituitary gonadotropins increases (Franchimont, *et al.*, 1970). Evidence supporting the primary ovarian aging theory is that the termination of the reproductive life-span is closely related to the fall in estrogen production and to the disappearance of all normal follicles, the source of estrogen (Jones, 1970). The failure of estrogen to rise in response to elevated gonadotropin secretion is evidence supporting primary ovarian failure (P. Aschheim, personal communication). However, Dilman (1971) reports a rise in phenolsteroids, which include estrogens and related steroids secreted by the ovary.

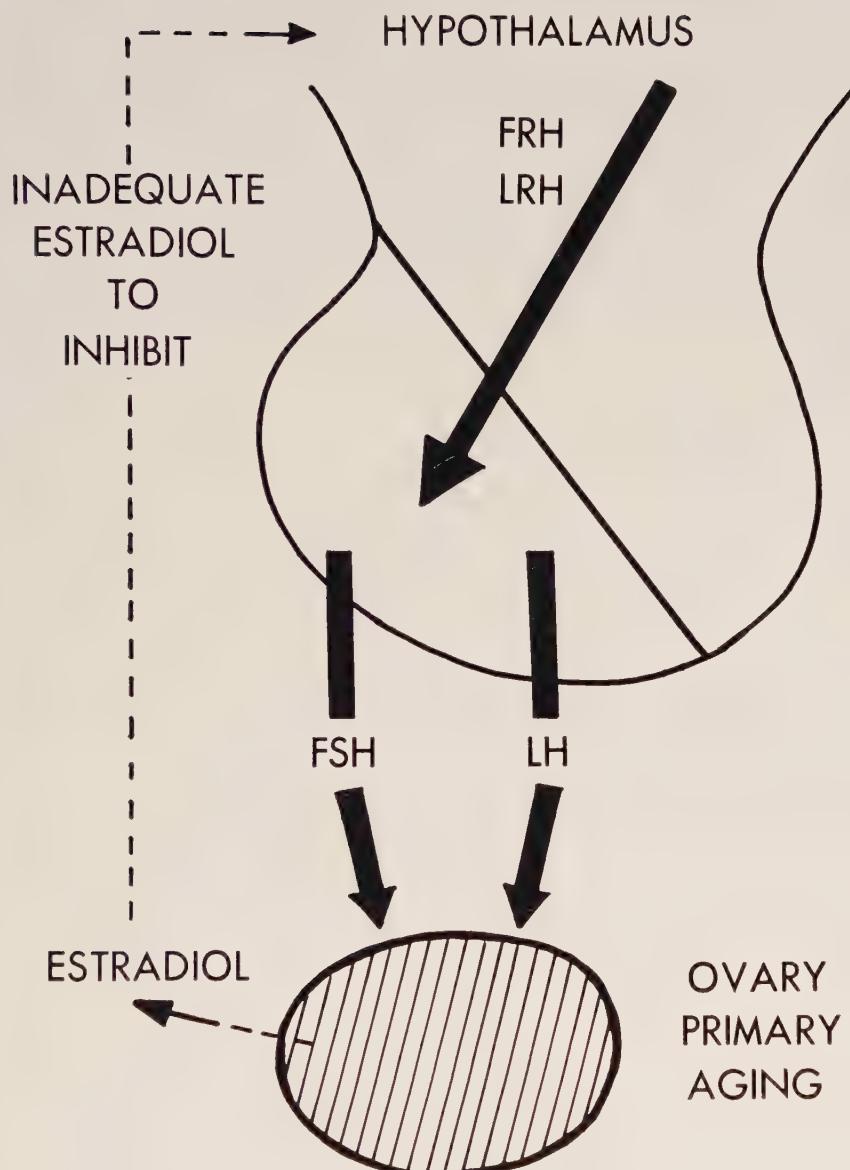


Figure 20-2. Primary aging of the ovary as the cause of the female climacteric. The amount of estradiol secreted by the aged ovary becomes too small to inhibit the hypothalamus and so the secretion of pituitary gonadotropins increases.

Changes in Pituitary Gonadotropin Secretion

Ovarian failure could be caused by pituitary overstimulation. It is logical to propose as a second hypothesis (Fig. 20-3) that hypersecretion of pituitary gonadotropins overworks the ovary, which, as a result, wears out prematurely. Overstimulation of the ovary by pituitary gonadotropins occurs after hemi-ovariectomy and leads to premature exhaustion of the ovary before the normal age of the menopause (Magendie, *et al.*, 1953). Most workers are of the opinion that the secretion of pituitary gonadotropins is increased before the menopause (Kohler, *et al.*, 1968; Coble,

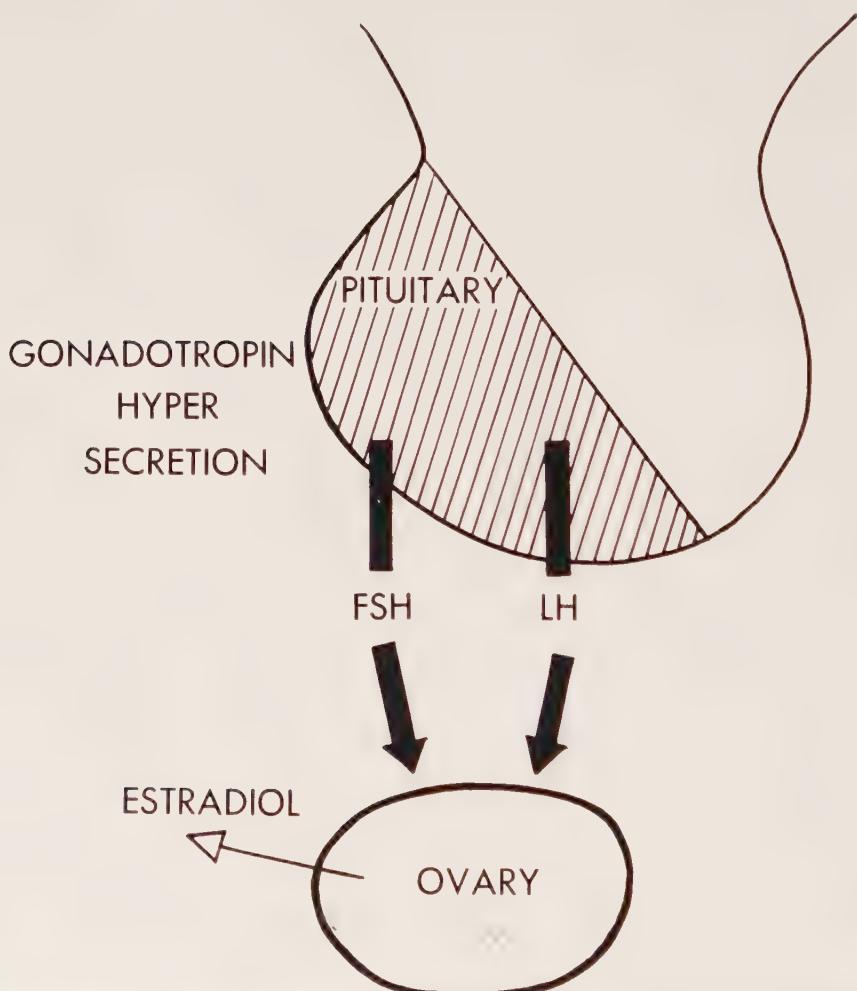


Figure 20-3. Hypersecretion of pituitary gonadotropins as the cause of the female climacteric. Overstimulation of the ovary by gonadotropins shortens its life leading to ovarian failure.

et al., 1969). In careful long term studies of individual premenopausal women, Baranov, *et al.* (1972) showed that there were no changes in the excretion of gonadotropins, estrogens and pregnandiol until the first missed menstrual period. At that time there was a remarkable increase in the excretion of gonadotropins from the early days of the abnormal period, while the levels of estrogen and pregnandiol remained low. Irregularity of the menstrual cycles with phases of high gonadotropin secretion is a feature of the climacteric. These bursts of high gonadotropin secretion must shorten the life of the ovary, if ovarian aging is accelerated by gonadotropic stimulation.

Another mechanism proposed is that the climacteric is due to an alteration in the ratio of pituitary FSH:LH. The FSH level falls, whilst LH is elevated (Llewellyn-Jones, 1971). As a result the graafian follicles are less well stimulated, leading to decreased estrogen secretion, failure of ovulation, marked reduction of progesterone secretion and irregularities of menstruation. These variations in gonadotropin secretion are probably due to changes in hypothalamic control.

Elevation of the Hypothalamic Threshold

Baranov and Dilman (1949) drew attention to the hypothalamic mechanism. Baranov, *et al.* (1972) postulated that the climacteric is due to age changes in hypothalamic centers controlling the secretion of pituitary gonadotropins. Dilman (1971) believes that aging of the hypothalamus gradually renders it less sensitive to inhibition by estrogen. The progressive elevation of the hypothalamic threshold (Fig. 20-4) thereby increases the output of pituitary gonadotropins which stimulate ovarian function (Dilman, Chap. 32).

It is suggested that the hypothalamic threshold to estrogen suppression rises not only at puberty (Donovan and Werff ten Bosch, 1959; Baker and Kragt, 1969) to switch on reproductive function, but also in middle age to switch off this function (Dilman, 1971). However, the studies of Odell and Swerdloff (1968) and Wise, *et al.* (1973) indicate that the sensitivity of the hypothalamic-pituitary axis to feedback control by estrogen remains essentially unchanged in postmenopausal women. Nevertheless, further studies will be required to settle this point because of the complexity of hypothalamic-pituitary-ovarian relationships.

Conclusion

Intrinsic aging of the ovary may be accelerated as a result of age changes in the hypothalamus which increase the secretion of pituitary gonadotropins and so hasten ovarian failure.

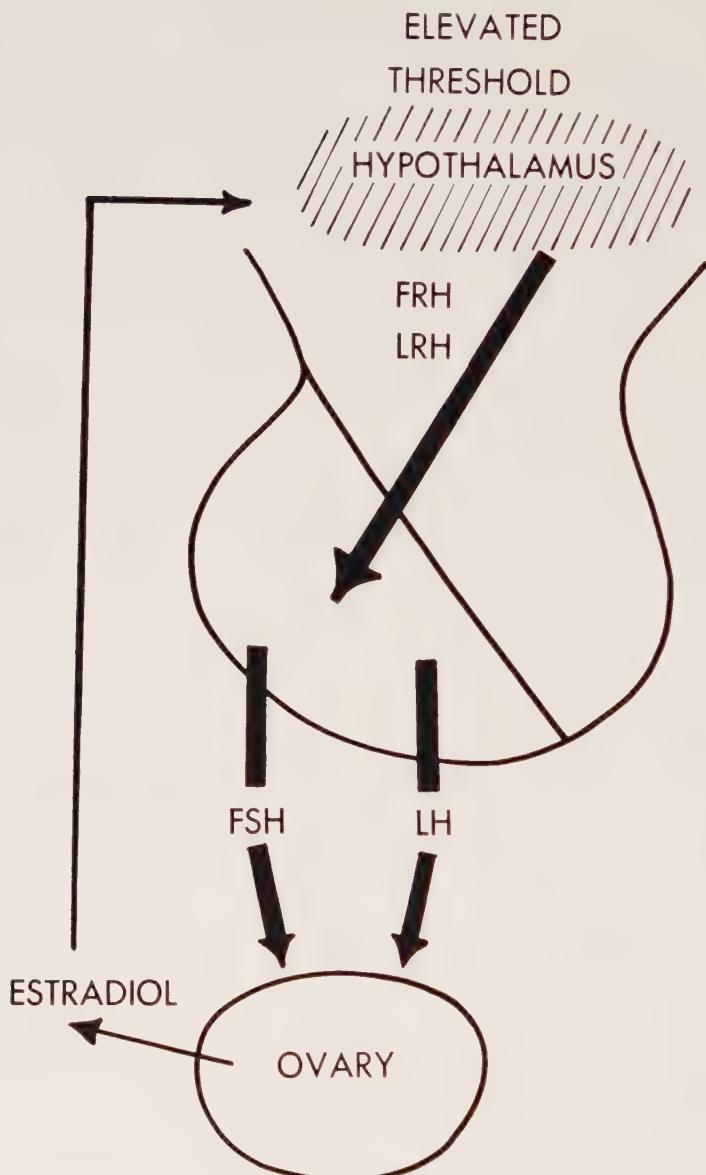


Figure 20-4. Elevation of the hypothalamic threshold to estrogen as the cause of the female climacteric. The hypothalamus is unable to inhibit the secretion of pituitary gonadotropins which rises as a result. Overstimulation of the ovary leads to premature exhaustion.

THE CLIMACTERIC AND AGING

As reproductive activity wanes there is a tendency to become obese and develop atherosclerosis, osteoporosis and neoplasms of the uterus and breast.

Atherosclerosis

There is little question that women enjoy some degree of protection from atherosclerosis prior to the menopause and that this "immunity" is subsequently lost. There is an accelerated incidence of coronary heart disease after the age of 50, which by the 70's equals that of the male. There is a large literature on the role of estrogens in atherogenesis, and the use of estrogens in the prevention of atherosclerosis (for a brief review see Pelkonen, 1971). However, there is little evidence to suggest that the administration of exogenous estrogens will protect the postmenopausal woman from atherosclerosis.

Osteoporosis

Postmenopausal osteoporosis of varying degrees occurs in practically all women. The degree of bone loss is directly related to the severity of ovarian failure and the duration of estrogen depletion (McEwen, 1969). The loss of bone is an age-related process beginning in the 5th decade in both sexes (Garn, *et al.*, 1967), however, this process is accelerated after the menopause (Albright, *et al.*, 1941; Pelkonen, 1971). A lack of estrogens is one of the key factors involved in osteoporosis, but the mechanism is poorly understood (Pelkonen, 1971). Estrogen treatment can be valuable in prevention of postmenopausal bone loss (Davis, *et al.*, 1966; Husman and Suchan, 1970; Schleyer-Saunders, 1971). However, the place of estrogens in the prophylaxis of osteoporosis remains controversial.

Skin

Estrogen is found to rejuvenate skin in postmenopausal women (Goldzieher, 1946; Drant, 1949; Schleyer-Saunders, 1971). As a result of estrogen lack the skin of postmenopausal women becomes tough, dry, scaly and inelastic (Wilson and Wilson, 1963).

Neoplasms

There is an increased incidence of cancer in association with a decline in estrogen production in middle age (Wilson, 1962; Krylova, *et al.*, 1972). However, this is probably a false association because of the long latent period between exposure to carcinogen and the clinical onset of cancer (Trichopoulos, *et al.*, 1972). There is evidence that ovarian hormones probably have delayed carcinogenic actions. The risk of breast cancer after one or more decades is low in women with menopause induced by cas-

tration before age 35, and increases with age at natural menopause (Feinleib, 1968; Trichopoulos, *et al.*, 1972). Thus ovarian function is seen as an etiological factor in human breast cancer. Dilman and co-workers have associated neoplasia in women with the secretion of nonclassical phenol-steroids by the ovary (Berstein, *et al.*, 1969; Dilman, Chap. 32) and luteinizing hormone (Krylova, *et al.*, 1967; Dilman, *et al.*, 1973).

CONCLUSION

There is a marked reduction in estrogen secretion by the ovary during the climacteric. Estrogen lack is associated with the increased incidence of coronary atherosclerosis and osteoporosis in postmenopausal women. Replacement therapy with estrogen not only abolishes the vascular symptoms (e.g. hot flushes) of the climacteric, but it also rejuvenates the genital tract and appears to retard the development of a number of pathological changes in postmenopausal women. However, exogenous estrogen does not prevent aging (Utian, 1973).

The increased incidence of cancer of the breast and genital tract in postmenopausal women may be associated with changes in ovarian and pituitary function.

Due to the increase in life expectancy there are now large numbers of emancipated, working postmenopausal women, many of whom are doctors. Thus the stage is set for more intensive research into the problems of prevention and/or treatment of postmenopausal deficiencies.

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CHAPTER 21

CANCER, AGING AND THE PITUITARY

ALBERT FREEDMAN

SUMMARY

CANCER INCIDENCE is remarkably constant, occurring at all ages, although the site varies with age and country. Carcinogenesis is a multistage progressive process from initiation, through a latent interval to cytological appearance. The incidence of most cancers occurs at certain age-peaks which vary from birth to senescence.

Endogenous (hormonal) factors determine mainly the site at each life epoch, while exogenous (social, environmental) factors acting in the latent period determine the geographical incidence.

The behavior of cancers, especially of breast and prostate can often be influenced by deliberate modification of the hormonal milieu. Induced hypopituitary states can cause regression of some disseminated breast and prostatic cancers, probably due to the dependence of neoplastic cells on pituitary tropic hormones and/or sex hormones.

Malignant cells may also aberrantly secrete polypeptides identical with pituitary hormones, especially if their histogenesis is related embryologically to the hypophysis.

The pineal hormone, melatonin, is inhibitory to many pituitary functions via hypothalamic releasing hormones. Early experiments in animals and man indicate that suppression of pituitary function with melatonin can induce regression in some hormonally dependent tumors.

DEFINITION OF CANCER

Cancer is malignant neoplasia, a process of nonreparative new growth characterized by invasiveness, destruction of contiguous tissues and metastatic spread.

Willis (1948) offers a description of neoplasia with etiological connotations "a tumour is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after the cessation of the stimuli which evolved the change."

The term cancer (Ca) will be used here to describe malignant tumors and will include carcinoma, sarcoma, and hemopoietic malignancies, myeloproliferative and lymphoproliferative.

CANCER AND AGING

It has long been known that cancer incidence and mortality increases, crescendo-like with age (Fig. 21-1). At 35 years, about 60 persons per 100,000 die of cancer per annum, at 60 years about 400 and at 75 years about 1,000.

The "cancer age" is commonly regarded as being over 40 years for females and over 50 years for males. This is incorrect as malignant disease occurs at any age from intrauterine life to extreme age, only the frequency and type of neoplasm is different.

The linear-logarithmic relationship between aging and cancer deaths is remarkably constant in European, American, Asian and Australian statistics (Fig. 21-2). On semi-logarithmic scale, this constant relationship becomes obvious (Bock and Dold, 1967).

The actuarial device of plotting logarithmic mortality rate against logarithmic age is known as the Gompertz function. The value of this statistical technique was recognized by Metcalf (1958) and more fully appreciated by Starr (1961).

The slope of the line for total cancer incidence in the Gompertz function is one in 2 to 3 before age 30 and one in 5.5 to 6.0 after 30 years (Fig. 21-3). "It is therefore very probable that the carcinogenic process, uncertain though it be, must evolve through at least two or three stages for tumors characteristic of early age and through five or six stages for can-

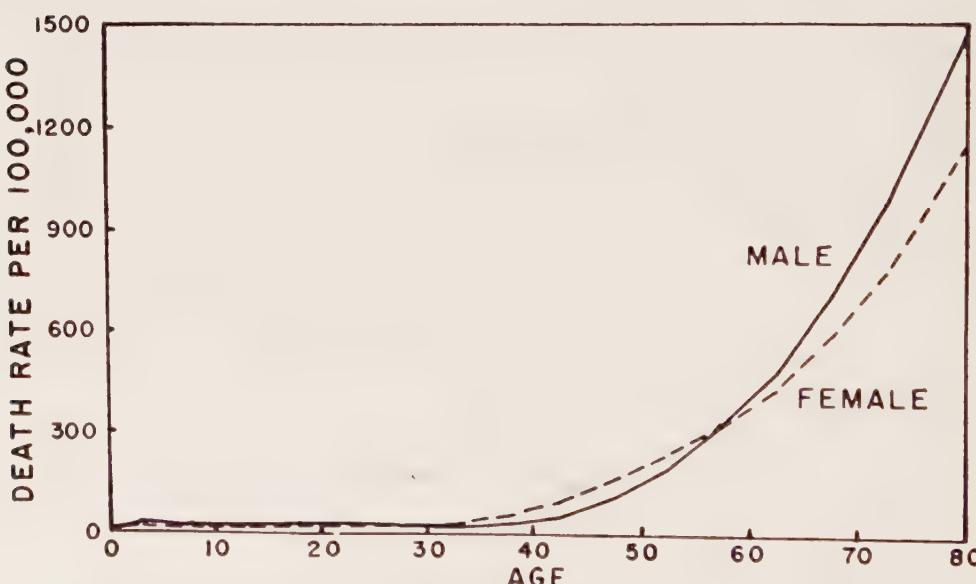


Figure 21-1. Cancer death rate and age (from Bock and Dold, 1967).

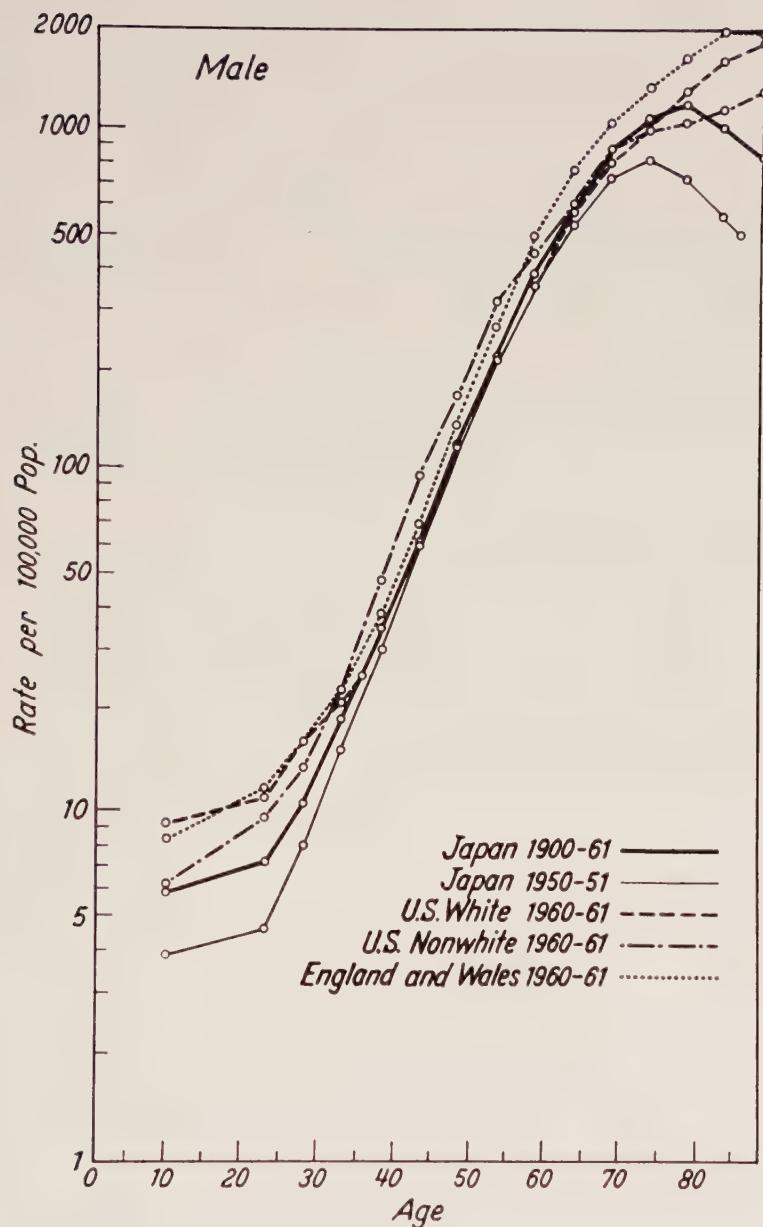


Figure 21-2. Cancer death rate and age in various countries (from Bock and Dold, 1967).

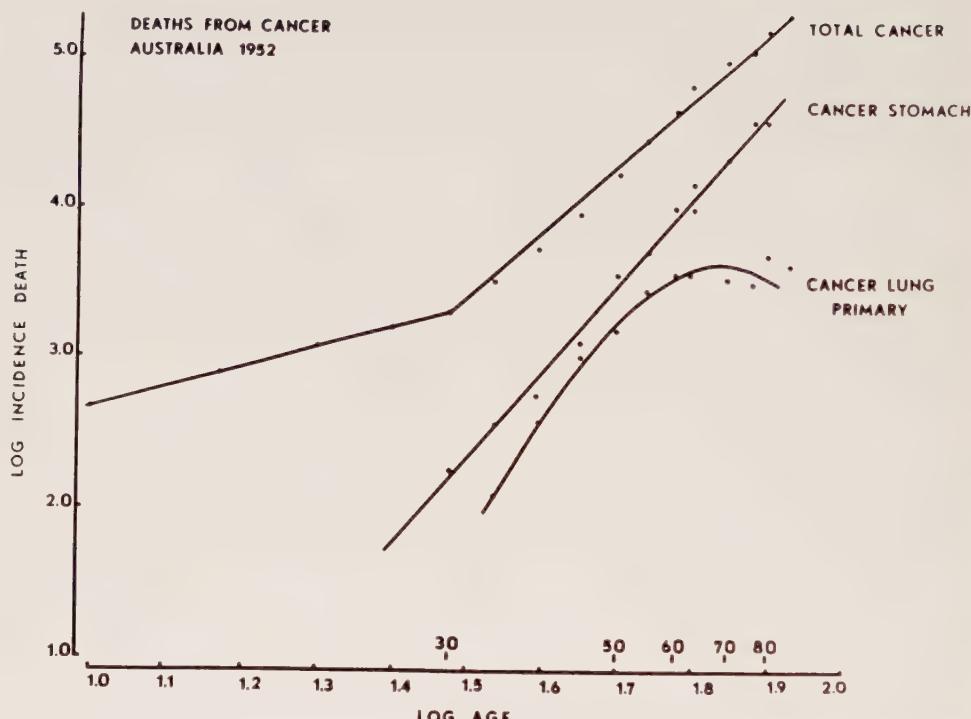


Figure 21-3. Gompertz function in relation to cancerous subpopulation in Australia (from Metcalfe, 1955).

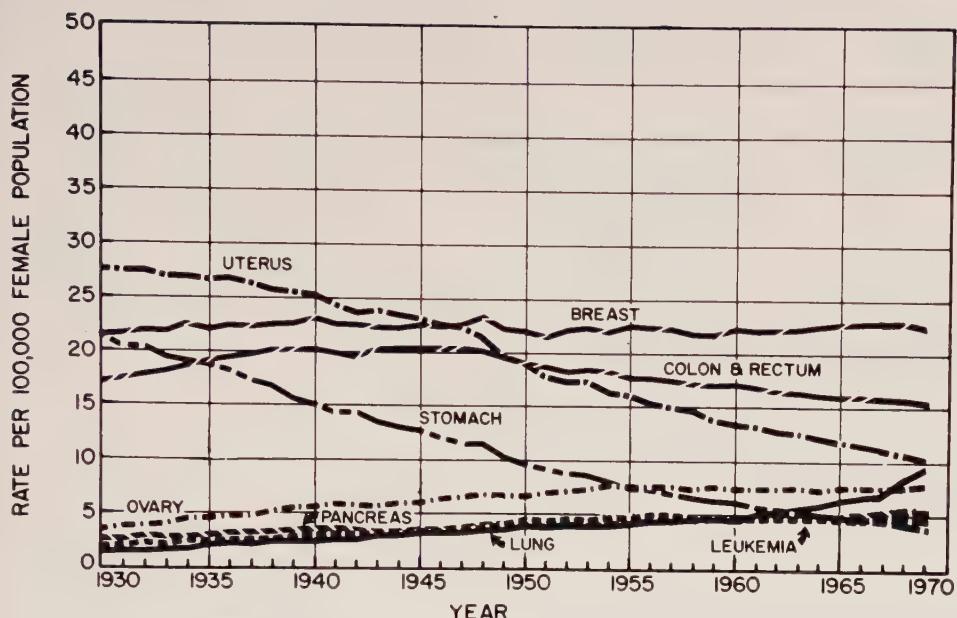
cers of the second half of man's biological life-span" (Starr, 1956). This observation is of the utmost significance for it may explain the difficulties which are being encountered in formulating a hypothesis based on a single cause and a single stage.

The Incidence of Cancers

Where reliable statistics are available over several generations, the incidence of cancers is remarkably constant with no suggestion of epidemics or increases (Bock and Dold, 1967). Whereas the total cancer rate within any country varies but little, the site of cancer varies greatly. The explosive rise in bronchial cancer among males is loudly quoted, yet the progressive and contrapuntal fall in stomach cancers in both sexes and the decrease in uterine cancers is nearly as significant (Fig. 21-4 and Fig. 21-5).

THEORIES OF CARCINOGENESIS

Experimental carcinogenesis indicates that there is an initiating factor and a latent interval before a recognizable lesion can be observed (Fig. 21-

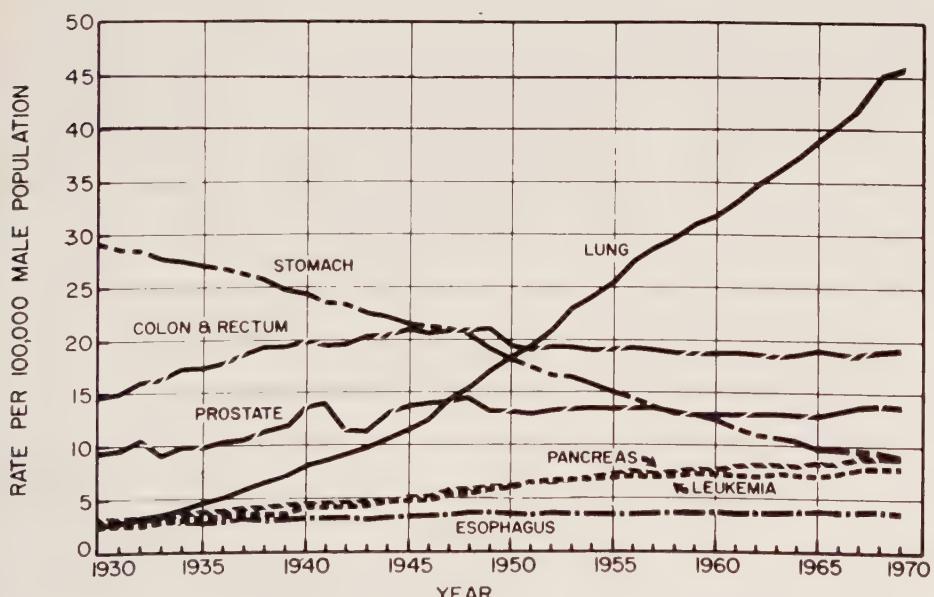


* Rate for the female population standardized for age on the 1940 U.S. population.

Sources of Data: National Vital Statistics Division and Bureau of the Census, United States.

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Figure 21-4. Female cancer death rates according to site in U.S.A. (from Cancer Facts and Figures, American Cancer Society, 1974).



* Rate for the male population standardized for age on the 1940 U.S. population.

Sources of Data: National Vital Statistics Division and Bureau of the Census, United States.

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Figure 21-5. Male cancer death rates according to site in U.S.A. (from Cancer Facts and Figures, American Cancer Society, 1974).

TABLE 21-I
PEAK AGE INCIDENCE OF CANCERS

	<i>Male</i>	<i>Female</i>
All cancers	62	62
Cervix uteri		55
Corpus uteri		58
Ovary		45-48
Bronchus, lung and pleura (combined)	56	63
Stomach	62	62
Rectum	68	68
Lip, mouth and tongue (combined)	68	63
Female breast		45-68
Esophagus	68	63
Pharynx	62	62
Prostate	67	
Skin	63-73	62-72
Soft tissue sarcomata	16 and 55	15 and 65
Osteogenic sarcoma	17, 40 and 60	7-15 and 42-70

From Hartnett, W. L., "A Survey of Cancer in London," British Empire Cancer Campaign, 1952.

6). Promoting factors act during the latent interval which is usually about one-sixth of the life-span of the species being tested (Berenblum, 1954). The pattern of cancer formation is that of a multistage progressive or processional process.

If the incidence of any particular type of cancer is plotted against age, the graph shows an individual pattern for each cancer, nearly always with a peak incidence at a characteristic age (Fig. 21-7). With the exception of soft tissue sarcomas and bone tumors, which have pubertal and post-menopausal double peak, tumors have a single peak incidence (Table 21-I). Breast, ovary and skin are plateau-like in incidence.

This feature may be represented graphically (see Fig. 21-7) using esoph-

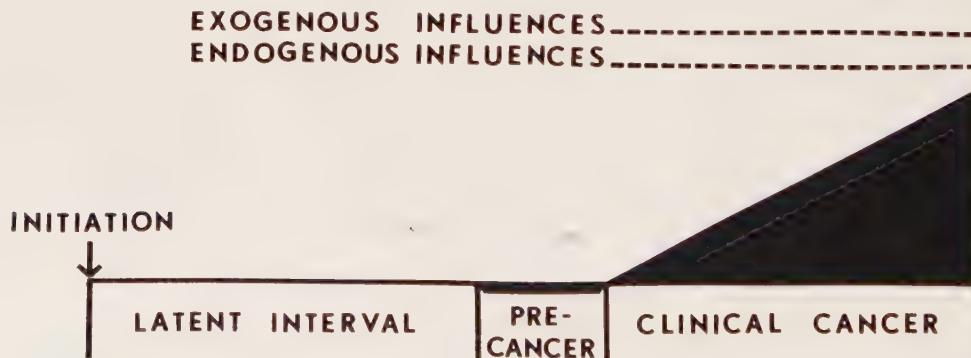


Figure 21-6. Carcinogenesis is a multistage progressive process.

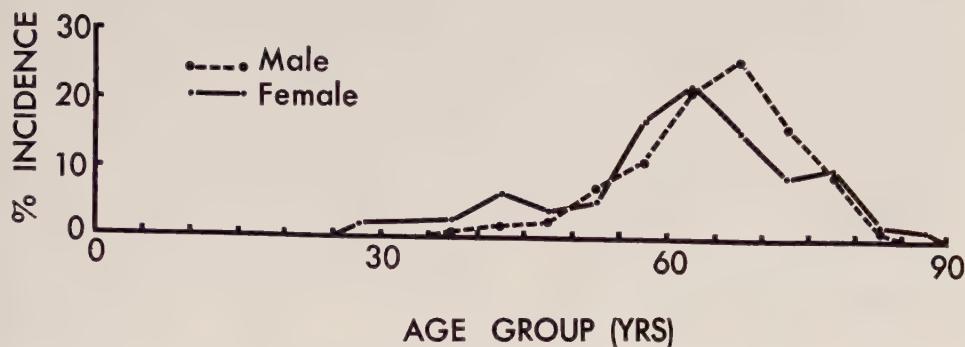


Figure 21-7. The peak incidence of cancer of the esophagus.

ageal cancer incidence as a typical example. This invites comparison with the processional process of carcinogenesis (Fig. 21-6).

In humans, the peak incidence of various cancers is quite variable, being a few months in the case of trophoblastic tumors and the mesenchymal cancers characteristic of early infancy. Occupational tumors, where carcinogens are identifiable, have long but widely varying latent intervals, ranging from 9 to 40 years. These include carcinogens such as radiation exposure, solar radiation, chromates, naphthylamine and innumerable other chemicals (Hueper and Conway, 1964).

Immunosuppressive therapy used in the prevention of organ graft rejection or in the control of auto-immune diseases, may induce lymphoproliferative disease such as reticulum cell sarcoma within a few months (Allison and Law, 1968; Lancet, 1969). Declining thymus function and immune responses in old age may be related to hemolymphatic cancers, as may be the depressed immune responses associated with the diseases such as auto-immune diseases or malignant disease itself (Metcalf, 1966; Miller, 1967, Burnet, 1967).

Nevertheless, the constancy of the peak age incidence of most cancers in so many countries with so many varying social and environmental factors speaks strongly in favor of endogenous causes. It is difficult to avoid the view that these endogenous causes are largely hormonal in origin since the essential patterns of human growth and development are themselves endogenous, biologically timed and hormonal in nature. It is the interplay of endogenous (hormonal) and innumerable exogenous (environmental) factors which yield the variations of cancer patterns in different places and among different socio-ethnic groups.

As pointed out by Starr (1956) in the morphogenesis of the embryo, it is the nervous system which superintends the development of other organs. The antiquity of the structure of the hypophysis in all species is equalled

by its constancy in function. In the human it appears early, the ectodermal anlage forming the anterior lobe and the hypothalamus remaining associated with the neural lobe. The pituitary hormones closely resemble the placental hormones at this stage and evoke a wide range of hormonal target effects on the developing fetus. How pituitary function affects the life-span is discussed in other chapters. In this chapter, the hormonal influences on cancer throughout various epochs from embryo to senescence will be examined. The possibility of influencing some cancers therapeutically by alteration of the hormonal milieu will also be described.

CANCER AND THE LIFE EPOCHS

Man, in common with all other species, passes through his predetermined life-span with recognizable stages in a sequential order from conception until senescent-death. Each of the organs and constituent tissues have their own time curve. It is obvious that the health of the body is dependent on the harmony of these time curves. As man passes through his several stages to senility, the type of cancer and its incidence also changes in a remarkably constant sequence, unfolding in symphonic manner (Fig. 21-8). The incidence, when viewed in relation to the major endocrine epochs of birth,

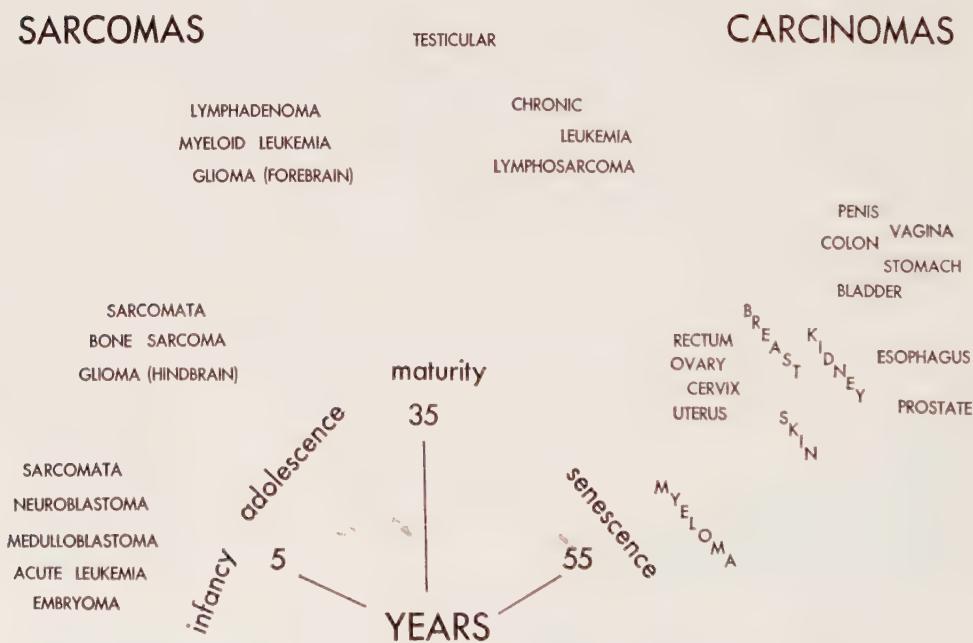


Figure 21-8. Age at peak-incidence of cancers.

puberty, maturity, menopause, etc., provide valuable clues to their evolution and possible hormonal relationship.

Childhood

Babies are rarely born with cancers although transplacental metastases from maternal malignancy occur occasionally. In Europe and North America, cancer ranks third (accidents excluded) to major respiratory disease and congenital malformations as a cause of death in infants and children under the age of five. From the age of five to fourteen years it is the major natural cause of death (Dargeon, 1940; Farber, 1950; Cohen and Lee, 1961).

Cancers in childhood differ significantly from those of adults in at least three major modes. They contain almost no carcinomata; nearly half of them are soft tissue sarcomata; many are unique to this age group. Before five years the following tumors are common:

1. Neuroblastoma of adrenal gland, sympathetic chain and retina. Their incidence is much higher in routine postmortems than in clinical experience, indicating a natural tendency to maturation and regression (Bill, 1968).
2. Wilm's tumor—nephroblastoma arising from intermediate cell mass.
3. Acute leukemia predominately lymphoblastic.
4. Medulloblastoma of hindbrain.
5. Sarcoma botryoides of vagina and bladder.

These tumors derive from primitive embryonal tissues and are much more malignant than tumors of later childhood.

The significant association of congenital abnormalities such as aniridia, hemihypertrophy and renal tract anomalies with nephroblastoma, indicate their genesis within the first trimester of gestation—the somite stage of embryogenesis (Miller, *et al.*, 1964). It is possible that these disorders may be more susceptible to malignant transformation by oncogenic viruses (Miller and Todaro, 1969), but the basic defect must be initiated at fertilization or in the presomite stage of embryogenesis.

After the critical post-natal years, bone sarcomas of osteogenic and Ewing type begin to appear. Hind brain gliomas, soft tissue sarcomas, lymphomas, hepatomas and adrenal tumors are characteristic of childhood. It is difficult to accept exogenous or environmental initiating and promoting factors in the etiology of childhood cancers (Peller, 1960).

Initiation at some point of development—most likely at the somite stage—would appear to be a reasonable hypothesis in the case of these tumors of infancy. This is in keeping with the clinical observation that the slope

of the time in the Gompertz equation is one in 2 to 3, comparable in two or three stages of promotion (See Fig. 21-3).

Puberty

The biological clock which determines the onset of puberty is unknown, but it chimes with hormonal notes on a symphonic scale. The body undergoes a major change in its endocrine milieu with corresponding increases in the size of the thyroid, pituitary and pineal gland and the maturation of the sexual organs.

There is a corresponding difference in the incidence and variety of malignant disease in these youthful years. The sarcoma carcinoma ratio begins to reverse, tumors of the female breast and reproductive organs appear with increasing frequency. During this phase of increased pituitary-hypothalamic activity, crops of pigmented nevi occur in greater frequency. The common brown mole is present in all humans, averaging about eighteen (Chorlton and Freedman, 1966) and consists of pigmented lesions situated at the junction of the epidermis and dermis. These junctional nevi are more numerous at puberty and pregnancy and decrease in senility. During the epoch of maturation, many nevi migrate away from the junction to occupy dermal and epidermal sites. A small percentage develop junctional activity and show the histological features of highly malignant melanomata. This frightening appearance may be present before puberty, but the lesions are biologically malignant and metastatic only after puberty. This symbiotic independent life cycle of tumor and host is evident also in many, if not all neoplasms of the body (Starr, 1961).

Pigmentation of acanthosis nigricans which heralds the formation of internal cancers appears after puberty. Liposarcoma is similar to melanoma in its post-pubertal malignant potential. Within this age group are to be found many of the early adult type tumors but they are mostly of low grade or borderline malignancy and include thyroid, renal, adrenal adenoma, breast fibroadenoma, colonic polypi, skin and bladder papillomata. Jaw tumors of mixed and controversial histiogenesis characterize this age. Post nasal fibromas grow in puberty and recede spontaneously with maturity.

The epoch contains the highest incidence of malignant bone sarcoma of the osteogenic and the Ewing type. Ewing tumors appear earlier than osteogenic sarcoma which are spread over ages 9 to 21 years (Dahlin, 1957). Chondroskeletal tumors—osteoclastoma and chondrosarcoma—including the genetically determined multiple hereditary eehondromata show a later peak while sarcomatous Paget's disease of bone and myelomatosis are post-maturity diseases. The temporal relationship of incidence with the epochal events in osteogenesis should have been emphasized. One only needs to re-

call the sequence of pubertal growth in the skeleton, the fusion of cartilagenous epiphyses in late adolescence and changes in bone marrow distribution during maturity and senescence. The influence of pituitary hormones on osteogenesis readily suggests a possible relationship of bone tumors and their response to hormones.

Maturity

Testicular tumors—seminoma, teratoma and mixed are characteristic of this epoch. Mesenchymal tumors in general and the lymphoproliferative and myeloproliferative tumors—lymphomata and leukemia—increase in frequency. Adamantinoma, epiphyseal osteoclastoma and astrocytoma are also features of the postpubertal period.

Pregnancy is the great endocrine happening in women with hormonal notes reaching their highest, loudest and usually happiest harmonies. It is unfortunately also the activator of many cancers of the maturity years. Breast tissue and skin melanoblasts resound to the new hormonal environment with accelerated growth and their corresponding neoplasms are most rapidly fatal. The trophoblastic tumors of the placenta are also highly malignant and restricted of course to this epoch.

Menopause and Senescence

The so called "cancer age" begins at about 40 to 45 years, before which carcinomata are rare, unusual and more likely to have been accelerated by exogenous causes. The characteristic peak frequencies (see Table 21-I) start to make their appearances and crowd the biological scene, each particular tumor having its distinctive range of age incidence and peak. Bladder, esophageal and prostatic cancers have a narrow range in age incidence while others such as kidney and breast are plateau-like. Skin cancers show a progressive rise with age. In males, bronchial and alimentary cancers are dominant while in females, reproductive and alimentary cancers are more frequent.

With menopause, cancers of the cervix uteri and corpus uteri recede slowly whilst those of breast continue to be prominent even in the sixties and seventies. The sarcomata reappear in late maturity, especially the myelomatoses and sarcoma of Paget's disease.

BREAST CANCER

The low incidence of tumors of the reproductive organs in childhood changes rapidly at puberty and reaches a peak shortly after menopause, after which there is a rapid decline. The frequency therefore, corresponds closely with the reproductive period. That geographic, racial and social habits are important is obvious when one examines these exogenous factors.

Rates for breast cancers are high in Caucasoids, and low in Chinese, Philipinos and Mexicans (Steiner, 1954). Mortality rates are highest in England, Wales, Denmark and U.S.A. (white), Netherlands, Australia and Canada, lower in Italy and least in Japan and Ceylon (Starr, 1961). The disease is significantly less frequent in married women than in nulliparous (Macklin, 1959; Chorlton, 1966). Failure to nurse for longer than three months is associated with a higher risk of breast cancer but this may be a reflection of degree of parity.

In the decade of the menopause there is a peak incidence with tendency to level off before again starting its continuous sharp rise. A woman derives no protection with increasing years.

The effect of long term contraceptive pills or long term low dosage estrogens (youth pills) is unproven.

Women who have undergone mastectomy for breast cancer are advised against any form of estrogen therapy. Even progesterone can occasionally accelerate some breast cancers (Kaufman, *et al.*, 1964).

Previous oophorectomy appears to reduce the incidence of breast cancer (MacMahon and Feinleib, 1960), but if a woman has had oophorectomy, then recurrence has a significantly worse prognosis (Moore, *et al.*, 1968).

When races migrate to different countries, it is a general rule that their first generation tend to retain their racial cancer patterns in their new environment. Subsequent generations tend to lose these racial patterns and adopt the cancer pattern of their new environment (Steiner, 1954). Thus the close harmony and association between environment (used in its widest sense of culture, habits, sexual mores, nutrition, climate, etc.) and reproductive activity is again emphasized.

The use of endocrine manipulation in the management of breast and prostatic cancer and to a lesser extent in endometrial and renal cancer constitutes the greatest advance in cancer control in the last decade.

Hypophysectomy and Breast Cancer

In 1952 surgical hypophysectomy was first performed and successfully induced palliation in advanced breast cancer (Luft, *et al.*, 1957). Its results were comparable with bilateral adrenalectomy and a large body of experience accumulated rapidly.

It was originally felt, on theoretical grounds, that this procedure should be more effective than adrenalectomy because:

- a. It removes the only source of adrenocorticotrophic hormone (ACTH),
- b. ACTH withdrawal would remove the stimulus to adrenal androgens and estrogens,
- c. It should also remove any other pituitary hormone or combination of hormones which appear to stimulate the breast directly such as growth

hormone (HGH), prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH). HGH can increase the rate of growth of metastatic breast cancer (Pearson and Ray, 1959), whereas a decrease in production should be beneficial in retarding growths.

There is increasing and frustrating evidence that it is extremely difficult to perform total, complete and lasting hypophysectomy by any means, whether by surgery, stalk section, yttrium implantation of the pituitary, external supervoltage irradiation, neutron beam therapy or destruction of the area in the median eminence where pituitary-releasing factors originate.

It must be assumed that in many of the large series of cases of hypophysectomy hitherto reported the hypophysectomy has been subtotal (Edelstyn, *et al.*, 1968).

Significant and useful palliation in about 40 percent of 77 cases (Moore, *et al.*, 1968), and 42 percent of 630 cases (Pearson and Ray, 1959), is nevertheless impressive even if complete pituitary ablation has not been achieved. The search for better selection of cases continues.

Hormonal Patterns in Breast Cancer

In the induction of mammary cancers in laboratory rodents, pituitary function plays a primary role. Hypophysectomy results in substantial slowing of growth in experimental mammary tumors of all varieties, hydrocarbon induced, estrogen induced or transplanted neoplasms (Moon, *et al.*, 1950; Mühlbock, 1958).

In human cancer, abnormally high concentrations of estriol have been found in the urine of postmenopausal women with breast cancer (Marmorston, *et al.*, 1965). This is due to the ability of the carcinoma tissue to utilize dehydroepiandrosterone (DHEA) for direct conversion to estriol (Adams and Wong, 1968).

Segaloff (1958) found that patients with breast cancer had lower excretion rates of urinary gonadotropins than would be expected from their endocrine status but no correlation could be found with remission rates from androgen therapy.

In contrast, using different methods of bioassay, Loraine, *et al.* (1957) found that cases with high urinary gonadotropins responded less favorably to diethylstilbestrol therapy.

Reduced hypophyseal function—"pseudohypophysectomy" as it is termed by Segaloff, occurs in many chronic debilitating illnesses but more commonly in breast cancer and even more so in cancer of the cervix uteri (Segaloff, 1967). Much debate exists as to whether the low levels of assayable gonad-stimulating hormones are due to reduced urinary excretion of these pituitary hormones or whether a "gonadotropin-inhibiting substance"

(GIS) is present. In some cases human urines do contain high titers of GIS which are capable of inhibiting gastric secretion in the assay animals. This may be due to contamination by urogastrone (Hahn and Albert, 1965; Segaloff, 1967), but observations to date are too few.

An alternative hypothesis seems more attractive. Since aging appears to be an endogenous factor in cancer development, age-associated hormonal changes may be important in their pathological influences. Dilman (1974) suggests that the cause of age-linked changes in the endocrine system is a failure of hormonal homeostasis. With increasing age there is decreasing sensitivity of the hypothalamus to feed-back suppression and a compensatory increase in the activity of peripheral endocrine glands. That this mechanism of carcinogenesis is possible is supported by the demonstration of homeostatic failure in many breast and endometrial cancers (Dilman, *et al.*, 1968; Dilman, 1971).

The Bulbrook-Hayward Discriminant

The beneficial results of oophorectomy, of adrenalectomy and of hypophysectomy were originally attributed to the abolition of estrogen secretion by ovarian ablation or removal of pituitary stimuli to the ovary. However, estrogens continued to be detected after endocrine ablation therapy and the estrogen content of urine did not correlate with good or poor remission rates (Bulbrook, 1968).

Since 1958, a series of papers have been emanating from Guy's Hospital, London, attempting to relate endocrine status with the effects of adrenalectomy and hypophysectomy in breast cancer (Atkins, *et al.*, 1968; Hayward and Bulbrook, 1965).

Retrospective analysis of the urinary steroid patterns of patients with advanced breast cancer who failed to respond to an adrenalectomy or to hypophysectomy (nonresponders) differed from the steroid pattern of responding patients.

In general, nonresponders to endocrine ablative procedures tended to excrete less androgen metabolites (dehydroepiandrosterone, androsterone and etiocholanolone) and more breakdown products of adrenal cortisol (17-hydroxycorticosteroids) than responders.

This androgenic/corticosteroid metabolite ratio is the basis of the discriminant factor (D) of Bulbrook and Hayward, and is expressed as follows:

$$D = 80 - 80 (17 \text{ OHCS}) + \text{Etio}$$

where 17 OHCS = 17-hydroxycorticoids mg 24 hr urine; Etio = etiocholanolone microgram 24 hr urine. No satisfactory basis has been offered for this finding.

Summarizing ten years of experience in 206 patients Atkins and his co-workers (Atkins, 1960; Atkins, *et al.*, 1968a and b) concluded that the negative discriminants generally have a lower remission rate than patients with positive discriminants, but factors such as disease-free interval and menopausal status modify the predictive value of D. Patients without previous mastectomy do not respond as well to adrenalectomy as they do to hypophysectomy. The combination of all these factors does however appear to identify a group who are not responsive to endocrine ablative treatment.

The Variable Results of Hypophysectomy

The reported remission rates, their duration, relation to endocrine status and previous treatments are quite variable and often contradictory. Regression rates as high as 50 percent (Kennedy and French, 1965), as low as 11 percent (Witt, *et al.*, 1963), and even 9 percent (Atkins, 1968), make evaluation of comparable cases most difficult. Features which add to this difficulty include:

- a. Evaluation of results. Very few studies use the criteria of the Cooperative Breast Cancer Group (Cancer Chemotherapy Reports, 1961; Hellestrom and Franksson, 1957). There is quite a deal of doubt about the reliability of urinary calcium excretion as a criterion of progression or regression. Hypophysectomy may alter the renal excretion of calcium. Further, without previous oophorectomy the frequency of visceral metastases must obviously be underestimated. Pain relief following craniotomy carries the additional possibility of frontal lobe disturbance.
- b. Selection of cases. From its earliest times, clinical experience showed that patients with predominantly visceral metastases fared badly after hypophysectomy.
- c. Castration failures and failures or relapse after androgens also tend to be excluded from hypophysectomy series.

If one excludes patients with predominantly visceral involvement, castration failures, but include those with partial control by androgens and steroids, the overall superiority of pituitary ablation compared with adrenalectomy plus oophorectomy is proven by clinical trials (Hayward, *et al.*, 1970; Stoll, 1969 and 1972).

Completeness of Hypophysectomy

Much of the preceding discussion relates collectively to various techniques of pituitary damage. These include transfrontal craniotomy, transnasal aspiration, internal irradiation by various modalities of radiation, isotope implantation, stalk section, stereotaxic cryosurgical destruction of

the gland or the site of production of releasing factors in the hypothalamus.

Complete hypophysectomy is rarely achieved and is perhaps unnecessary to produce regression in breast cancer (Van Buren and Bergenstal, 1960; Luft, *et al.*, 1957). Attempts at the most meticulous removal of the pituitary tissue is not accompanied by correspondingly better regression rates. It is probable that what is needed is a state of hypopituitarism—not apituitarism. The inability of a diminished pituitary gland to respond at its former level to releasing factors may well be the necessary state for regression of breast cancers. Diabetes insipidus as a complication of surgery need not be present, but when present acts as a marker for satisfactory hypopituitary state with nonresponse to releasing factors (Lazarus and Young, 1966; Bleasel and Lazarus, 1965).

Pharmacological Hypophysectomy

L-dihydroxyphenylalanine (L-dopa) suppresses prolactin secretion and raises growth hormone and FSH levels but has no effect on TSH levels (Stoll, 1972; Minton and Dickey, 1972).

Stoll obtained 3/7 responses of 50 percent breast cancer regression when he combined L-dopa with estrogens. These same patients had previously not responded to estrogen therapy alone.

Minton and Dickey (1972) obtained relief of bone pain in a case of premenopausal metastatic breast cancer when they suppressed serum prolactin with L-dopa. Pain returned on stopping L-dopa. In another case, the prolactin levels were not adequately suppressed by levodopa and no pain relief was obtained.

The European Breast Cancer Group (1972a) found no more than 18 percent objective tumor regressions in 19 postmenopausal patients using 2-Br- α -ergocryptine in doses now regarded as suboptimal. This same group of investigators (1972b) have completed another trial with a still more potent inhibitor of prolactin secretion CG 603 1-(morpholinomethyl)-4-phthalimido-piperidindione, 2, 6. Only 1 in 23 postmenopausal patients obtained a tumor regression. The selective suppression of prolactin secretion alone holds little therapeutic promise and is not comparable to incomplete hypophysectomy.

CANCERS AS ABERRANT ENDOCRINE ORGANS

In recent years, there has been increasing recognition that many patients suffering from malignant disease also have disturbances of body metabo-

lism which are not explained by the anatomical situation of the tumor or of its metastases. Numerous hormone-like substances have been isolated from a wide variety of tumors which can secrete them in amounts that cause the cancer to behave like an aberrant endocrine organ. These hormones are functionally identical with hormones of endocrine and neuroendocrine glands and also with tissue hormones such as serotonin, calcitonin, insulins, kinins, prostaglandins, etc. These "Humors of tumors" may be partly regulated by the general hormonal environment (Freedman, 1966; Myers, *et al.*, 1966; Ross, 1968).

Among the earliest of the paraneoplastic syndromes to be recognized are those of bronchial origin which cause hypokalemic alkalosis and even more florid Cushing's syndrome (Azzopardi and Williams, 1968). Perhaps up to 10 percent of lung cancers produce ACTH-like peptides which can alter body metabolism. Many tumors, especially those of bronchial origin secrete multiple hormones (O'Neil, *et al.*, 1968). Others can alter the metabolism of various steroids to produce estrogens (Adams and Wong, 1968).

Tables 21-II and 21-III summarize some of the hormonal effects of the tumors. The list is not exhaustive and excludes metabolic changes which could be derived from antimetabolites, anti-hormones or immunological disturbances.

TABLE 21-II
PARANEOPLASTIC SYNDROMES
(a) Classical Hormones

<i>Syndrome</i>	<i>Hormone</i>	<i>Tissue</i>
Precocious puberty	GTH	Trophoblastic tumors Presacral dermoids Hepatoma Adrenocortical carcinoma
"Clinical" Cushing's	ACTH	Carcinoma lung, colon, stomach, pancreas, prostate, thymus, lymphomas and others
Hyperthyroidism	TSH	Trophoblastic tumors Carcinoma testis and ovary
Pigmentation ? Acanthosis nigricans	MSH	Many cancers—especially stomach and pancreas
Hyponatremia	ADH	Carcinoma bronchus, testis, kidney
Hypercalcemia	PTH	Carcinoma bronchus, breast, kidney, ovary and pancreas. Carcinoma breast
(a) Low serum PO ₄ (b) Normal serum PO ₄	7-dehydrocholesterol	
Hypocalcemia with flushing	Calcitonin	Medullary carcinoma of thyroid, parafollicular carcinoma of parathyroid and mediastinum

TABLE 21-III
PARANEOPLASTIC SYNDROMES
(b) Tissues Hormones

<i>Syndrome</i>	<i>Hormone</i>	<i>Tissue</i>
Erythrocythemia	Erythropoietin	Carcinoma kidney, benign renal cysts, cerebellar hemangioblastoma. Uterine fibroids, carcinoma ovary
Hypoglycemia	Insulin-like ± Glucagon	Retroperitoneal fibrosarcoma. Hepatoma, carcinoma caecum, adrenocortical carcinoma
Hypertension	Catecholamines Renin	Phaeochromocytoma Neuroblastoma Nephroblastoma (Wilm's)
Carcinoid syndrome	Serotonin Bradykinin	Carcinoma bronchus, carcinoids of bronchus, gut
Hyperperistalsis, flushing and migraine	Prostaglandins and aminopeptides	Ganglioneuroma Medullary carcinoma thyroid Carcinoma bronchus

Ontology and Pathogenesis of "Endocrine" Tumors

All cells have genetic capacities for reproduction and for synthesis of end-products. Normal cellular maturation involves selective and extensive repression of totipotentiality of its DNA. Cancer cells may be described as being largely "depressed" in respect of their synthetic capacities.

TABLE 21-IV
EMBRYOLOGY OF TUMOR SYNDROMES

<i>Anlage</i>	<i>Tumor</i>	<i>Hormones and Syndrome</i>
Branchial cleft	Pituitary Thyroid Lung Medullary carcinoma of thyroid, parathyroid and mediastinum	Various classical syndromes Thyroxin ADH, GH, MSH, ACTH, GTH Calcitonin ± prostaglandins
Endoderm	Retroperitoneal sarcoma Carcinoma liver, caecum and pancreas Carcinoma stomach	Insulins Insulins Gastrin, Zollinger-Ellison
Foregut and midgut	Carcinoma bronchus Intestinal carcinoids	Serotonin, kinins and prostaglandins
Mesonephric ridge	Carcinoma kidney Carcinoma ovary Nephroblastoma (Wilm's)	Erythropoietin Erythropoietin Renin hypertension
Neural crest	Phaeochromocytoma Neuroblastoma Melanoma Ganglioneuroma	Catecholamines Catecholamines Catecholamines Catecholamines and prostaglandins
Skin ectoderm	Carcinoma breast	Hypercalcemia of provitamin D

It is of interest, therefore, to examine some of the paraendocrine tumors in the light of their embryological origin.

It would not seem unreasonable to regard many cancers as expressing persistence of such polypeptide-synthesizing capabilities as were characteristic of their embryonic anlage.

It makes it a little easier then to understand why cancers of the lung, which derive from the same branchial cleft as the parathyroid and pituitary gland can and do synthesize so much and so many pituitary and parathyroid hormones.

Tumors of endodermal origin, such as liver, cecum and retro-peritoneal tissues produce many of the digestive hormones, those of mesonephric ridge produce erythropoietin and renin. Still further, tumors ectodermally derived, be they sympathetic nervous system or the migrating melanocytes, produce neural hormones such as serotonin and catecholamines (Hinterberger, *et al.*, 1967).

Review of cancers in terms of embryonic and fetal endocrinology has not yet been systematically undertaken.

FURTHER INVESTIGATION OF ENDOCRINE STATUS

In order to investigate the possibility of detecting a cancer diathesis, preliminary surveys of the hormonal status of cancer patients with suitable

TABLE 21-V
GROWTH HORMONE LEVELS AND CANCER SITE/TYPE

Cancer Site/Type	Number of Cases	Growth Hormone Level ($\mu\text{g}/\text{ml}$)	Range
		Mean Level ^a	
Buccal Cavity	5	8.3	2.0-13.5
Colon/rectum	17	4.0	0.2-12.0
Glioma	8	3.8	0.4- 7.5
Sarcoma	16	3.7	0.2-11.4
Stomach	6	3.5	0.4- 7.2
Reticulosis	18	2.8	0.0- 8.5
Lung	14	2.5	0.4- 7.5
Ovary	10	2.4	0.5- 6.5
Melanoma	36	2.4	0.2- 8.0
Breast	61	1.8	0.0- 4.6
Cervix/uterus	16	1.7	0.3- 4.4
Miscellaneous ^b	17	4.5	0.2-13.0
All Cancers	224	2.6	0.0-13.5
Benign	27	1.8	0.5- 7.0

^a Mean level for 50 normal patients of 5-80 years is 2.4 $\mu\text{g}/\text{ml}$ with a range of 0.6-7.7 $\mu\text{g}/\text{ml}$ (Bleasel and Lazarus, 1965).

^b Includes 4 prostatic, 4 bladder, 2 hepatic, 2 renal, 2 thyroid, 1 testicular, 1 pancreatic and 1 bile duct cancers.

Carried out at the Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney. Radioimmunoassay method of Lazarus and Young, 1966. Supported in part by a grant from the New South Wales State Cancer Council.

TABLE 21-VI
LUTEINIZING HORMONE LEVEL AND CANCER SITE/TYPE

<i>Cancer Site/Type</i>	<i>Number of Cases</i>	<i>Luteinizing Hormone Level (mU/ml)</i>	
		<i>Mean Level^a</i>	<i>Range</i>
Cervix	2	27.0	26.5-27.5
Breast	16	12.2	2.5-32.0
Reticulosis	9	8.0	1.2-27.8
Sarcoma	10	7.4	1.6-15.5
Ovary	11	7.3	1.8-16.5
Lung	7	6.1	1.8-12.5
Colon/Rectum	7	5.8	2.5-10.0
Melanoma	9	5.7	1.2-10.0
Miscellaneous ^b	20	8.9	1.6-26.2
All Cancers	91	8.7	1.2-32.0
Benign	7	9.7	2.0-27.8

^a Range for eugonadal males 2.8-10.8 mU/ml and for eugonadal females (except at ovulation) 3.6-11.3 mU/ml (Lazarus and Young, 1966).

^b Includes 4 prostatic, 3 bladder, 2 renal, 2 thyroid, 2 stomach, 2 testicular, 1 uterine, 1 anal, 1 hepatic, 1 brain and 1 pancreatic cancers.

Carried out at the Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney. Radioimmunoassay method of Lazarus and Young, 1966. Supported in part by a grant from the New South Wales State Cancer Council.

controls have been carried out at the Special Unit at the Prince of Wales Hospital, Sydney (Starr, Chorlton and Freedman, 1966; Wynne, Salasoo and Starr, 1966; Wynne, Salasoo and Chorlton, 1968). Sera were collected at 10:00 A.M. from patients who were fasting and resting overnight. Assays were carried out in duplicate on two occasions before commencement of therapy (See Tables 21-V and 21-VI).

There is a significant but unexplained increase in the mean levels of growth hormone in a limited number of cancers of buccal cavity, colon, rectum and stomach.

One may note the possibly significant elevation of luteinizing hormone in a small number of advanced breast and ovarian cancers.

Whether these findings are consistent and whether they represent increased pituitary secretion or tumor secretion is not yet determined. Further work is in progress.

PINEAL-PITUITARY RELATIONSHIPS

Although Galen originally described the pineal gland and Descartes located the seat of the soul in the "conarium of Galen," the pineal has been regarded generally as useless or functionless—an unsung member of the cephalic choir. In 1967, Rodin and Overall showed a direct correlation between human pineal size and body weight and a significant correlation of these with age.

Figure 21-9 (after Rodin and Overall) demonstrates that the pineal age-weight relationship is not linear. It has a postpubertal rise (or prepubertal fall) and size and weight increase with maturity and aging. Pineocytes are similar in size from ages 2 till 91 years. A similar distribution of size with age was found in patients dying of cancer. Rodin and Overall found that glands were generally larger but with no special histological differences.

Barone and Das Gupta (1970) studied the effect of pinealecotomy on metastases in rats transplanted with Walker 256 carcinoma. Using untreated and sham operated controls, metastases occurred in 79 percent of pinealectomized rats compared with 31 percent of sham operated and 36 percent of control animals. Pineal function apparently involves resistance to transplanted Walker 256 carcinoma.

The mammalian pineal has the unique ability to synthesize methoxyindoles such as melatonin and methoxytryptophol. In particular, melatonin appears to act as a hormone, being secreted into the cerebro-spinal fluid. It is rapidly extracted from the blood by most tissues and is hardly detectable in the urine (Wurtman and Axelrod, 1966; Axelrod, *et al.*, 1961).

Melatonin inhibits gonadal function by suppressing pituitary FSH and LH. It antagonizes the effect of pituitary MSH on melanophores. It

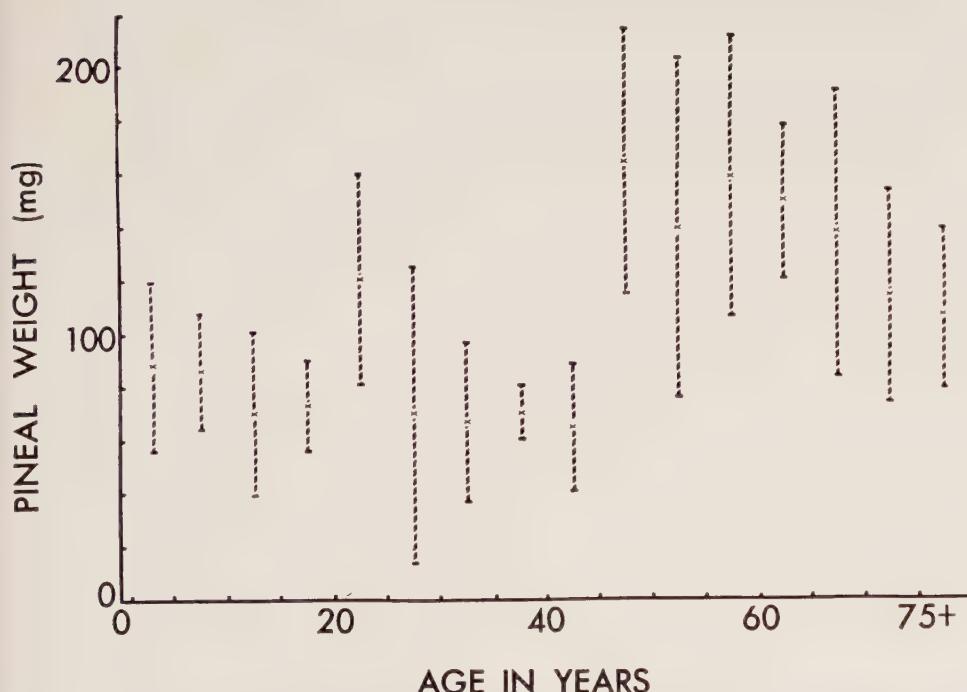


Figure 21-9. Relation between age and weight of the pineal gland. The vertical lines indicate standard deviations. Reconstructed from data of Rodin and Overall (1967).

probably suppresses pituitary TSH and has actions also on the gut, adrenal cortex and brain (Novales and Novales, 1965; Thieblot, 1965; Wurtman, *et al.*, 1964).

One must therefore regard the pineal gland as a neurohumoral complex, unique in its ability to suppress or inhibit many of the neurosecretory functions of the pituitary. Pineal secretory activity is related to activity of habenular and parataenial nuclei. The preoptic area, the hypothalamus, periventricular grey matter of the thalamus, the lateral ventricles and superior cervical ganglion also participate in the control of the pineal secretion. Hypophyso-pineal connections are both intercentral and neurohormonal in nature (Thieblot, 1965).

With these meagre and rather incomplete data, and with a belief that an increase in the number of pineal cells denotes physiological activity and significance, some preliminary and exploratory work has been carried out at the Special Unit, Price of Wales Hospital, Sydney. The effect of infusion of melatonin on several tumor types was studied. There was no established dose for humans. Incremental doses were given, and it was found that, in general 1.0 to 3.0 mg Kg 24 hr of melatonin given by constant intravenous infusion was sufficient to induce significant depression of growth hormone (Fig. 21-10) and luteinizing hormone.

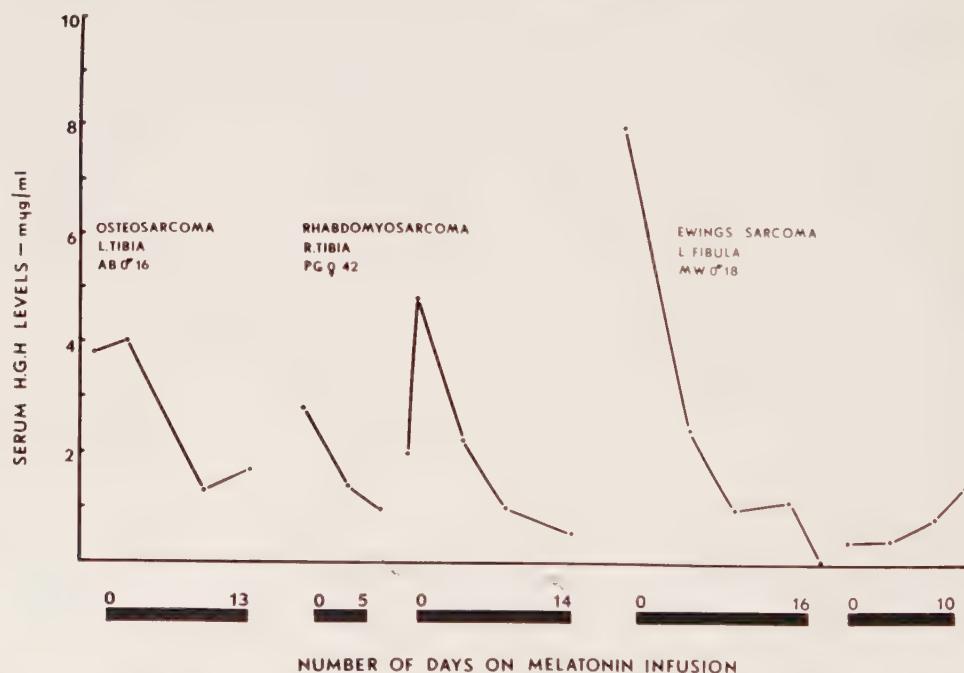


Figure 21-10. Effect of infusion of melatonin in normal saline at rate of 1.0 mg/kg/24 hours on serum growth hormone (HGH) levels in 3 cancer patients. Blood was taken at 9:00 A.M. from the resting and fasting patient.

No toxicity or metabolic effects were noted and the hormone appears to have no significant side effects.

Early Clinical Studies with Melatonin

In some of the early studies with advanced cancers, no influence of melatonin could be detected. In some cases of advanced melanoma and one prepubertal girl with osteogenic sarcoma, acceleration of metastases was observed. However, quite startling regression was measured during and after 10 to 14 days of continuous intravenous infusion of melatonin in two cases of adult rhabdomyosarcoma, adult squamous carcinoma and one adult synovioma. We were surprised and delighted as were several independent and hitherto skeptical observers.

Three patients with metastatic prostate cancer were each treated with melatonin infusion.

Case 1: Male—J. C. age 55 years; recurrence of adenocarcinoma of prostate with extension into bladder and laterally to pelvic wall. No response was found with stilbestrol administration and subsequent orchidectomy. Partial regression for 4 months was obtained with radiotherapy to a tumor dose of 4,500 rads over 8 weeks. After recurrence, he received 3.0 mg/kg of melatonin for 24 hours by subclavian venous infusion for 20 days. Repeat cystoscopic examinations showed complete disappearance of the tumour. He received weekly IMI 50 mg of melatonin in 5 ml propylene glycol for 3 months, and for 6 months IMI 100 mg melatonin in 5 ml propylene glycol every month. Cystoscopy every 3 months for a period of over 14 months showed no evidence of recurrence.

Case 2: Male—A. A. age 79 years; with recurrent prostatic carcinoma involving thoracic and lumbar vertebrae. No response to orchidectomy, TACE or cytotoxic therapy using ThioTEPA and cyclophosphamide. Pain relief within three days of melatonin infusion 3 mg/kg/day by subclavian vein infusion for 22 days. A control period of 3 days with normal saline infusion was associated with recurrence of pain for 3 days—then relief on reinstituting melatonin infusion.

Radiologically there has been no change in x-rays but formerly raised acid phosphatase of 80 K.A. units fell after infusion to 10 K.A. units and has not increased since.

Two other infusions of melatonin at the same dosage for 14 days were given at 3 month intervals and he appears to be in a phase of arrest.

Case 3: Male—A. B. age 74 years; had widely disseminated prostatic carcinoma, not responsive to estrogen therapy. He was hypertensive—B.P. 190/110—with advanced renal failure. He had marked pancytopenia with bone marrow aspiration at 3 different sites all showing marrow packed with malignant cells. He required blood transfusions every 14 to 18 days in order to maintain hemoglobin levels of above 9.5 g%. After 22 days of infusion of melatonin 3 mg/kg/day his transfusion requirements were reduced to one transfusion every 40 to 50 days for a period of 3 months. Bone pain was relieved permanently, but he died of cerebral hemorrhage 4 months after his first melatonin infusion.

In the case of the girl with prepubertal osteosarcoma, since melatonin had accelerated the malignant growth, and since the effect could have been

mediated by neurohypophyseal suppression, ACTH was infused with prompt reversal and temporary quiescence of the local bone sarcoma. The beneficial effect of high dose ACTH in two rather similar infant girls with rhabdomyosarcoma of bladder is in contrast with the generally poor effects of adrenocortical steroids given in comparable situations.

CONCLUSION

It is possible that alterations of the hormonal milieu, especially of the "higher" or tropic hormones, may influence a wider variety of cancers than breast, prostate, endometrium and kidney. In particular, much research is anticipated to elucidate the role of the pituitary hormones in mesenchymal tumors of children.

A study of the fetal hormone pattern could be rewarding since it is in this very different primordial endocrine state that the initiation of the carcinogenetic process probably begins. The fetal adrenal gland secretes very different androgens than its postnatal, mature and senile counterparts. The field is unexplored, inviting further investigation and therapeutic promise.

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CHAPTER 22

LEYDIG-CELL FUNCTION IN OLD AGE

A. VERMEULEN

SUMMARY

IT IS NOW EVIDENT that there occurs a progressive decrease in Leydig cell function in man with aging, which finds its biochemical expression in decreased plasma testosterone and apparent free testosterone concentration. This decreased function appears to have a primary testicular origin, the pituitary responding to the decreased testosterone levels with increased gonadotropin secretion; indeed both LH and FSH are significantly higher in elderly males than in younger adults. The pituitary response to LRH is increased, confirming the intact function of the gonadotrophs. The testicular response to HCG is decreased in absolute, but normal in relative value. Estrogen levels in old age are higher than in younger adults; most probably this is the consequence of a change in androgen metabolism.

INTRODUCTION

It is still a matter of controversy whether in old age there is a decrease in testicular function.

Hollander and Hollander (1958) reported a decrease with age of testosterone levels in human spermatic vein blood. Coppage and Cooner (1965), Kent and Accone (1966) as well as Gandy and Peterson (1968) on the other hand reported that plasma testosterone levels remain within the same range from adolescence until old age. Kirchner and Coffman (1968) again reported significantly lower plasma testosterone levels in a small group of males between 55 and 65 years old, as compared to young adults.

We ourselves have been interested in testicular function in old age (Vermeulen, *et al.*, 1972) and have determined plasma testosterone levels, the apparent free testosterone concentration (Vermeulen, *et al.*, 1971) and the binding capacity of the testosterone binding globulin in 200 male subjects age 10 to 95 years.

TESTOSTERONE SECRETION AND AGE

We observed (Fig. 22-1) that plasma testosterone (T) levels decrease progressively from age 50, and that especially after age 70, there is a rapid decline in plasma T levels, with however wide individual variations, some

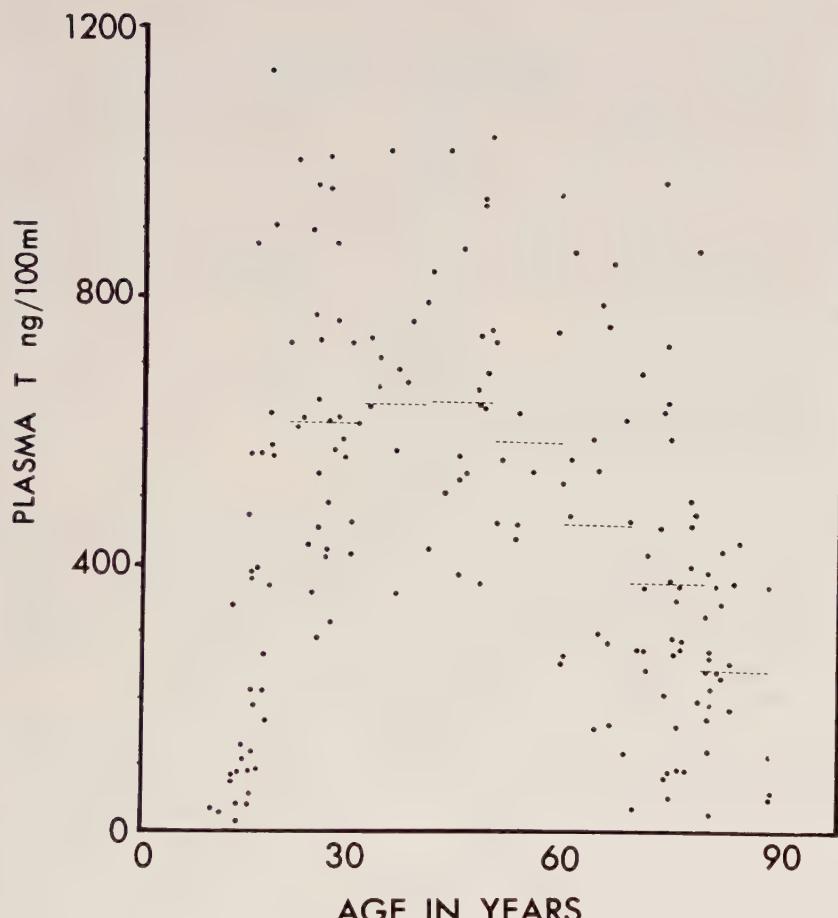


Figure 22-1. Influence of age on plasma testosterone in normal males.

elderly males having testosterone levels as high as in young adults, others having levels as low as in the female. Parallel with this progressive decrease in plasma testosterone levels, we observe a progressive increase of the testosterone binding capacity (Table 22-I) from $3.2 \times 10^{-8} M$ to $8.9 \times 10^{-8} M$. As a consequence the free testosterone fraction decreases from a mean of 2.5 percent to a mean of 1.2 percent, and as a corollary an even more pronounced decrease of the free testosterone concentration from a mean value of 10.6 ng/100 ml in the age group 20 to 50 to a mean value of 3.6 ng/100 ml in patients over 65 years old.

With increasing age, there is also a decrease in the metabolic clearance rate from $640 \text{ l/m}^2/24 \text{ hr}$ in young adults, against $530 \pm 35 \text{ l/m}^2/24 \text{ hr}$ in the elderly. Moreover there exists a positive linear correlation between the free testosterone fraction and the MCR ($r = 0.64, n = 22$). As the blood pro-

TABLE 22-I
TESTOSTERONE LEVELS, BINDING CAPACITY AND FREE TESTOSTERONE IN MALES

	<i>< 50 Yrs.</i>	<i>> 65 Yrs.</i>	<i>Statistical Significance of Difference p</i>
Plasma testosterone (ng/100 ml)	487 ^a (407-583)	264 (210-331)	< 0.001
Testosterone binding capacity (10^{-8} M)	3.2 (2.3-3.6) ^b	8.9 (7.3-11)	< 0.001
Free testosterone fraction (%)	2.5 (2.4-2.6)	1.2 (1.0-1.4)	< 0.001
Apparent free T concentration (ng/100 ml)	10.6 (9.3-12.0)	3.6 (3.1-4.3)	< 0.001

^a Mean.

()^b: 95 percentiles.

duction rate of a steroid is the product of the metabolic clearance rate and the mean testosterone concentration, it is evident that one will observe a decrease of the testosterone production from a mean value of 6.6 ± 0.5 mg/24 hr in the young, to a mean of 4.0 ± 0.6 mg 24 hr in the elderly.

ETIOLOGY OF LEYDIG CELL AGE CHANGE

From these results it is evident that there occurs a gradual decrease of the Leydig secretion in old age. The question then arises whether this decreased secretion has a primary testicular origin, or whether it has a primary pituitary origin.

In order to investigate this problem, we measured the plasma gonadotropin levels in elderly males and their variation after maximal stimulation of the gonadotrophs with LRH.

The Leydig cell reactivity on the other hand was studied by measuring the plasma testosterone response to HCG stimulation.

Moreover, in order to investigate whether the increase in testosterone binding capacity might be the consequence of increased estrogen levels, we measured by radioimmunoassay (Verdonck and Vermeulen, 1974) estrone (E_1) and estradiol (E_2) in plasma.

Gonadotropin Secretion

As far as basal LH and FSH secretion are concerned it can be seen (Table 22-II) that in elderly males both these levels are significantly higher than in younger adults.

LRH injection (200 µg I.V.) induced a marked increase in plasma LH and to a lesser extent in FSH. The LH levels attained were higher in the

TABLE 22-II
LHRH TEST (200 µg LHRH I.V.)

	<i>Young</i> (< 50 Yrs.)	<i>Elderly</i> (> 65 Yrs.)	<i>Difference</i> <i>p</i>
	<i>Male Adults</i> (n = 14)	<i>Male Adults</i> (n = 11)	
LH (mIU/ml)			
Basal value (95% Conf. limits)	12.5 (9.1-17.1)	22.5 (15.8-31.7)	< 0.05
20 min.	62.5 (47-83)	62.5 (46.8-88.3)	—
40 min.	69.2 (54.8-87.5)	78.3 (60.4-94.6)	
60 min.	55.6 (45.2-68.3)	75.8 (62.0-95.7)	< 0.1
120 min.	33.8 (23.8-48.1)	55.6 (35.5-87.1)	—
FSH (mIU/ml)			
Basal value	6.8 (5.1- 9.2)	15.0 (10.1-22.0)	< 0.01
20 min.	9.1 (6.3-13.2)	18.1 (12.6-25.9)	< 0.05
40 min.	11.6 (9.1-14.9)	19.6 (13.6-28.4)	< 0.1
60 min.	10.9 (8.7-13.6)	19.9 (13.8-28.8)	< 0.05
120 min.	9.0 (7.3-11.3)	14.2 (9.0-22.6)	—

elderly than in younger adults, but the differences were just below the limit of significance; increases in FSH levels were significantly higher in the elderly males.

Hence it may be concluded that the decreased testicular function is not the consequence of a decreased gonadotropin secretion, and that the reactivity of the gonadotrophs to a maximal LRH stimulation is intact.

Testicular Response to Gonadotropins

Upon HCG stimulation (1500 IU for 3 days), plasma testosterone levels in elderly males reach values which are significantly lower than those observed in younger males (Table 22-III), the percentage increase being rather similar in both groups of subjects.

From these results it may be concluded that the decreased testicular function in old age has a primary testicular origin, basal gonadotropin secretion being significantly increased and the response of the gonadotrophs to LRH stimulation being normal or even increased, whereas the response of the Leydig cells to HCG stimulation is decreased.

TABLE 22-III
HCG STIMULATION TESTS (1500 I.U./DAY FOR 3 DAYS)
IN YOUNG AND ELDERLY ADULTS

	Plasma Testosterone Levels (ng/100ml Geometric Mean)							
	D + 1 day		D + 2 days		D + 3 days			
	Basal	abs. val.	bas. val.	abs. val.	bas. val.	abs. val.	bas. val.	% of
Young adults (n = 8)								
geom. mean	520	722	147	948	182	1,265	242	
95% conf. limits of mean .	422-640	592-906	122-177	831-1,082	149-222	1,036-1,545	215-273	
Elderly males (> 65 yrs.)								
(n = 11)	262	399	120	504	169	566	185	
95% conf. limits of mean .	134-511	256-623	98-145	333-760	120-238	343-933	140-244	
Difference p	< 0.1	< 0.05	ns	< 0.02	--	< 0.02	< 0.1	

Estrogen Production

Determination of estrogen levels in young and elderly males (Table 22-IV) finally revealed that both estradiol and estrone levels are slightly but statistically ($p < 0.001$) higher in elderly males, when compared to younger adults.

As estradiol finds its origin in the peripheral conversion of testosterone and to a lesser extent in testicular secretion, the increased estradiol level (notwithstanding decreased testosterone levels) suggests either a shift in the secretion of the Leydig cells or, more probably an increased peripheral conversion, as a consequence of a change in peripheral metabolism. Changes in testosterone metabolism in old age have been observed by us previously (Vermeulen, *et al.*, 1972). Increased estrone levels are probably a consequence of increased androstenedione levels (Gandy and Peterson, 1968) and increased conversion. Whether the increased estrogen levels, decreased testosterone levels, or a change in the estrogen androgen ratio are

TABLE 22-IV
PLASMA ESTROGENS AND ESTROGEN/TESTOSTERONE RATIOS IN MALES

	< 50 Yrs.	> 65 Yrs.	Statistical Significance of Difference p
Pl. estradiol (E_2) (ng/100 ml)	1.5 ^a (1.3-1.8)	2.2 (1.7-2.6)	< 0.01
Pl. estrone (ng/100 ml)	3.9 (3.3-4.6) ^b	5.3 (4.5-6.2)	< 0.02
100 E_2/T	0.32 (0.26-0.36)	0.73 (0.54-0.98)	< 0.001

^a Mean.

()^b: 95 percentiles.

responsible for the increased TeBG levels is unclear for the moment but is presently investigated in our laboratory.

Senile Changes in the Testis

Given the primary testicular origin of decreased gonadal function in elderly males, a question arises as to the origin of this decrease. Sargent and MacDonald (1948) reported a gradual decrease of Leydig cell weight with advancing age. Harbitz (1973) observed a gradual decrease in Leydig cell mass with age only in patients with benign nodular prostate hypertrophy with or without carcinoma. These authors point out however that Leydig cell mass diminishes with protracted (> 7 days) terminal illness, hence interpretation of autopsy finding may be difficult. Sasano and Ichijo (1969) observed that senile changes in the testis showed a distribution pattern closely related to alterations in blood supply secondary to arteriosclerosis.

CONCLUSION

From these data, it may be hypothesized that the decreased gonadal function in old age is secondary to a decrease in blood supply to the testes, leading to tubular degeneration and Leydig cell atrophy.

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GROWTH HORMONE AND AGING

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SUMMARY

A NUMBER OF STUDIES suggest that the secretion of growth hormone (GH) declines with age in man. Serum GH levels are high in the newborn and in young children, but fall to a level which remains relatively unchanged from youth until old age in men; the level decreases further in menopausal women. In elderly subjects of both sexes sleep-related serum GH peaks are usually absent, and as a result the 24-hour secretion of GH is diminished.

Despite the fall in the secretion of this anabolic hormone in old age there is no good evidence from animal studies that senescence is due to a lack of GH. This hormone improves survival in the young animal, but apparently not in the older. It reduces the mortality of hypophysectomized rats and dwarf mice during the first half of life. In the normal middle-aged rat long-term treatment with GH does not alter the general course of physiological aging. On the other hand, excessive amounts of GH may hasten the development of terminal diseases (diabetes mellitus and neoplastic, renal and cardiovascular diseases) both in man and experimental animals and so reduce life expectancy.

INTRODUCTION

There is a relationship between growth and aging. It is well known that the longer the period of growth continues the greater is the duration of life (Northrop, 1917; McCay, *et al.*, 1935; Backman, 1940; Saxton, 1945; Lansing, 1948; Everitt, 1959; Comfort, 1964). This conclusion is based on comparative animal studies and the effects of food restriction which slows growth and prolongs life in rodents and invertebrates. Aging characteristically follows growth cessation and may even be a consequence of growth cessation (Lansing, 1948; Kohn, 1965). Although the pituitary growth hormone is a major factor in growth, its significance in aging and longevity is poorly understood (Everitt, 1959; Alvarez, 1964). This chapter will review present knowledge on age changes in growth hormone secretion and the possible effects of growth hormone on aging and longevity.

GROWTH HORMONE SECRETION AND AGE

In the 1940's the question was asked: are growth cessation and the onset of aging phenomena due to a decline in pituitary growth hormone secretion? At that time it was not possible to measure GH secretion; only recently have suitable techniques been developed.

Measurement of Growth Hormone Secretion Rate

The early methods of studying GH secretion rate by measuring pituitary GH content or serum GH level are of limited value. This is because the pituitary content of GH is a balance between GH production and secretion by the pituitary. Similarly, the serum GH level is a balance between the secretion of GH by the pituitary into the bloodstream and its removal from the circulating blood by metabolism and excretion.

Nevertheless these studies do provide indirect evidence of age changes in growth hormone secretion.

The secretion rate of GH can now be determined from metabolic clearance studies using I^{125} or I^{131} labeled GH in man (Taylor, *et al.*, 1969) and in the rat (Frohman and Bernardis, 1970). Also it may be measured in human subjects by serial HGH measurements at 20-minute intervals over a 24-hour period to estimate HGH secreted in episodes (Finkelstein, *et al.*, 1972).

Pituitary GH Content and Age

The GH content of the pituitary increases during growth in the young rat (Birge, *et al.*, 1967; Burek and Frohman, 1970) and young mouse (Yanai and Nagasawa, 1968). However, when pituitary GH content is expressed in relation to total body weight it decreases with age in both male and female pigs (Baird, *et al.*, 1952; Baker, *et al.*, 1956), in the cow (Armstrong and Hansel, 1956) and in the male rat (Bowman, 1961), but not in the female rat (Solomon and Greep, 1958). These studies suggest that the pituitary storage of GH in relation to body weight is reduced as the animal grows older. However, as mentioned above, this does not necessarily mean that GH secretion decreases with age.

Human studies of pituitary GH content have been performed only on small numbers of glands removed from patients with a variety of diseases (Gemzell and Heijkenskjöld, 1956; Gershberg, 1957; Daughaday, 1968). No significant changes in pituitary GH content with age are evident from these studies.

Fasting Serum GH Level and Age

Radioimmunoassay techniques now permit the accurate measurement of serum GH levels (Glick, *et al.*, 1963; Lazarus and Young, 1966), although

obviously only radioimmunoassayable peptides are measured. The fasting GH level in serum appears to be high in the newborn (Glick, *et al.*, 1963 and 1965) and in children (Girard, *et al.*, 1961; Greenwood, *et al.*, 1964), but falls to a level which remains relatively unchanged from childhood to old age (Lazarus and Young, 1966). In elderly subjects, fasting serum GH levels are reported to be the same as in young adults (Lazarus and Young, 1966; Cartlidge, *et al.*, 1970; Dudl, *et al.*, 1973), although a significant decrease is reported for women aged 40 to 59 years (Vidalon, *et al.*, 1973). The recent studies of Dilman (Chap. 32, Table 32-X) indicate that the basal serum GH level falls progressively with age.

Serum GH levels are highest during the period of most rapid growth soon after birth in man and also in the pig (Machlin, *et al.*, 1968; Siers and Hazel, 1970) and the Holstein bull (Purchas, *et al.*, 1970). The maintenance of similar GH levels from late childhood until old age, indicates that GH is secreted after growth cessation.

Stimulation of GH Secretion and Age

In human subjects GH secretion is stimulated by hypoglycemia, exercise, fasting, stress, arginine, proteins, etc. (Glick, *et al.*, 1965). The elevation of serum GH after stimulation appears to be greater in young subjects than in older, thereby suggesting a higher secretion rate in childhood and youth. Using the Bovril (meat extract) stimulation test Buckler (1969) found a significant rise in serum radioimmunoassayable GH in young men, but not in men older than 30 years. The rise in plasma GH due to the hypoglycemia which develops 3 to 4 hours after a meal, is greater in children than in adults (Hunter and Rigal, 1966).

In elderly subjects the GH response to insulin hypoglycemia is found to be normal (Root and Oski, 1969; Cartlidge, *et al.*, 1970; Sachar, *et al.*, 1971; Kalk, *et al.*, 1973), although Laron, *et al.* (1970) reported it subnormal. In each study only small numbers of subjects were used. Elderly men appear to be more responsive than women (Kalk, *et al.*, 1973). Thus the hypothalamic-pituitary secretory mechanism remains intact in normal old age. This is not so in progeria patients who do not release GH in response to hypoglycemia (Villee, *et al.*, 1969).

Suppression of GH Secretion and Age

Hyperglycemia is known to suppress the secretion of GH (Glick, *et al.*, 1965). In middle aged subjects Dilman (1971 and Chap. 32) reported that a standard glucose load failed to reduce the serum GH level. Although confirmed by Sandberg, *et al.* (1973), it is not supported by the work of Benjamin, *et al.* (1970) and Dudl, *et al.* (1973). Further studies are required to settle the controversy.

HGH Secretion Rate and Age

The metabolic clearance study of Taylor, *et al.* (1969) established the normal metabolic clearance rate for HGH as 229 ml/min and the production rate as 347 μg HGH/min. This study was carried out on 22 normal human subjects aged 17 to 75 years and failed to demonstrate any sex or age differences. However, the study was not designed for testing age changes.

Two later studies (Finkelstein, *et al.*, 1972; Carlson, *et al.*, 1972) suggest that there is a reduction in the spontaneous secretion of growth hormone in old age. Finkelstein and his co-workers studied the age-related change in spontaneous HGH secretion rates and secretory patterns in a group of normal prepubertal children, adolescents and young and older adults. They determined the concentration of HGH in plasma samples obtained at 20-minute intervals over a 24-hour period. Having shown a rise in secretion rate from 91 $\mu\text{g}/\text{day}$ in prepubertal children to 690 $\mu\text{g}/\text{day}$ in adolescent children, these workers demonstrated a reduction in HGH secretion rate in young adults to 385 $\mu\text{g}/\text{day}$, while in older adults (47-62 years) the total 24-hour secretion of HGH decreased and approached zero in three of five subjects (Table 23-I).

Similarly Carlson, *et al.* (1972) have shown that the well-demonstrated physiologic sleep-peak of HGH, occurring early in the course of an undisturbed sleep in normal young people, failed to occur in four of six subjects over the age of 50 years. These workers have suggested that, since there is a persistence of normal HGH responses in insulin-induced hypoglycemia in elderly persons (Sacher, *et al.*, 1971), the age-related loss of

TABLE 23-I
THE EPISODIC SECRETION OF GROWTH HORMONE IN YOUNG
AND OLDER ADULTS DURING A 24-HOUR PERIOD

Age Group	Age (Years)	Sex	Secretory Episodes (μg)		Secretion Rate ($\mu\text{g}/\text{day}$)
			Awake	Asleep	
Young	23	F	189	73, 344	606
	24	M	0	328	328
	24	M	79, 114	298	491
	28	F	65, 76	74	215
	33	F	76, 41, 238, 111	190, 113	769
Older	47	F	0	0	0
	51	F	0	0	0
	51	F	109, 146, 81, 54, 115	0	505
	62	M	0	57	57
	62	M	0	0	0

From J. W. Finkelstein, *et al.*, "Age-related change in the twenty-four-hour spontaneous secretion of growth hormone," *Journal of Clinical Endocrinology*, 85:665 (1972).

HGH sleep-peaks is not due to a generalized hyporesponsiveness to all HGH provocative stimuli. At the 4th International Congress of Endocrinology in Washington (1972) these workers agreed that the absence of such sleep-induced peaks in elderly people could be related to the metabolic effects of aging.

In Vitro Secretion of GH and Age

The *in vitro* synthesis of GH by the anterior pituitary gland has been studied by several workers. During growth there is an increase in GH secretion by the anterior pituitary *in vitro* in the young male rat, but there is no change in the female (Burek and Frohman, 1970; Yamamoto, *et al.*, 1970). In an earlier study on the secretion of GH by pituitaries from female rats *in vitro* Meites, *et al.* (1962) failed to demonstrate any change in secretion between 25 days and 2 years. When GH production was calculated per mg of pituitary, the young pituitary secreted 3 to 5 times more GH than the old. It must be emphasized that these *in vitro* studies measure the basal secretion of GH independent of hypothalamic stimulation.

Hypothalamic GRH Activity and Age

Hypothalamic GRH activity probably diminishes with age. Pecile, *et al.* (1965; Chap. 5) reported a high GRH activity in the hypothalamic extracts of 30-day-old rats and a much lower activity in extracts of 2-year-old rats. Schally, *et al.* (1967) have demonstrated GRH activity in the hypothalami of middle-aged and old men.

Pecile, *et al.* (1965) also showed that the pituitary gland of the 2-year-old rat was less responsive to hypothalamic GRH than the pituitary of a 30-day-old rat. Thus aging had also occurred in the pituitary.

TISSUE RESPONSE TO GROWTH HORMONE IN OLD AGE

The early concept that GH is concerned only with the phase of growth is unfounded. GH is clearly active on many metabolic processes throughout life.

In old age many tissues are able to respond to growth hormone in much the same way as in the young animal. Asling, *et al.* (1952) remarked that GH "confers strangely youthful proportions on the nitrogen, fat and water components of the body, even in old animals." After growth cessation in middle-aged rats it is possible to restart growth by giving injections of GH both in the female (Emerson, 1955) and the male rat (Everitt, 1959). However, progressively greater doses of GH are required to achieve continuous growth (Emerson, 1955), suggesting a loss of tissue response.

With increasing age there is a diminished responsiveness of adipose tissue in the rat to GH as measured by the rise in serum free fatty acids (Jelinkova and Hrúza, 1964). Also the incorporation of H^3 cholesterol into

aorta after GH administration is greatly reduced in old rats (Hrúza, Chap. 24, Fig. 24-10).

In small numbers of elderly human subjects the effects of human growth hormone (HGH) have been shown to be similar to those on young subjects in causing nitrogen retention, lowering the blood urea nitrogen, increasing plasma free fatty acids and decreasing urinary sodium (Beck, *et al.*, 1960; Root and Oski, 1969). The administration of GH to 7 elderly subjects more than 70 years old significantly increased TmG, but did not affect glomerular filtration rate (Marelli, 1968).

Impaired responsiveness to HGH in elderly subjects was observed by Root and Oski (1969), who found that HGH failed to inhibit glucose consumption by red cells *in vitro* and did not induce hydroxyprolinuria.

Thus certain tissues lose their responsiveness to GH in old age. However, the loss of response to GH is minor compared with the severe loss in progeria patients, who exhibit premature senescence (Villee, *et al.*, 1969).

GROWTH HORMONE AND PHYSIOLOGICAL AGING

Although GH secretion appears to fall in old age in man, there is no good evidence that GH is the youth hormone or "elixir of youth" that early workers had suggested. There is no convincing evidence that GH has a general effect on physiological aging as distinct from growth. The administration of moderately large doses of GH (5 mg bovine GH per day for 200 days) to middle-aged rats increased body weight and raised the fasting blood sugar level (Everitt, 1959).

However, neither this treatment, nor treatment with physiological doses of GH, had any significant effect on the course of physiological aging in 6 metabolic, 4 hematological and 3 electrocardiographic parameters (Everitt, 1959). Furthermore, large doses of GH failed to prevent the terminal loss of body weight, which is observed as rats approach death (Everitt, 1959). Therefore a lack of GH is not the cause of physiological aging in the old rat.

Skeletal growth and aging are accelerated by GH (Silberberg, Chap. 12). On the other hand, collagen aging appears to be retarded by GH treatment in the hypophysectomized rat (see Fig. 11-10). However, Panigraphy and Patnaik (*Exp Gerontol*, 10:85, 1975) showed that GH increased the content of insoluble (aged) collagen in bones of lizards).

GROWTH HORMONE AND LONGEVITY

A normal secretion of growth hormone appears to be necessary for a normal life-span. Hypophysectomy reduces the life expectancy and GH replacement increases survival in hypophysectomized toads (Jørgensen and Larsen, 1963) and male rats (Everitt, 1971). In the hypophysectomized

rat GH reduces mortality until middle age (400 days), but this protective effect is then lost (Fig. 23-1). GH is essential for survival in rats hypophysectomized at the age of 6 days (Walker, *et al.*, 1950), in order to stimulate growth of the skull and so prevent death from compression of the brain which continues to grow. GH also increases the survival of dwarf mice (Fabris, *et al.*, 1972) by an immunological mechanism.

In man a lack of GH does not necessarily lead to a short life, since Rimonin, *et al.* (1966) described a 77-year-old dwarf, whose GH deficiency obviously started in childhood.

The long-term treatment of intact middle aged male rats with moderately large doses of bovine GH prolonged the period of growth, but resulted in a 10 percent reduction in life duration which was not statistically significant (Everitt, 1959). Similarly, treatment of old female rats with GH and prednisolone did not affect their survival (Emerson and Emerson, 1962).

Oversecretion of GH in man causes gigantism in childhood and acromegaly in adults. The mortality of 194 acromegalic patients was found by

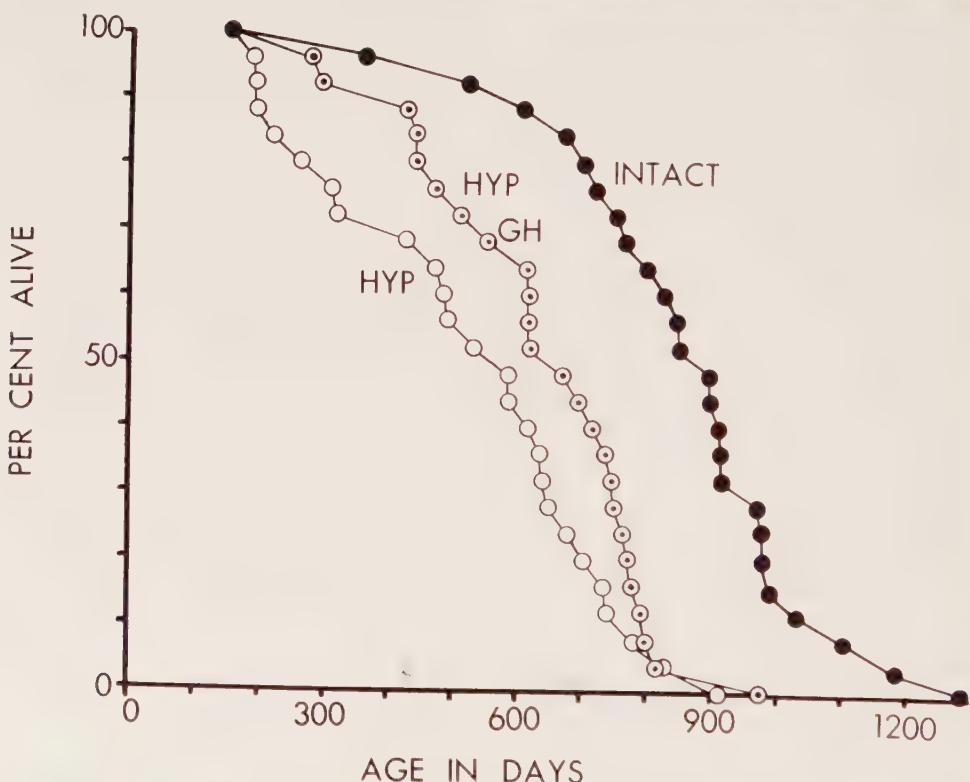


Figure 23-1. The effect of growth hormone in reducing the mortality of the hypophysectomized rat, until middle age (400 days). The intact controls were exceptionally long-lived; the mean life duration is usually 750-800 days.

Wright, *et al.* (1970) to be twice that expected from the general population of the same age and sex structure. Acromegalic patients had an increased number of deaths due to cardiovascular, cerebrovascular and respiratory diseases.

A study of the longevity of very tall men by the Metropolitan Life Insurance Company (1937) revealed an increased mortality in men whose height was 6 ft 5 in (195 cm) or more. Giants, as a group, are short lived (Cheah, 1970). The average age at death was 34 years in 7 giants whose heights ranged from 7 ft 6 in (229 cm) to 8 ft 7 in (262 cm) (Metropolitan Life Insurance Company, 1937).

Comber (1968) discussed the reduced longevity of the giant in relation to his physiology as follows: "With one and a half times the height of normal man he could be expected to weigh at least three times as much. But the larger bones and muscles would only be twice as strong to support and move this weight. The heart, too, though 2½ times as powerful as a normal man's, would have to work five times as hard to keep the blood flowing round this enormous body. Small wonder that dwarfs tend to live longer than giants."

Thus, longevity data in man, although fragmentary, indicate that an excess of GH may shorten life.

GROWTH HORMONE AND THE DISEASES OF OLD AGE

The long-term treatment of intact female rats with GH produces gigantism (Evans, *et al.*, 1948) and increases the incidence of neoplasms in pulmonary and lymphatic tissues, adrenal glands and reproductive organs (Moon, *et al.*, 1950). However in hypophysectomized rats treated with growth hormone there is an absence of such tumors (Moon, *et al.*, 1951). Similar induction of tumors did not occur in mice (Moon, *et al.*, 1952), although Takakura, *et al.* (1967) report that GH accelerates the development of plasma cell tumors and lymphosarcomas in mice. Growth hormone was found by Tipnis and Sirsat (1969) to shorten the latent period of induction of skin cancer with 20-methyl cholanthrene, to increase the lateral spread and enhance the invasive capacity of this tumor in mice.

In 223 patients with acromegaly and gigantism Mustacchi and Shimkin (1957) could not find any increase in the incidence of cancer, after corrections were made for age and sex. GH is apparently not an important etiological factor in human cancer. In rats, however, GH probably plays a supporting role in tumorigenesis.

Selye (1951a) reported that GH produces nephrosclerosis, periarteritis nodosa and hypertension in female rats sensitized by unilateral nephrectomy and high NaCl intake. These effects of GH appear to be mediated by the adrenals, since they are abolished by adrenalectomy (Selye, 1951b).

CONCLUSIONS

There is no evidence that the pituitary growth hormone is the "elixir of youth," although it does appear to reduce mortality until middle age in the hypophysectomized rat. However, an excess of growth hormone can induce pathological changes similar to the diseases that terminate life.

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CHAPTER 24

LIPID METABOLISM AND AGING ENDOCRINE ROLE

ZDENEK HRUZA

SUMMARY

DURING AGING the amount of fat in the tissues of man and animals increases. In older animals, there is a decreased catabolism of neutral fats in adipose tissue and in the aortic wall after lipolytic stimulation, as for example after stress or after the administration of growth hormone or catecholamines. The decreased response of the adipose tissue to lipolytic agents is partly caused by the fat cell containing more fat and thereby less lipolytic enzymes during aging. In older animals there is a slower cholesterol turnover which is caused by a slower excretion of cholesterol and a slower exchange of cholesterol between the blood and the tissues. The disturbances in cholesterol metabolism during aging may be related to the decreased activity of the pituitary gland because hypophysectomized animals, like old animals, handle cholesterol less efficiently. In old hypophysectomized animals, the decrease of cholesterol turnover is smaller than in young hypophysectomized animals. This may be explained either by reduced sensitivity of the target organs to pituitary hormones, mainly growth hormone and thyrotropic hormone during aging or by diminished secretion of these hormones. In hypophysectomized animals, growth hormone speeds up cholesterol turnover but its action is much less in older animals. Sensitivity to hormones handling cholesterol (thyroxine, growth hormone, insulin) decreases during aging and there seems to be also some evidence that the secretion of several lipid-mobilizing hormones decreases with aging. Pituitary age changes, together with the decreased sensitivity of the adipose tissue and aorta to these hormones and reduced efficiency of the cholesterol-handling mechanisms may contribute to the development and progression of obesity and atherosclerosis in older individuals.

CHANGES IN LIPID METABOLISM DURING AGING

The Composition of Adipose Tissue

The amount of fat in the body increases with aging in man as well as in animals (Figs. 24-1 and 24-2). The most important increase in the fat

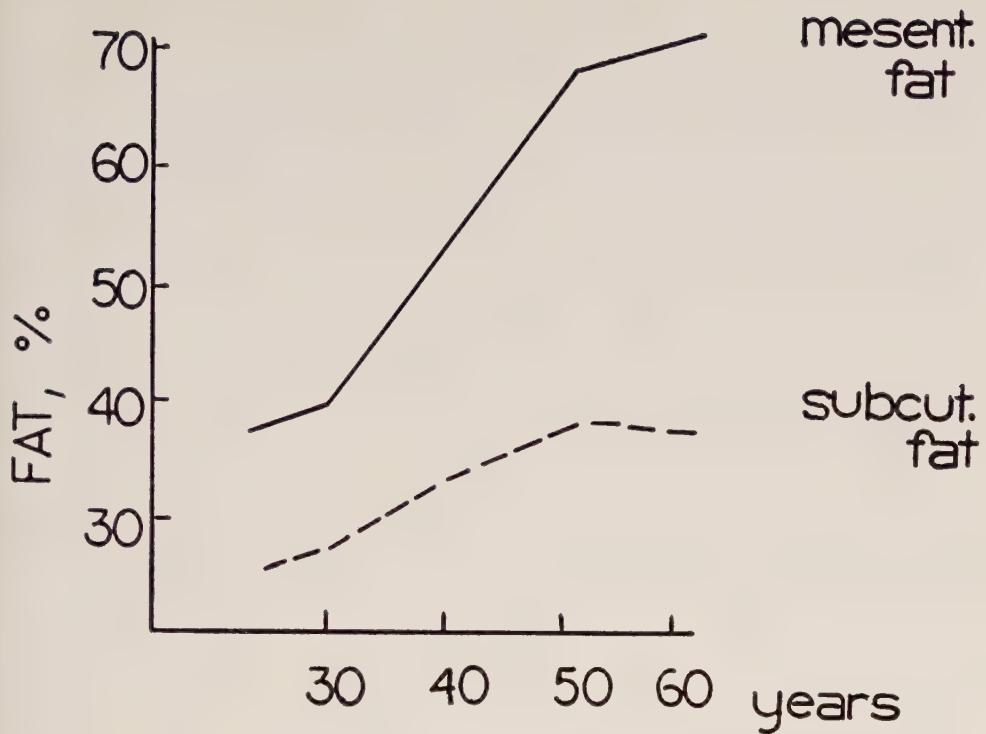


Figure 24-1. Aging changes in percentage of fat in mesenteric and subcutaneous tissue in women (After various sources).

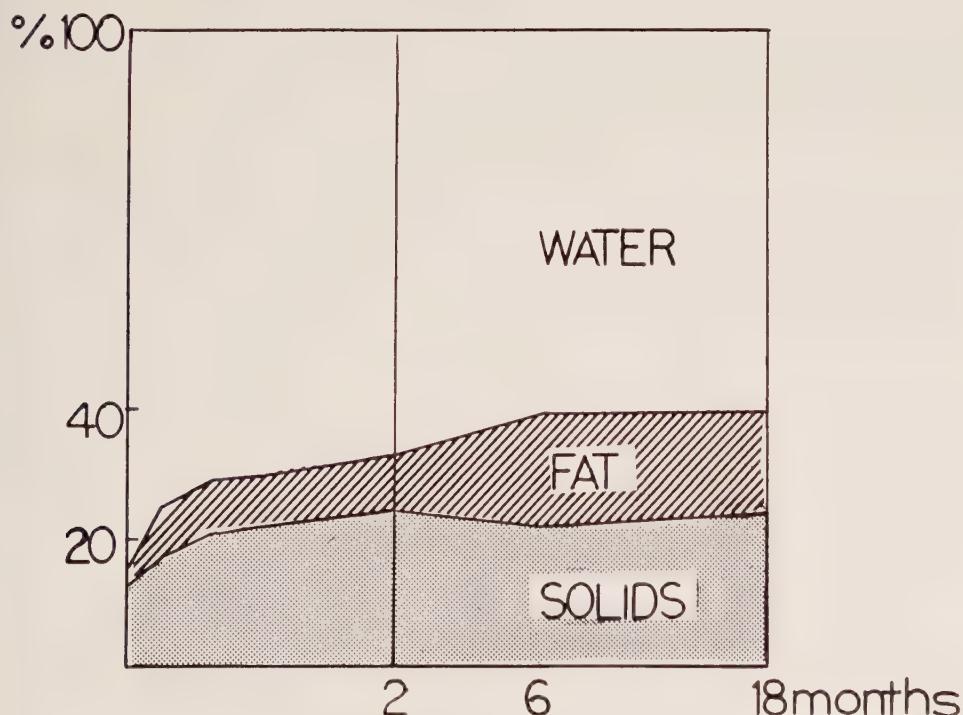


Figure 24-2. Changes of fat and water content in the body of rats during development and aging. Water is replaced by fat during development. Party after Jelinek (Babkova sbirka 3, 1961, State Health Publ. Czechoslovakia).

content of the rat takes place between the 2nd and 6th month. It is difficult to ascertain the role of individual factors which influence the storage of energy in the form of fat. In the rat the storage of fat is increased mainly at the time when rapid growth stops, that is, around the 3rd month. However, the utilization of energy decreases at an earlier age. The peak of the basic oxygen utilization occurs after 30 days then it decreases rapidly and thereafter remains approximately constant. These measurements, however, have not been continued long enough to assess these parameters in old animals. According to several authors the oxygen utilization in old rats, expressed on the basis of body surface, increases again to a certain extent (Benedict and Sherman, 1937). This increase, however, could be due to pathological states which are accompanied by decreased body weight in old animals. The fat vacuole in the fat cell of rats increases after 2 months (Jelínková, *et al.*, 1965) and the amount of fat in the cell increases. At the same time the relative weight of the fat bodies also increases (Fig. 24-3). Spontaneous food intake decreases with aging (Harte, *et al.*, 1948; Everitt, 1958). The only explanation for the increase of fat in the body during

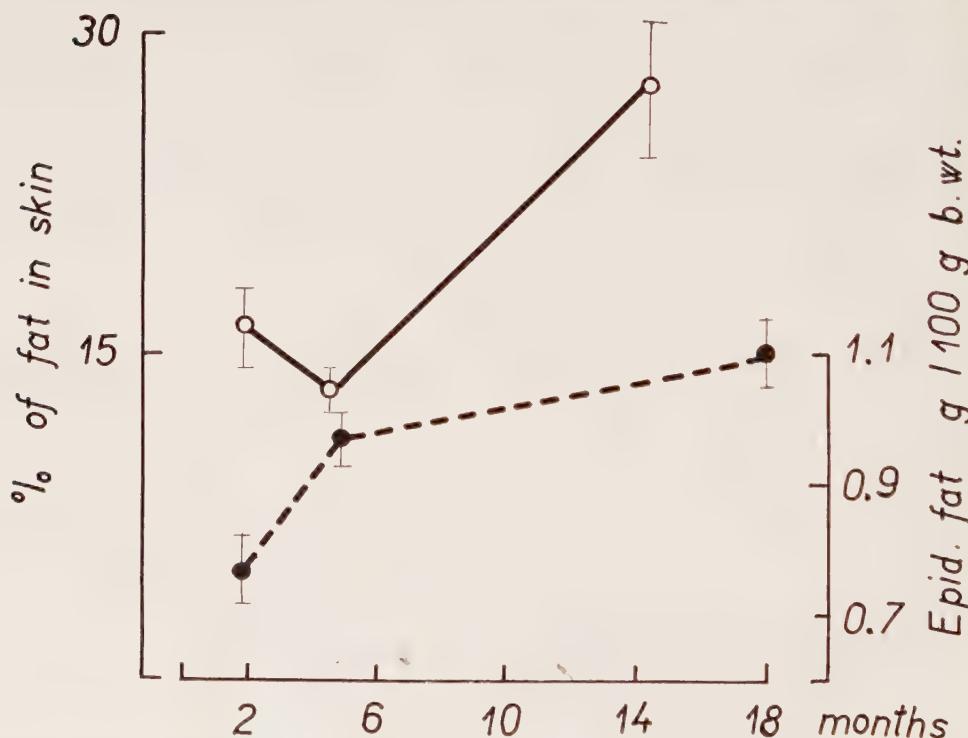


Figure 24-3. Changes of the amount of fat in skin (—) and of the weight of epididymal fat (---) during ontogenesis in rats. Means \pm S.E. as in all other figures (From Hrúza (1967), reproduced by permission of Symposia Soc Exp Biol).

aging is a slowing down of spontaneous activity resulting in a positive energy balance. The early observations of Slonaker (1907) showed that spontaneous activity in rats substantially decreases with aging.

The storage of fat is not necessarily the same in different sites of the body. Fat storage is due to deposition, to changes in the adipose tissue itself, or to the changes in endocrine regulation. Skerlj (1959) reports that in man it is the internal body fat (the fat in and on the organs and in the abdominal cavity) which increases first with age, and that it is far more difficult to influence this fat by diet than the subcutaneous adipose tissue. The subcutaneous adipose tissue also increases but the amount can easily be changed by diet. We have shown on our own rats that fat is deposited at different sites in the body at different rates. Figure 24-3 shows that the amount of epididymal fat rises mainly up to 5 months of age and later only slightly, whereas the increase in the amount of fat in the skin occurs principally after 5 months of age.

The total lipid content of adipose tissue increases with age, whereas the content of other components, such as protein, water and nitrogen, decreases (Placer and Slabochova, 1961; Benjamin, *et al.*, 1961). The free fatty acid (FFA) level in the adipose tissue, however, does not change with age (Altschuler, *et al.*, 1962a, b). The percentage of short-chain fatty acids (C_{10} - C_{16}) is higher in young rats than in the old ones. According to Angerwall (1960) the lipolytic activity of fat tissue in chicks is about 4 times higher than in hens.

The Metabolism of Adipose Tissue During Aging

Benjamin, *et al.* (1961) found that ^{14}C -acetate was incorporated into epididymal fat seven times more rapidly in young rats than in the old ones *in vitro* and so concluded that the synthetic capacity of the adipose tissue decreases with age. This is also supported by the finding that the oxidation of ($1-^{14}C$) glucose compared with the oxidation of ($6-^{14}C$) glucose is significantly less in old animals (Benjamin, *et al.*, 1961). The higher utilization of glucose in the pentose cycle in young rats leads to a higher production of NADPH₂, which is an important factor in lipid biosynthesis. They have also shown that the incorporation of palmitate into triglycerides is higher in young rats, especially when glucose is present in the medium (to provide a source of α -glycerophosphate which is necessary for esterification). The oxidation of palmitate to CO₂ by the epididymal adipose tissue does not change however (Benjamin, *et al.*, 1961). Hagen and Ball (1961) found that oxygen utilization by the adipose tissue decreases with the weight of the animal and, therefore, also with age. These experiments showed a generally decreased metabolism of adipose tissue of older ani-

mals *in vitro*. Similar changes, that is, reduced reactivity of adipose tissue of old rats, were also found during the incubation of adipose tissue with hormones. While studying the influence of insulin on glucose utilization by adipose tissue, Ball, *et al.* (1959) found that CO₂ production decreases in relation to the increasing weight and hence the age of the rat. The activity of lipoprotein lipase in adipose tissue decreases with age also (Chlouverakis, 1965).

Many other studies also indicate a close relationship between the process of aging and changes in fat metabolism. Becker, *et al.* (1950) and Herzenstein, *et al.* (1953) found that postprandial lipemia in elderly men is markedly higher and the time necessary for the return of the lipemia to normal values is many times longer. This was not confirmed by other authors (Grüner and Hilden, 1953). Block, *et al.* (1951) found that the clearing effect of heparin on chylomicronemia is much smaller in atherosclerotic subjects than in the normal ones and in old people compared with young. The same holds for young and old rats (Hruza, 1967). The changes in lipid metabolism during aging are reviewed by Hrachovec (1958), Gellhorn and Benjamin (1965) and Hruza (1967).

Fat Catabolism During Aging

During lipolysis, triglycerides are split into glycerol and FFA. These are partly oxidized, but a greater part of the FFA is reesterified by the liver where the fat content increases. Later, triglycerides are released from the liver to the blood causing lipemia. Fat catabolism can be measured by different techniques at different steps of the lipolytic process. The most common technique for the measurement of lipolysis, under both *in vivo* and *in vitro* conditions, is the estimation of FFA after catabolic stimulation. FFA are estimated in the blood, in adipose tissue or in the incubation medium if lipolysis is being measured *in vitro*. Using these techniques, we have found that fat catabolism decreases during aging. This can be demonstrated when lipolysis is stimulated by stress (Fig. 24-4) or the injection of epinephrine or norepinephrine (Fig. 24-5). This decrease in lipolysis during aging was found under both *in vivo* and *in vitro* conditions (Jelínková and Hruza, 1963; Hruza and Jelínková, 1963; Hruza, *et al.*, 1965; Jelínková and Hruza, 1964). In part, the decrease in fat catabolism during aging can be explained by a decrease in the amount of the fat-catabolizing enzymes in the fat cell (Fig. 24-6).

During aging the fat cell contains more fat and therefore there is less cytoplasm containing the enzymes. Under *in vivo* conditions, there is a further decrease in fat catabolism from early adulthood to old age which cannot be explained on the basis of the morphological change in fat tissue

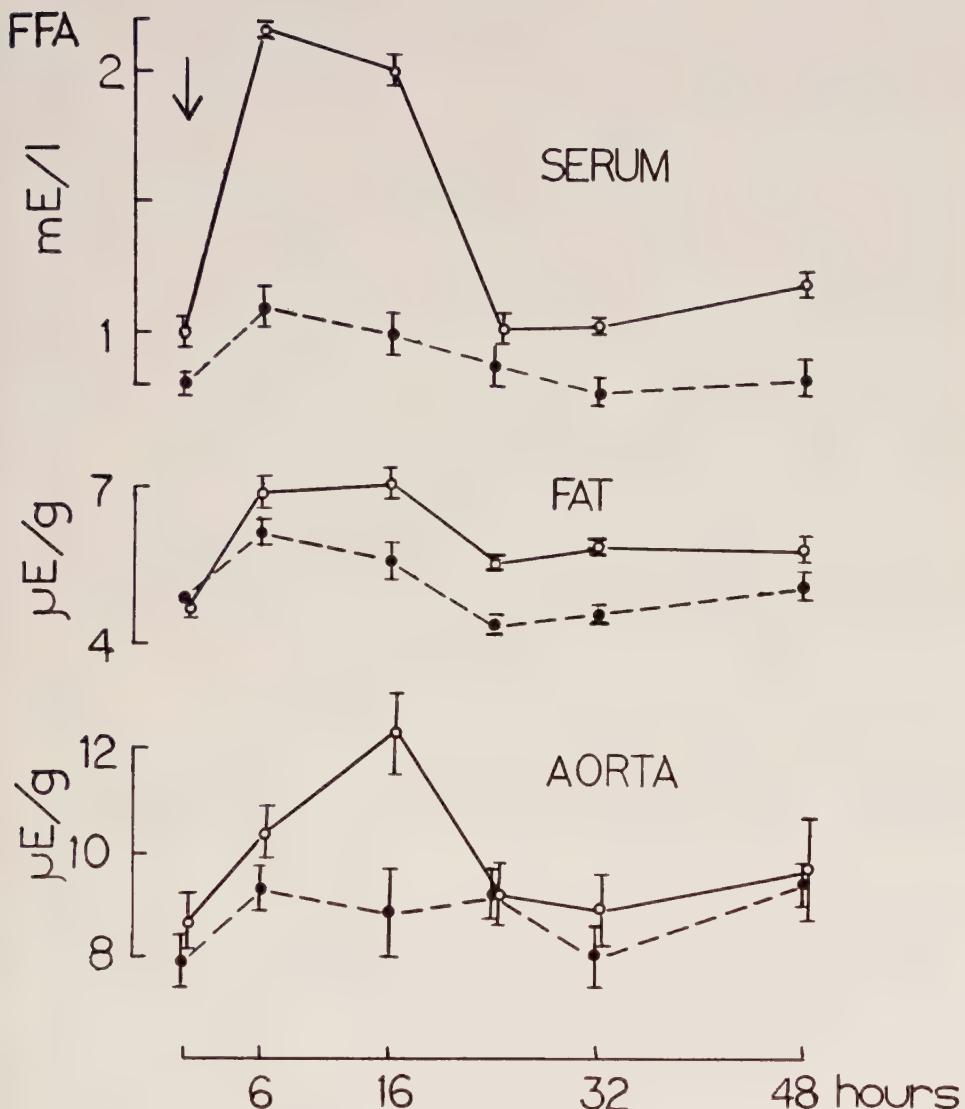


Figure 24-4. Free fatty acids in serum, periovary fat and aortic wall after turpentine abscess in young (o — o) and one-year-old (· · · ·) rats (From Hruza, *et al.* (1965), reproduced by permission of Pergamon Press).

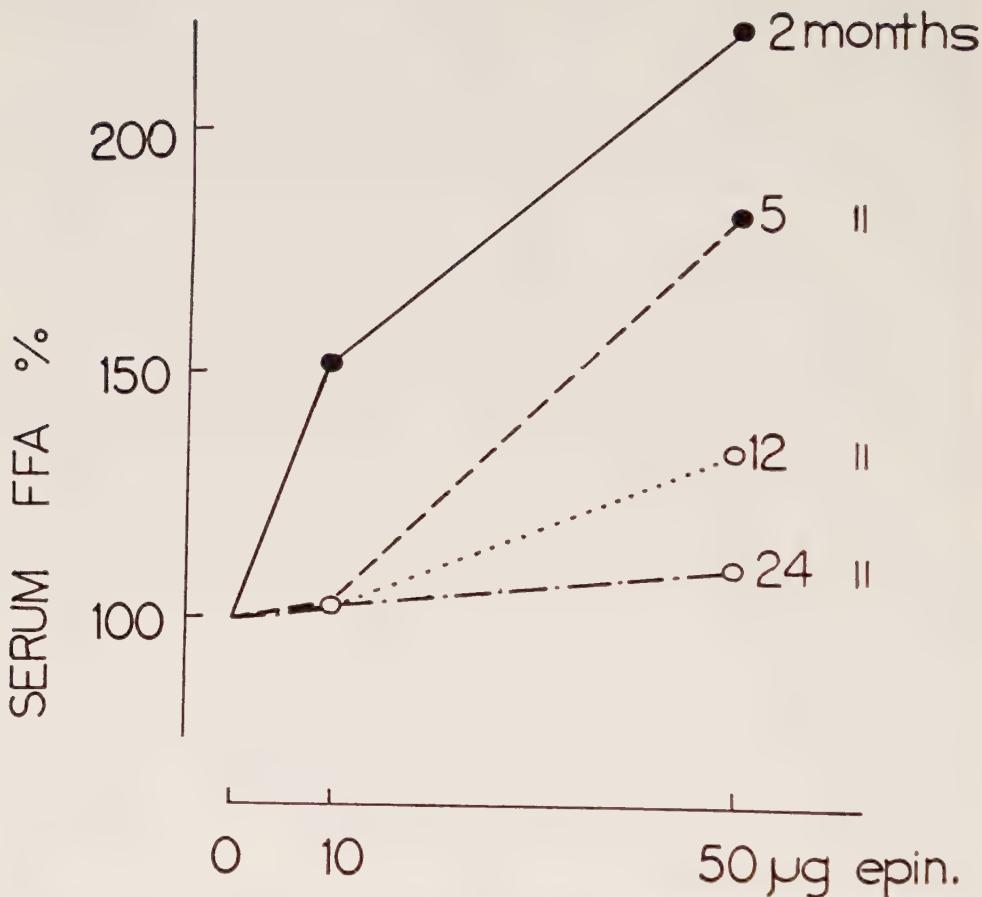


Figure 24-5. Free fatty acids in serum after 10 and 50 μg epinephrine injected intraperitoneally in young, adult, 1- and 2-year-old rats 1 hour after injection (From Jelíneková-Tenorová and Hruža (1963), reproduced by permission of S. Karger, Basel).

alone. This further decrease of fat catabolism is probably caused, at least in part, by the decrease with age in the secretion of sex hormones, which normally stimulate fat catabolism. As can be expected, the decreased responsiveness of fat tissue to catabolic stimulation is evident after the administration of fat-catabolizing hormones of the hypophysis, as will be shown later.

We have also found that a similar decrease with age in the reactivity to fat-catabolizing substances in both adipose tissue and aorta (Fig. 24-4). This may be significant for the development of atherosclerosis in older individuals.

It has been shown in many studies that the blood cholesterol level in-

creases during aging. Although the amount of cholesterol in the arterial wall increases substantially during aging and the relationship between cholesterol deposition and atherosclerosis is obvious, there has been no systematic investigation of the causes of the increased blood cholesterol level during aging. In the rat, we found that the blood cholesterol level also increases with age and that the cholesterol turnover is sharply decreased (Hruza and Wachtlová, 1969). Cholesterol turnover in the aorta and in some other tissues also decreases sharply in older rats (Fig. 24-7). One of the factors responsible for the slower cholesterol turnover in aging is apparently the reduced activity of the thyroid gland since thyroxine administration increases the turnover of cholesterol in old animals. Thyroidectomy decreases cholesterol turnover in young animals but is almost ineffective in the old ones (Hruza, 1971).

In man the decrease in fat catabolism and cholesterol turnover during aging may cause the slower disappearance of fat and cholesterol deposits in old subjects compared with young. In other words, such deposits should be more reversible in younger subjects. Some of the fatty streaks in the aortic wall which are common in 3- to 17-year-old children may disappear because the localization of fatty streaks differs from the prevalent locali-

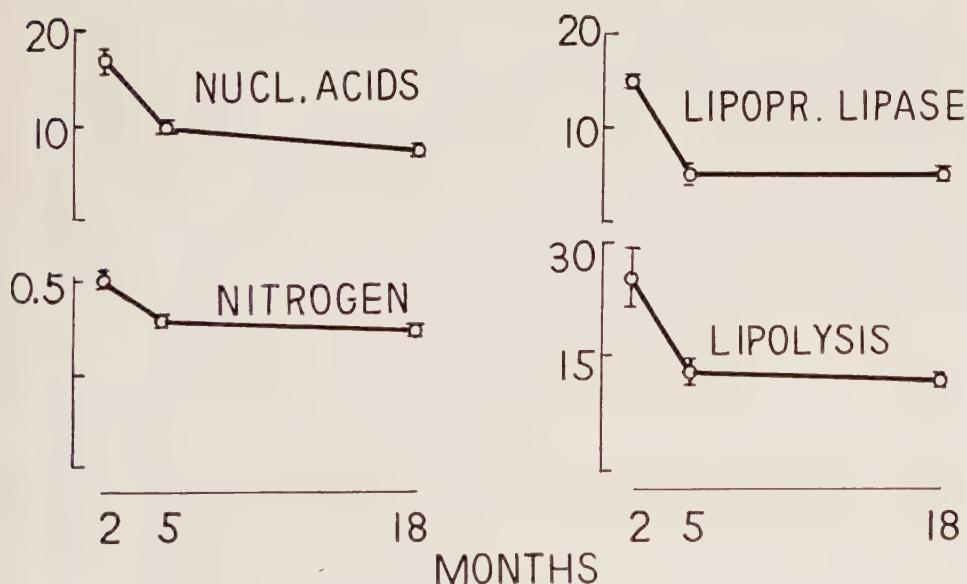


Figure 24-6. Composition of epididymal fat tissue in rats 2 to 18 months old. Nucleic acid P, total N, lipoprotein lipase and lipolytic activity, all related to tissue weight (From our own material, partly published in Jelímková, *et al.*, 1965). Reproduced by permission of *Physiologia Bohemoslovaca*.

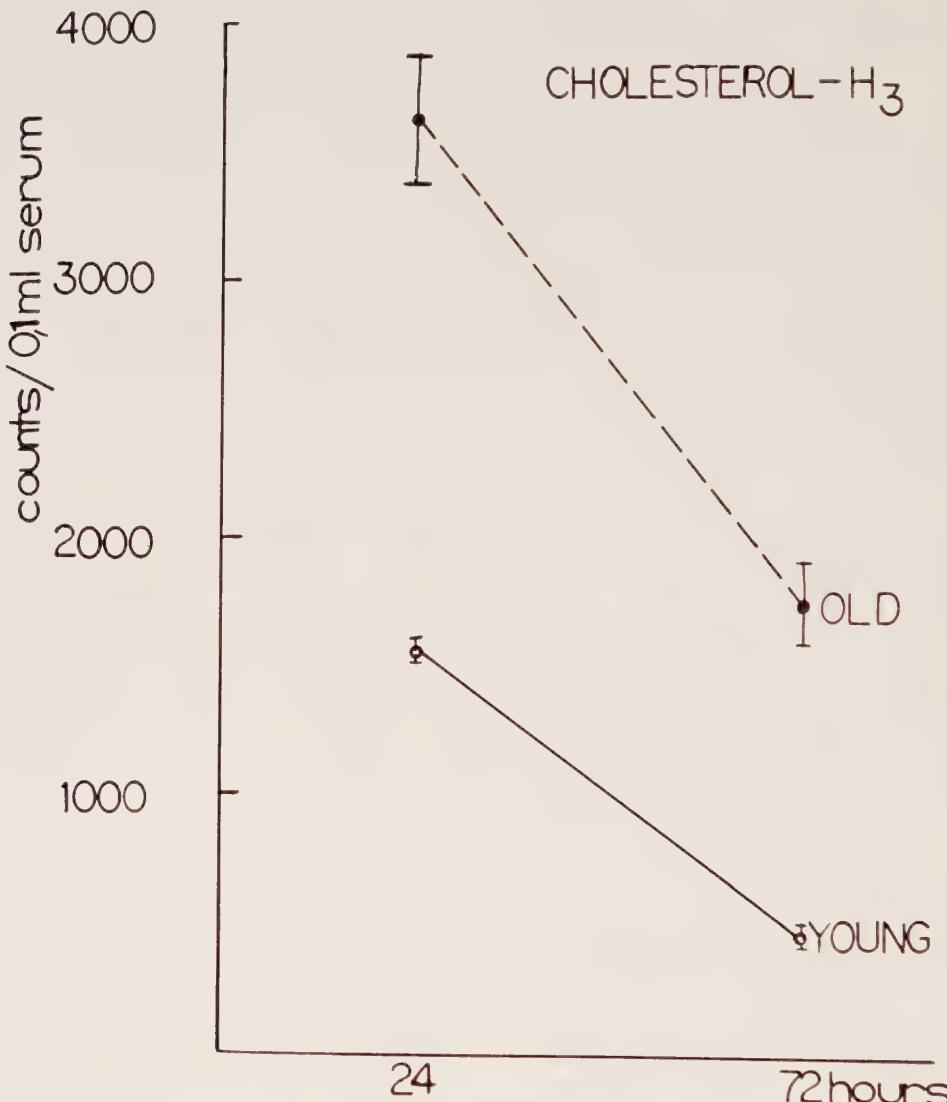


Figure 24-7. Cholesterol- 3H in the serum of young and older male rats, 24 and 72 hours after i.v. injection of the isotope, the same dose according to body weight. Scale: counts/min/0.1 ml serum (From Hruza and Wachtlová (1969), reproduced by permission of Pergamon Press).

zation of aortic plaques. If the streaks do not disappear, an atherosclerotic focus may develop. These factors may be important in explaining the higher incidence of atherosclerosis and obesity in older individuals.

HORMONAL REGULATION OF LIPID METABOLISM WITH PARTICULAR REFERENCE TO THE EFFECT OF THE PITUITARY HORMONES

The synthesis of lipid is stimulated mainly by insulin. The action of insulin is linked with the increased conversion of glucose into fatty acids and a decreased release of FFA from adipose tissue (for review see Jeanrenaud, 1968 or Winegrad, *et al.*, 1965). Estrogens, prolactin F, oxytocin and lysine-vasopressin have a much smaller effect on lipid synthesis. Oxytocin stimulates FFA release in some species. However in dogs, rabbits and women it diminishes FFA release although in men it is without effect (Mirsky, 1965). Lysine-vasopressin has a similar action on lipid synthesis as oxytocin. In pigeons, prolactin F has been shown to increase hepatic lipolysis, with subsequent deposition of fat in adipose tissue (Goodridge and Ball, 1967).

There are many more hormones having a lipolytic activity. Epinephrine, norepinephrine, ACTH, TSH, STH, glucagon and FSH all increase catabolism not only *in vivo*, but also with *in vitro* experiments using intact adipose tissue. These hormones also increase the reesterification of FFA with the exception of STH which increases only lipolysis (for review see Steinberg, 1963).

Nevertheless, the increase of FFA release is greater than the increase of FFA esterification and the net result is an increased catabolism of fat. The catabolic effect of catecholamines, ACTH and other hormones on adipose tissue is mediated by the formation of 3'-5'-adenosine-monophosphate from ATP which activates the lipolytic enzymes. Therefore the decrease in AMP degradation also stimulates lipolysis. The formation of 3'-5'-adenosine-monophosphate after the application of hormones is fast. On the other hand, the action of STH takes several hours, maximum lipolysis is reached after 4 hours. Actinomycin D blocks the effect of STH whereas it is without effect on the lipolytic action of other hormones, as e.g. ACTH (Jeanrenaud, 1968). STH apparently stimulates the synthesis of lipase directly and this takes a longer time (Fain, 1967). Glucocorticoids are needed for the action of STH on lipolysis (Jeanrenaud, 1968). ACTH stimulates lipolysis *in vitro* in mice and rabbits, is less active in rats and is ineffective in monkeys and dogs. Stimulation of lipolysis *in vivo* requires much greater, or pharmacological doses of the hormone.

The melanocyte-stimulating hormone has a lipolytic effect on adipose tissue *in vitro* in the rabbit and guinea pig but not in the rat and dog. Pep-

tide I from the adenohypophysis and peptide II from the neurohypophysis (identical with fraction H of Rudman, *et al.*, 1962) are lipolytic only in rabbit adipose tissue. Fraction L, isolated from sheep pituitary gland, also has a fat mobilizing action. Several fractions of prolactin (C and R) are lipolytic, but fraction F is insulin-like in stimulating lipid synthesis. Arginine-vasopressin is lipolytic (for review of the lipolytic hormones of the hypophysis see Raben, 1965; Astwood, 1965). Synthetic peptides with a similar composition to ACTH also have adipokinetic activity. A lipid-mobilizing substance (LM) which has been isolated from the pituitary, is active in rats and mice (Weil, 1965). The activity of the extract from the human hypophysis containing the lipid-mobilizing factor is depressed by the γ -globulin fraction from the serum of obese people, but not by normal serum (Trygstad and Foss, 1967). Several other peptides with lipid-mobilizing activity have been described. Some of them have been isolated from the hypophysis, others from serum or urine, as for example, the factor described by Beaton, *et al.* (1965), the Chalmers factor and others.

Several hormones do not have a direct, but only a "permissive" action on fat catabolism. The effect of epinephrine is for example increased by glucocorticoids or thyroxine. The same is probably true for testosterone. Castration obesity is well known in many animal species.

The blood cholesterol level increases after the administration of cortisone, ACTH or testosterone and decreases after treatment with estrogens or thyroxine (Katz and Pick, 1967). *Sterolin* from the posterior lobe of the hypophysis increases the blood cholesterol level in dogs, rats and men and *desterolin* has the opposite effect (Wachtel, 1961).

The hypophysis has a predominantly fat-catabolizing action. When hypophysectomized animals are fasted glycogen is lost much more rapidly and fat is lost more slowly. There is considerably less mobilization of FFA from the adipose tissue of hypophysectomized animals and Gibson and Nalbandov (1966) showed that obesity develops in hypophysectomized cockerels even with a reduction of 50 percent in food intake. Hypophysectomized animals accumulate much more cholesterol in the liver and blood when fed a cholesterol-rich diet. The formation of bile acids and their turnover is smaller after hypophysectomy. Hypophysectomized animals are unable to handle an excess of cholesterol (Beher, *et al.*, 1967). On the other hand, hypophysectomy has been reported to arrest the progression of atherosclerosis in severe diabetes (Malinov, 1963).

Fat Catabolism After Repeated Stress

Since the hypophysis is intimately connected with the stress reaction, the effect of stress on fat metabolism will be discussed. Some data in the literature point to a relationship between repeated stress and a greater incidence

of coronary disease. American jet pilots autopsied during the Korean War had a surprisingly high incidence of coronary disease, which used to be very rare at such an early age. The emotional stress associated with flying and overfeeding may be causative factors here. American physicians in general are of the opinion that the emotional situation has a bearing on coronary disease. Russek (1964) reported an increased incidence of coronary disease in professions with emotional stresses. Byers, *et al.* (1962) noted more myocardial infarction in excitable, career-minded people who are constantly under pressure. These people have a reduced blood coagulation time, a higher blood cholesterol level and a higher secretion of catecholamine metabolites. Russek (1964) and Morris (1964) have grouped physicians according to the responsibility and excitability of their practice. Surgeons and general practitioners are the most infarct-prone whilst dentists and dermatologists are the least. Cathey, *et al.* (1962) showed that responsibility, difficult employment and emotional conflicts are significantly correlated with coronary disease. Dock (1959) found atherosclerosis 40 times more frequent in animals in the Philadelphia zoo during a period of overcrowding which appeared to evoke social stress. These animals also showed more rapid coagulability of blood and higher serum cholesterol levels. Gillman and Hathorn (1959) recorded more coronary lesions in female discard-breeders than in nonbreeders. Myocardial infarcts and higher blood cholesterol levels were also found by Sobel, *et al.* (1962) in old dogs after stress or ACTH therapy and similar results have been reported by Chomulo (1964). Epinephrine is one of the mediators of the stress reaction which can cause lesions of the vessels. It is not necessary to use doses of epinephrine which are large enough to cause medio-calcinosis. Much smaller doses of epinephrine will cause lipid deposition in the media (Kellaway, *et al.*, 1963). After epinephrine administration Shimamoto (1963); Lorenzen (1963) and Jagannathan, *et al.* (1964) found edema, fat and mucopolysaccharide deposition and greater sulphate incorporation into vessel walls. Fibroblasts cultivated *in vitro* in serum from stressed animals undergo fatty infiltration (Kask, 1965). Sobel (1962) supposed that the effect of repeated stress on the vessel wall is primarily related to lipid metabolism, that is hypercholesterolemia, lipemia and poor fat tolerance. In earlier experiments, we have shown that repeated traumatization of rats or repeated administration of the mediators of the stress reaction, adrenaline or ACTH, causes a decrease in the reactivity of adipose tissue to the lipolytic action of epinephrine (Hruza, *et al.*, 1963, 1964). We observed a similar situation in man; in old individuals or in young people exposed to repeated stress (jet pilots) lipolysis is less after epinephrine injection than in young male controls (Fig. 24-8). Therefore essentially the same phenomenon, a slowing down of fat catabolism,

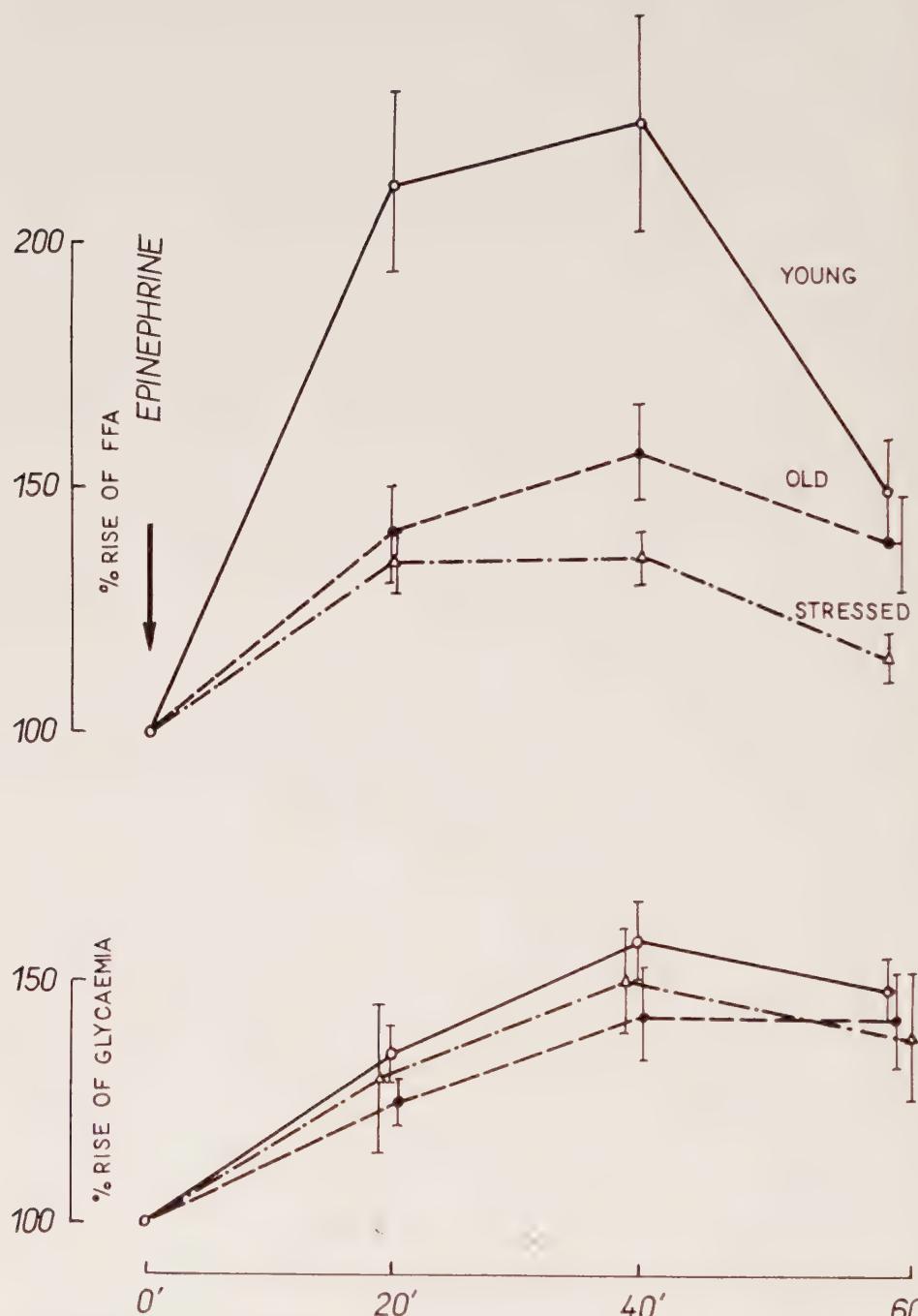


Figure 24-8. Rise of free fatty acids and glucose after injection of epinephrine in young (o—o), old (----) and stressed young men (jet pilots, Δ--Δ) (From Hruza (1967), reproduced by permission of Society for Experimental Biology).

which has been previously found in aging was also found in young animals and people exposed to repeated stress.

HYPOPHYSIS AND LIPID METABOLISM DURING AGING

It has not been clearly shown if the hypophyseal hormones regulating lipid metabolism undergo significant changes during aging. Morphological changes in the aging pituitary gland show cell atrophy and reduced vascularity. Some of the pituitary hormones obviously undergo a period of increased secretion, especially the gonadotropins after the menopause. The blood level of thyrotropic hormone decreases after the menopause (Bottari, 1959). The pituitary hormones have been measured either in the pituitary gland or in the blood. The unchanged level of the hormone in the blood may be, in general, merely an expression of a decreased secretion combined with a decreased destruction as seems to be true for thyroxine in aging (Gregerman, *et al.*, 1962). The unchanged level of the hormone in the gland does not necessarily mean that its secretion does not change because the release from the gland may be different. Also the sensitivity of the target organ to hormones may decrease in aging as may occur with thyroxine and insulin. There are conflicting reports on changes in the blood levels of TSH and ACTH in elderly people and there is perhaps a decline in the STH level in the pituitary (for review see Everitt, Chap. 23; Everitt, 1964; Lockett, Chap. 15 of this monograph). However, some authors, as e.g. Baker, *et al.* (1956) in swine and Bowman (1961) in rats did not find significant differences in the total content of STH in the pituitary between adult and older animals.

An example from our own work shows a decreased sensitivity of the target organ to pituitary hormones with increasing age (Fig. 24-9). In this case, the reactivity of adipose tissue to the lipolytic action of STH was tested *in vivo*. It can be clearly seen, that there is a marked decrease in FFA release after STH in older animals. Changes in FFA were demonstrated in blood but the same changes can be shown in adipose tissue. The decreased sensitivity of adipose tissue to the lipolytic action of STH during aging is essentially the same as for other lipolytic agents described previously, because the properties of the target organ (adipose tissue) change with age. We can reasonably expect that the same would be found if other hypophyseal lipolytic hormones were tested, because most of them appear to act via 3'-5'-adenosine-monophosphate like epinephrine which has been tested thoroughly. Since STH acts in a different manner to epinephrine, that is, via the synthesis of lipase, our results with STH are probably related to the reduced synthesis of RNA and lipase during aging. There are of course some other possible explanations for the decreased sensitivity of adipose tissue to lipolytic agents with aging. In our previous papers, we

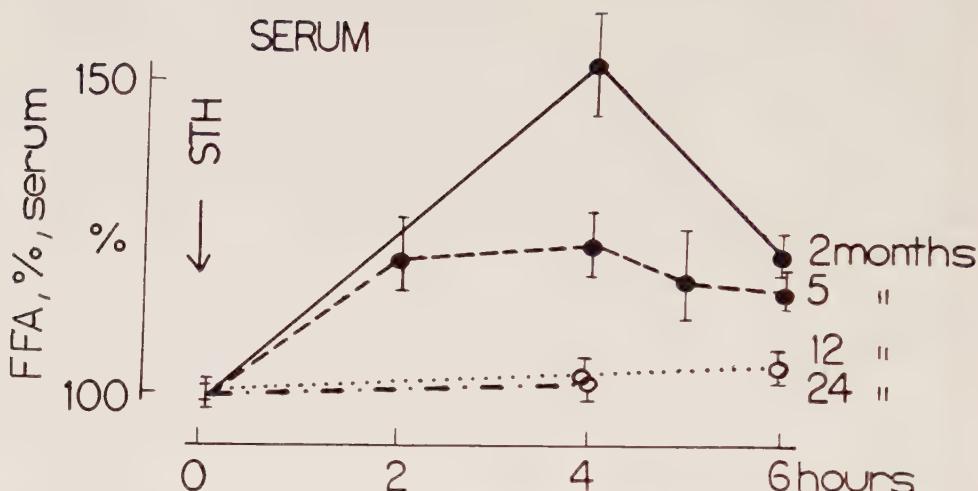


Figure 24-9. Serum fatty acids after the administration of growth hormone to rats of different ages (Jelínková and Hruza, 1964). Reproduced by permission of *Physiologia Bohemoslovaca*.

have excluded the effect of a decreased secretion of thyroxine but we could not exclude the effect of a decreased testosterone secretion, which has a permissive action on lipolysis stimulated by other hormones. Further it is not clear if repeated stress which decreases the reactivity of adipose tissue to lipolysis also contributes to the decreased reactivity of adipose tissue to lipolytic agents during aging and whether this may occur via the accumulation of naturally occurring repeated stresses during the life-span.

The metabolism of cholesterol is regulated mainly by the pituitary and thyroid gland. Except for the hypothetical sterolin and desterolin which were described just by one author (Wachtel, 1961), there are two main hypophyseal hormones speeding up cholesterol metabolism, i.e. thyrotropic hormone and growth hormone (STH). The slowing down of cholesterol catabolism is greater after hypophysectomy than after thyroidectomy (Hruza, 1971) which suggests an action of growth hormone or a hormone, other than thyrotropic hormone. Byers, et al. (1970) found that the hypercholesterolemic effect of thyroidectomy can be abolished by growth hormone alone and speculated that thyroidectomy may cause hypercholesterolemia due to a lack of stimulation of growth hormone secretion by thyroxine. In our own experiments (unpublished) metabolism of cholesterol which was slowed down after hypophysectomy returned to normal after the administration of growth hormone. However, there was not a significant change in the metabolism of cholesterol (i.e. speeding up) after the administration of growth hormone to normal animals. In these animals, on the other hand, thyroxine speeded up the metabolism of chole-

terol markedly. Therefore, the effect of thyroid hormone seems to be more important than the effect of growth hormone.

Old rats handle cholesterol less effectively than the young ones (Hruza and Wachtlová, 1969, Fig. 24-7, Table 24-I). Hypophysectomy slows down cholesterol turnover more in young than in old animals (Table 24-I). The turnover of cholesterol was measured in these experiments by counting the radioactivity of ^3H -cholesterol 3 days after the I.V. injection of ^3H -cholesterol. The higher radioactivity of serum shows that ^3H -cholesterol was either excreted more slowly in the bile or was more slowly exchanged with tissue cholesterol or both. Since the radioactivity of ^3H -cholesterol in the tissues was corrected to different levels of ^3H -cholesterol in the blood, lower radioactivity in the tissues (aorta) shows slower exchange of blood and tissue cholesterol. After hypophysectomy, radioactivity of cholesterol in the blood of young animals increased by 55 percent and in the aorta decreased by 12.5 percent. In old animals, the increase in the radioactivity of blood cholesterol after hypophysectomy was 40 percent, but there was not a significant change in the aorta. This shows that cholesterol turnover is less affected by hypophysectomy in the old animals than in the young ones. The explanation may be that there is a smaller secretion of pituitary hormones affecting cholesterol metabolism in old animals and therefore a smaller change occurs in these animals after hypophysectomy. Another explanation may be that the target organs of old animals are less sensitive to hormones affecting cholesterol metabolism and if the hormones are not present, cholesterol metabolism changes less in old rats. The second expla-

TABLE 24-I
EFFECT OF HYPOPHYSECTOMY ON CHOLESTEROL TURNOVER IN
YOUNG AND OLD RATS

Group	No. of Animals	Serum Radioactivity dpm \pm S.E.	% Change, Significance	Aorta dpm/mg/Serum Counts \pm S.E.	% Change, Significance
1. Young (140 g) controls	9	369.9 \pm 52.2		14.08 \pm 0.86	
2. Young Hypophy- sectomy	13	615.3 \pm 34.5	+ 55% $P_{1,2} < 0.0005$	12.30 \pm 0.58	- 12.5% $P < 0.05$
3. Old (500 g) controls	9	733.7 \pm 40.5		9.14 \pm 0.42	
4. Old Hypophy- sectomy	11	1,026 \pm 106	+ 40% $P_{3,4} < 0.025$ $P_{1,3} < 0.0005$ $P_{2,3} < 0.025$	8.46 \pm 0.46	Nonsignif. $P_{1,3} < 0.0005$

From Z. Hruza "Effect of Endocrine Factors on Cholesterol Turnover in Young and Old Rats," *Experimental Gerontology*, 6:199 (1971).

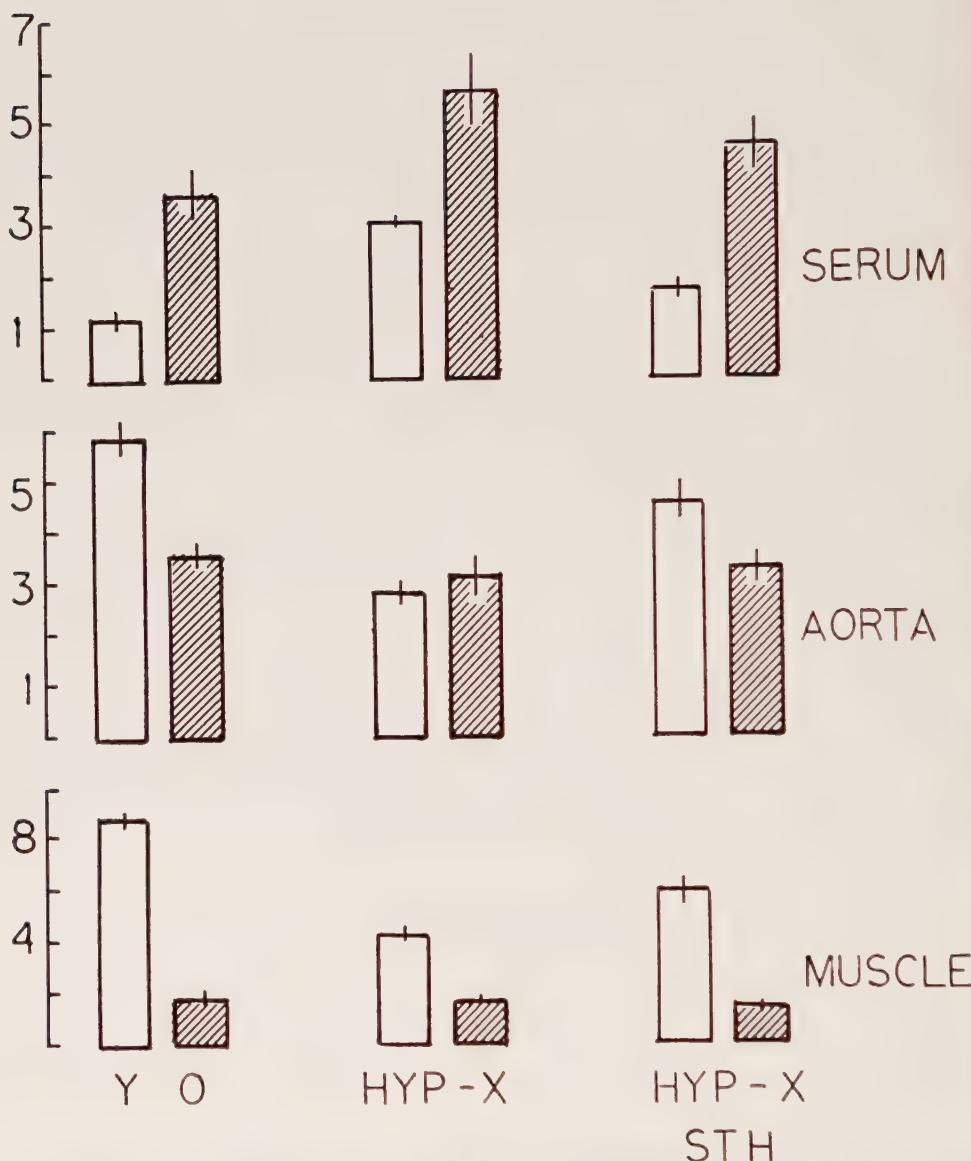


Figure 24-10. ^3H -cholesterol level 72 hrs. after i.v. injection in young (□) and old (■) rats. Means \pm S.E. Controls, hypophysectomized and hypophysectomized animals with application of growth hormone (STH) 0.3 mg/100 g body weight twice a day for 3 days. Upper part: dpm in serum, $\times 10^3$. Middle part: dpm in aorta per mg cholesterol, divided by serum dpm. Lower part: same for muscles (Zbužkova and Hruza, in preparation).

nation is more probable because cholesterol metabolism is speeded up in old animals much less after injection of other hormones affecting cholesterol metabolism, like thyroxine or insulin (Hruza, 1971). A combination of reduced pituitary secretion of growth hormone and thyrotropic hormone and diminished sensitivity of target organs to the pituitary hormones in old animals is the most probable explanation of our results.

Some of our own results showing the effect of growth hormone on the turnover of cholesterol in hypophysectomized young and old rats are presented in Figure 24-10 (Zbuzkova and Hruza, 1974). Growth hormone speeds up the disappearance of ^3H -cholesterol from the blood of hypophysectomized young and old animals (serum radioactivity 3 days after injection increases). This decreased radioactivity of blood after the injection of growth hormone is a result of a combination of a faster excretion of cholesterol and a faster exchange between blood and tissue cholesterol. The incorporation of ^3H -cholesterol into the tissues (aorta and muscles) increases after the administration of growth hormone to hypophysectomized animals but only in the young group. In old animals, the turnover of cholesterol in the tissues is not influenced by growth hormone (Zbuzkova and Hruza, 1974). This points to a reduced sensitivity of the target organs to growth hormone in old animals which is similar to the reduced sensitivity to insulin and thyroxine we described before (Hruza, 1971).

CONCLUSIONS

The decreased secretion of hypophyseal lipolytic hormones which may occur with aging, together with a decreased sensitivity of adipose tissue to these hormones may contribute to the pathological manifestations of aging. These factors may be important in the development and progression of such typical diseases of aging as obesity and atherosclerosis.

Similarly the decreased turnover and decreased excretion of cholesterol during aging is rather like the situation in hypophysectomized animals. The decreased secretion of hypophyseal hormones may partly explain the defective handling of cholesterol in older individuals. The decreased sensitivity of tissue lipids to lipolysis and the slower turnover of tissue cholesterol means that lipid and cholesterol deposits, once laid down will disappear only with difficulty in older individuals. Whereas in the young individual, these deposits may be transitory and more easily removable under the appropriate regime.

All our conclusions are speculative and many links in this hypothesis are still missing. Nevertheless, a relationship between experimental atherosclerosis, repeated stress, aging and the decreased sensitivity of adipose tissue and the aorta to lipolysis and the inefficient handling of cholesterol is apparent.

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CHAPTER 25

DIABETES MELLITUS AND AGING

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SUMMARY

THREE IS A progressive rise in glucose intolerance with increasing age. A review of studies of this aspect of metabolic homeostasis reveals the need for reassessment of the presently used "normal" figures with regard to aging for the diagnosis of diabetes mellitus, and emphasizes the value of the continuing longitudinal studies to assess the true incidence of this condition related to the glucose tolerance pattern with aging.

The etiology of this progressive change now appears most likely to be related to a progressive decrease in insulin release by the pancreatic beta cell with age, but this finding and interpretation is by no means universally accepted at this time.

A number of other possible contributing factors including increased peripheral insulin resistance, progressive caloric deprivation, and changes in growth hormone secretion must be assessed further.

It is considered that the rising incidence of carbohydrate intolerance, and particularly of the diabetic state may of itself increase the rate of aging. However, an objective assessment of the effect of these changes on the rate of physiological aging is presently associated with considerable difficulty.

INTRODUCTION

It has been well demonstrated over the past 50 years that there is a deterioration of glucose tolerance with aging (Crockford, *et al.*, 1966; Metz, *et al.*, 1966; Andres, 1971), but the interpretation of this change has remained difficult. While it is generally accepted that there is an increase in the incidence of diabetes mellitus with aging, the degree of increase has varied in estimate depending on the diagnostic tests used and their subsequent interpretation.

Thus Streeten (1965) reported an "abnormal" oral glucose tolerance test in 77 percent of a series of subjects over 70 years of age. This result emphasizes the need for further appropriate studies to allow the decision as to whether a degree of glucose intolerance at a certain age represents true diabetes mellitus (a pathological state), or an acceptable physiological aging process.

While some increase in the incidence of this common disease might be expected with progressive aging of the β cells of the pancreas, perhaps associated with repeated metabolic stresses (a degenerative phenomenon), yet a gradual increase in blood sugar level with aging (Crockford, *et al.*, 1966) might well be seen as an adaptive phenomenon, similar to the accepted "normal" rise in blood pressure or serum cholesterol reading, and as such not necessarily a pathological change.

In this chapter it is planned to review some of the extensive work on the measurement of glucose tolerance, and to consider the possible etiology behind the reported changes in glucose tolerance with aging, with particular reference to possible pituitary etiology, for example of growth hormone changes with age.

THE INTERPRETATION OF TESTS OF GLUCOSE TOLERANCE WITH AGING

In any discussion of the effect of aging on glucose tolerance, the work done in the area of interpretation of the commonly used tests is of obvious importance.

In this field a prolonged and continuing study by Reuben Andres and colleagues at the Gerontology Research Center, National Institute of Health, Baltimore, has been of great interest. These workers have reported studies of the influence of age on performance in standard oral glucose tolerance tests, the cortisone-glucose tolerance test (Pozefsky, *et al.*, 1965); the intravenous tolbutamide response test (Swerdlow, *et al.*, 1967); the intravenous glucose tolerance test (Andres, 1971); and have devised an original method of assessing the result of the study by comparing with a constructed nomogram the results with those of similarly aged subjects.

The Oral Glucose Tolerance Test

A review of a number of studies of glucose tolerance in apparently normal populations, and in general relying on a mean value to express the results, will quickly show that the effect of age is large and consistent (Andres, 1971).

Thus in the Bedford Survey (Butterfield, 1966) in a community near London, a 50 gram glucose load was given and capillary blood glucose concentration was measured at 1 and 2 hours. The 1 hour levels rose from 125 mg per 100 ml in the 20- to 29-year-old subjects to 194 mg per 100 ml in the 70- to 79-year-old group, an increase of 14 mg per 100 ml per decade. The 2 hour levels were 86 and 121 mg per 100 ml in the two age groups, an increase of 7 mg per 100 ml per decade.

Other English surveys have provided a similar result (Diabetes Survey, 1963; Boyns, *et al.*, 1969), both in the degree of age change, and in the de-

crease in this effect noted between 60 and 120 minutes. This latter effect becomes less pronounced when a larger dose (e.g. 100 grams) of glucose is used initially.

A study on a larger scale was the National Health Survey conducted in the United States from 1960 to 1962. A 50 g glucose load was given to 111,000 people without regard to time of day or time of the previous meal, and a 1 hour venous sample was taken. The mean 1 hour glucose level rose from 100 mg per 100 ml in the 18- to 24-year-age group linearly and progressively to 166 mg per 100 ml in the 75- to 79-year-old age group—again an increase of about 14 mg per 100 ml per decade of life (US National Center, 1964).

A larger glucose load of 100 g was used to study a Michigan community (Hayner, *et al.*, 1965), and again at 1 hour the mean venous blood glucose levels rose linearly from 100 mg per 100 ml at 16 to 19 years to 177 mg per 100 ml at 70 to 79 years, an increase of about 13 mg per 100 ml per decade of life.

In an Australian study of 1,170 subjects in the community of Busselton (Welborn, *et al.*, 1969) a 50 g glucose load was used, and venous blood was collected at 1 hour; mean levels rose from 86 mg per 100 ml in the 21 to 29 year age group to 131 mg per 100 ml in the group over the age of 70, a rise of about 10 mg per 100 ml per decade of life.

A study at the Gerontology Research Center, Baltimore (Andres, 1971) was performed giving a test load of 1.75 gram glucose per kg body weight. Tested under basal conditions the 1 hour blood glucose levels in 313 men rose from 144 mg per 100 ml in the 20 to 29 year age group to 174 mg per 100 ml in the 70 to 79 year age group, a rise of about 6 mg per 100 ml per decade, and a similar rise was demonstrated in this group at 2 hours after the test load (1971). Andres has pertinently noted that the glucose concentrations quoted in these studies were mean values, and that since these mean values for older subjects are approximately equal to what may be considered the upper limits of normality, half the subjects could be called diabetic.

In an attempt to place these results in better perspective Andres and his colleagues (Andres, 1967) have constructed a nomogram from their own data (Fig. 25-1). This allows the determination of a percentile ranking of each subject which takes age into account, and an individual's performance is judged against those of his cohorts. Although this device still does not provide certainty of diagnosis, a simple method has been suggested by the authors in which 2 percent of 20-year-old subjects be classified as abnormal, 3 percent of 30-year-olds, and so forth to 7 percent of 70-year-olds, with an equal number arbitrarily judged to be borderline. This scheme thus allows the percentage of abnormal tests to increase with age,

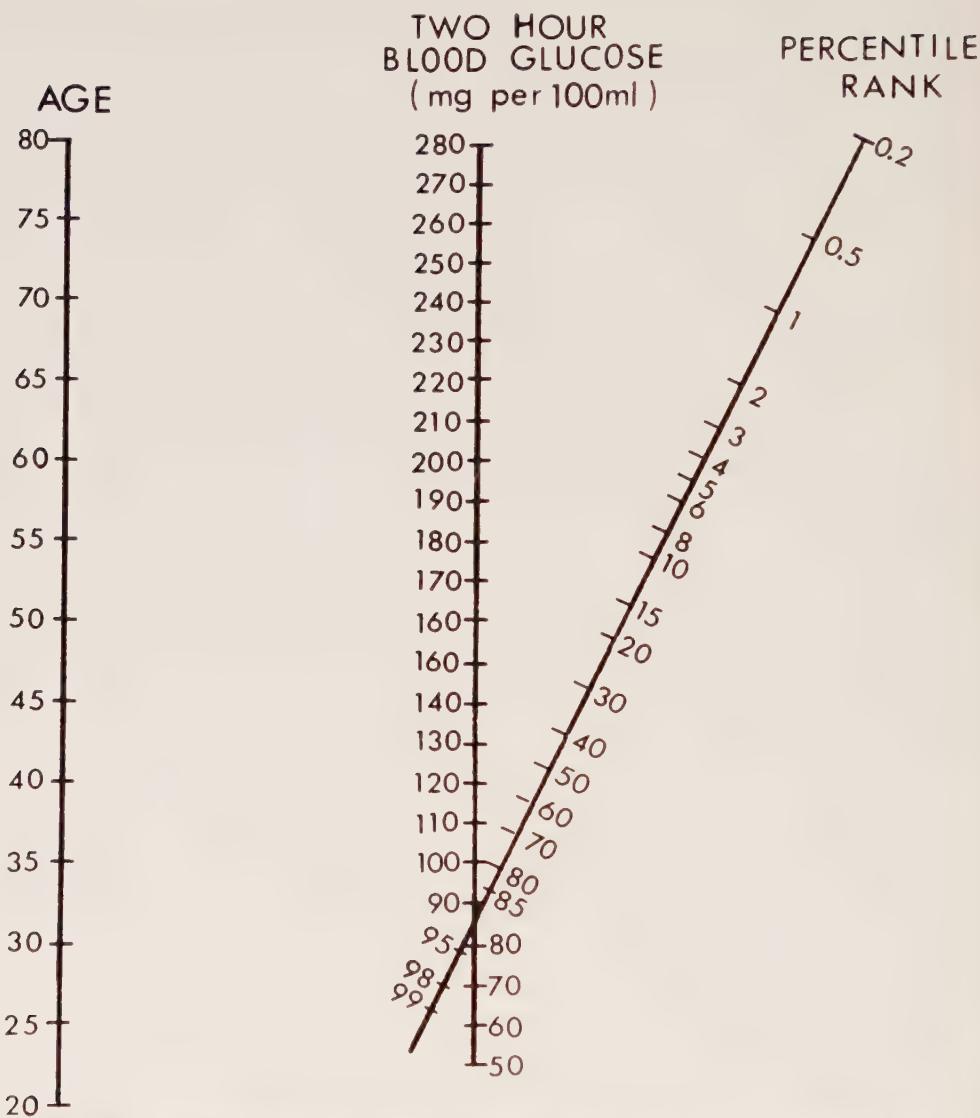


Figure 25-1. Nomogram for judging performance on the oral glucose tolerance test related to age (From Andres (1971a), reproduced with permission of the Medical Clinics of North America).

and defines another group for careful follow-up. The authors state "the implication of this correction for age is that some deterioration of performance with age is permissible" (Andres, 1971).

It should of course be possible to determine the accuracy of this technique at some later time using the information provided by the longitudinal studies being performed by this group of workers. However, for the

moment it would seem to provide a useful and practical approach to interpretation of results of this common and essential investigation.

Future studies of glucose tolerance with aging will probably provide most information if the insulin response to the glucose load is measured, and particularly when these measurements are made at sufficiently frequent intervals to allow the acute and delayed responses of the beta cell to be clearly defined.

The Intravenous Glucose Tolerance Test

Among the other tests of carbohydrate tolerance, probably the intravenous glucose tolerance test has provided the most information with regard to age changes. A number of workers have shown a clear deterioration in the handling of a glucose load administered in this way with progressive age (Silverstone, *et al.*, 1957; Crockford, *et al.*, 1966; Andres and Tobin, 1972). Andres has noted that in general, K values of 1.00 and over have come to be regarded as normal when the glucose dose is 25 gram, and with old subjects in several studies showing a mean K value close to this limit, glucose tolerance in about half of these old subjects would probably be judged abnormal.

Probably one of the most important facts to emerge from these studies is the continued evidence for carbohydrate intolerance when the glucose load is given in this unphysiologic way, thus bypassing the effect of the entero-beta cell axis.

Other Tests for Carbohydrate Intolerance

Although other forms of testing carbohydrate tolerance using the cortisone-glucose tolerance test, or the intravenous tolbutamide response test seem to be used less frequently since the widespread use of insulin measurements, detailed work on these two tests has been published in relation to aging. This work has largely emanated from the Andres group (Pozefsky, *et al.*, 1965; Swerdlow, *et al.*, 1967), and both confirmed the decline of carbohydrate tolerance with aging, and also allowed these authors to further develop the use of their nomograms, referred to earlier, in the interpretation of results.

ETIOLOGY OF AGE CHANGES IN CARBOHYDRATE TOLERANCE

Many different causes have been implicated in the search for the etiology of the gradual deterioration in carbohydrate tolerance with aging, including a diminished or altered insulin response, an elevated or paradoxical hypersecretion of growth hormone, chronic caloric deprivation, and changes in peripheral tissue response to insulin.

Insulin Response to Glucose with Aging

Reports of Impaired Insulin Response

Although conflicting theories have been advanced to explain the primary pathogenetic defect in diabetes mellitus, current evidence favors the concept that a deficiency in the insulin secretory mechanism of the beta cell is the predominant or primary lesion. Therefore a detailed review of studies related to the insulin response with aging is necessary.

Studies on insulin secretory response patterns at different ages still seem to be sufficiently incomplete or inconclusive to allow continued discussion by workers who describe changing glucose tolerance with age with a normal or elevated insulin response by the pancreas (Streeten, *et al.*, 1965; Langs, *et al.*, 1966; Welborn, *et al.*, 1966 and 1969; Chlouverakis, *et al.*, 1967; Dilman, 1971) and those who feel that the reverse is true (Crockford, *et al.*, 1966; Williams, 1968; Joffe, *et al.*, 1969; Felig, 1971; Dudl and Ensinck, 1972).

Much of the earlier work in this field may be altered in the light of recent studies on the importance of the acute insulin response pattern or two pool system. The report by Dudl and Ensinck (1972), one of the first specifically relating the acute insulin response to aging, would seem to support the view that there is a diminished insulin response in aging which may be responsible for the increased incidence of carbohydrate intolerance.

In this study three glucoregulatory hormones were evaluated in relation to body composition in 44 healthy men from 22 to 81 years old. Following a high carbohydrate diet for 3 days, the subjects were tested with an intravenous pulse of glucose (20 g 20 sec) and an infusion of arginine (0.5 g/kg/30 min) on consecutive mornings, and plasma samples obtained. Insulin, growth hormone and pancreatic glucagon were measured by specific radioimmunoassays and percent adiposity was calculated from measurements of whole body K⁴⁰.

Although no difference was present in the subjects' weights, the percent adiposity was significantly increased in the older age group. With advancing age, the basal glucose rose from 80 to 100 mg per 100 ml but basal insulin, pancreatic glucagon and growth hormone were unaltered. After the glucose pulse the log of the glucose disappearance (k_g) decreased from 2.51 to 1.29 with age, and the acute insulin response (AIR) as measured by the percent change in the mean three-to-five minute levels, also declined. Following the arginine infusion the response of insulin and growth hormone decreased with age, while glucagon release remained unchanged.

These workers concluded that since the AIR has been shown to be high-

ly correlated with k_g , the decrease in k_g with age may reflect the diminished AIR in their subjects. They also felt that in the fasting state, the rise of glucose with age and increased percent adiposity may be explained by inappropriately low levels of insulin, elevated levels of pancreatic glucagon, or both, and finally that while beta cell function and growth hormone release appear to decrease with advancing age, alpha cell function is not perceptibly altered.

These findings relating to a diminished insulin response to a glucose stimulus with aging would agree with earlier similar statements by Williams (1968) that the plasma insulin level decreases with aging, and Felig (1971) who commented in a review of the pathophysiology of diabetes mellitus "it is now recognized that glucose tolerance diminishes with increasing age, primarily as a result of a decrease in insulin secretion," and further "the secretory capacity of the beta cell is thus affected not only by genetic factors but by the normal aging process as well."

Similarly in 1969 Joffe, *et al.*, had published a study of insulin reserve in elderly nonobese healthy subjects, which demonstrated a sluggish immunoreactive insulin (IRI) response initially of the elderly group after acute beta islet cell stimulation compared to a control group. These workers found that whereas the young subjects reacted promptly with a rapid outpouring of insulin which reached its peak after about 5 minutes and then fell away, the response in the elderly group was more gradual; however, after 30 minutes the insulin levels in the two groups were not significantly different—unless related to the higher blood glucose levels evoking them in the older group, when even at 30 minutes an impaired response may be shown.

Reports of Normal or Elevated Insulin Response

In contrast to these findings, there have been several reports over the same period of time which have appeared to show that an impaired insulin response is not the cause of the decline in carbohydrate tolerance with aging. Thus in 1966 Langs, *et al.* reported that serum insulin concentration during cortisone glucose tolerance tests remained the same during the first hour of the test in both the old and young subjects, blood glucose levels also remaining similar in the two groups. However blood samples were only collected every 20 minutes in this study. It is also interesting that at 120 minutes they showed a much higher insulin level in the old age group, in the face of clearly higher glucose levels. They therefore concluded that the age-related decrease in glucose tolerance could not be attributed to inadequate insulin release, and that the elevated glucose concentrations despite hyperinsulinemia in older subjects suggested that de-

creased tissue sensitivity to insulin is a factor in the decrease of glucose tolerance with advancing age.

Previously, in 1965, Streeten, *et al.* had measured insulin-like activity of plasma (on the rat epididymal fat pad) during intravenous glucose tolerance tests and found that there was a rise with increasing age; while Welborn, *et al.* (1966) measured serum IRI during oral glucose tolerance tests in 45 people over an age range of 18 to 52 years, and found that there was no difference between the 8 oldest and 8 youngest subjects.

In 1967 Chlouverakis, *et al.* reported measurements of serum IRI, together with serum nonesterified fatty acids (NEFA) during oral glucose tolerance tests in a group of borderline diabetics compared with a control group. They showed an increase in IRI with increasing age in the absence of a notable change in glucose tolerance or serum NEFA, and concluded that older people maintained the same blood-sugar levels as young ones only under the influence of elevated circulating insulin levels.

Finally in a second study by Welborn, *et al.* (1969) serum insulin levels were measured in 1,770 subjects 1 hour after a 50 gram glucose load, and they were able to demonstrate a direct association of increasing age with increasing serum insulin level at 50 years of age and over. However, they also noted that blood sugar levels taken at 1 hour after this glucose load which exceeded 160 mg per 100 ml were associated with declining insulin levels, and indeed they concluded "that a one-hour blood sugar level that exceeds 160 mg per 100 ml indicates insulin deficiency as a general rule in this population sample."

Proinsulin Measurements with Aging

Recent studies directly measuring levels of plasma proinsulin in normal and diabetic subjects have shown that age appears to be a significant factor in influencing immunoreactive proinsulin content in normal subjects. Older normal subjects (40 to 60 years old) had significantly higher levels than the 20- to 40-year-old normal subjects, and also a greater ratio of immunoreactive proinsulin content to total immunoreactive insulin (Duckworth, *et al.*, 1972). The importance of this finding in the etiology of the progressive carbohydrate intolerance with aging is not yet clear, but the possible importance in explaining the finding of hyperinsulinism in some studies of glucose loading with aging, as quoted above, might be significant. Indeed Joffe, *et al.* (1969) had suggested that the hyperinsulinemia may be due to the excessive secretion of biologically inactive proinsulin which is not distinguishable from immunoreactive insulin by techniques used, but which has much less biologic activity than insulin (Kitabchi, 1970).

Conclusion

At present despite these widely varying results in the studies on insulin secretion with aging, the weight of evidence would now seem to support a gradual reduction in the response of the pancreas in insulin secretion to a glucose load with aging. The explanation for the divergent views already reported may include the measurement of proinsulin as already discussed, the use of different methods in assessing insulin levels, and varying groups of subjects.

Aging Changes in the Pancreas

To complement the physiologic and functional studies of the pancreas, and in particular of insulin secretion, morphologic studies have also been reported. Various changes in the pancreas with aging have been reported including regressive histological changes commonly found at necropsy in the pancreatic islets of old people (Robbins, 1962), a progressive decline in mean pancreatic weight with aging (Seige, *et al.*, 1958), degenerative changes in the islet blood-vessels with aging (Houcke, *et al.*, 1964) and an apparent decrease in the beta/alpha cell ratio (Seifert, 1954). This latter finding is interesting in view of the report by Dudl and Ensinck (1972) described earlier in this chapter in which β cell function was found to decrease with advancing age but α cell function was not perceptibly altered.

Thus changes in structure of the pancreas which might be considered to be due to the same causes as underlie those of aging changes in other body tissues, may thus result in functional changes in pancreatic secretion.

Basement Membrane Thickening

In recent years a number of electron microscopic studies have shown that in general capillary basement membranes are thicker in diabetics than in nondiabetics (Siperstein, *et al.*, 1968; Kilo, *et al.*, 1972), and that the capillary bed of most, if not all, tissue is affected to varying degree (Vrakko, 1970).

Siperstein, *et al.* (1968) have suggested from their studies that microvascular disease and insulin deficiency may be independent phenomena, or even that vascular disease is the primary event which in some way causes carbohydrate intolerance. If this were so, any close relationship between increasing basement membrane thickening and aging would be of interest. Although Siperstein, *et al.* failed to show such a relationship, Kilo, *et al.* (1972) in a study of muscle capillary basement membrane in 154 control subjects and 151 diabetics were able to show in both groups significant thickening as an aging phenomenon in both sexes.

Chronic Calorie Deprivation

Other possible causes for the increasing degree of carbohydrate intolerance with aging have been suggested.

Turtle (1969) has suggested that a state of chronic mild caloric deprivation may be present in the elderly person, and an oral glucose load of either 50 or 100 grams representing 200 to 400 K calories, may be greater than the patient would ever encounter in a physiological situation. As the daily caloric intake of aged persons is usually about one third the intake of an average healthy adolescent male, or approximately 1,000 K calories, this may be sufficient caloric deprivation to be responsible for the apparent carbohydrate intolerance. In support for this view Turtle quotes the work of Yalow, *et al.* (1965) who demonstrated the insulin secretion response pattern in the elderly resembles that seen during glucose administration after prolonged fasting.

Tissue Resistance to Insulin

Lack of response to insulin due to relative resistance at the tissue receptor sites could well be another cause for carbohydrate intolerance.

Apart from disturbances in the level of hormones opposing the action of insulin, such as growth hormone in acromegaly or cortisol in Cushing's syndrome, the most likely situation in which this may play a part is in the increasing incidence of obesity with aging. Apart from frank obesity, there is an increase in total fat content of the body in both sexes between the ages of 30 and 70 years (Young, *et al.*, 1963). Certainly obesity results in peripheral resistance to insulin, both endogenous and exogenous (Rabinowitz and Zierler, 1962; Karam, *et al.*, 1963), necessitating augmented insulin secretion (Kipnis, 1970).

Growth Hormone Secretion with Aging

Growth hormone has long been considered a diabetogenic hormone in man, and its influence on glucose homeostasis has been frequently demonstrated both in clinical situations such as acromegaly (Lawrence, *et al.*, 1970), and in experimental situations (Schalch, *et al.*, 1965). Thus a disturbance in growth hormone secretion with aging, and more particularly an elevation of growth hormone release might be a satisfactory explanation for the progressive glucose intolerance with aging. Dilman (1971) has indeed suggested a paradoxical rise in growth hormone after a glucose load in older patients. However other studies of growth hormone secretion with aging have suggested that the response to insulin-induced hypoglycaemia has been normal or diminished (Cartlidge, *et al.*, 1970), and a study by Dudl, *et al.* (1973) demonstrates the preservation of pituitary release of

growth hormone with aging. In this study growth hormone secretion in the elderly was similar to that encountered in younger subjects when evaluated under "basal" conditions following attempted suppression with glucose and after stimulation by arginine. Although there was a tendency to a lowered response to arginine in older subjects, the authors suggest that the concomitant increase in adipose mass may be an explanation for this marginal diminution of growth hormone output. This study would therefore not support the suggestion by Dilman that an elevation of growth hormone secretion is responsible for the progressive glucose intolerance with aging.

Autoimmunity, Aging and Diabetes Mellitus

Finally an attempt to study autoimmunity and aging was made by Whittingham, *et al.*, and in 1971 they reported that if the development of auto-antibodies is an indication of aging (Comfort, 1969) it can be claimed that in certain insulin-dependent diabetics the aging process is advanced by some 20 years over that in controls. Whether this finding may be associated with etiology of the diabetes, or be seen as evidence of the effect of diabetes on hastening aging changes is not clear.

CONCLUSION

The progressive rise in glucose intolerance with aging is now a well established fact. However, the interpretation of the glucose figures may provide a considerable problem and it still remains unclear as to whether this is a normal physiologic process of aging, perhaps as an adaptive phenomenon, or whether it is evidence for an increasing pathological change towards the disease state of diabetes mellitus. This problem should finally be solved by the completion of the longitudinal studies now in progress in several centers, and it would seem likely that a combination of both explanations may be the likely solution to this problem.

Meanwhile the use of revised "normal" figures, or more particularly the use of devices such as the nomogram of Andres, *et al.* (1967) will provide the greatest assistance.

Presently the explanation for this decrease in glucose tolerance seems to be primarily due to a progressive reduction in the release of insulin from the beta cell of the pancreas. However, other factors may also play a role in producing this effect. A direct disturbance of the hypothalamic-pituitary axis does not presently seem to be responsible directly for this change in glucose homeostasis, but the interrelationship between this metabolic parameter and other metabolic changes affected by the hypothalamic-pituitary axis justifies a continued study of this area.

The possible role of diabetes mellitus in increasing the rate of aging

could also be considered in this context. There is certainly a higher and probably earlier incidence of vascular disease in diabetics, particularly in long standing diabetics and it is possible to see this pathology hastening the aging process (Ipsen, *et al.*, 1969). However, to objectively measure aging, using for example a battery of tests as suggested by Comfort (1969), in a group of normals is surprisingly difficult. Indeed after initiating such a study in a large diabetic clinic, it became increasingly clear to this author that many of the measurements being used, such as blood pressure, serum cholesterol, tests of visual acuity, and of peripheral nervous system, could all be affected specifically by the disease process and would not therefore be a true objective measurement of "physiological" age in man. This immediately raised the problem of methods of measuring the physiological age in people with some pathological process apparent, and suggests that either further parameters may need to be included to replace those thus eliminated, or that all parameters being retained, a false measurement of aging may be obtained.

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CHAPTER 26

THE THYROID GLAND, METABOLIC RATE AND AGING

ARTHUR V. EVERITT

SUMMARY

THYROID HORMONES stimulate metabolic processes, and when administered to rats for long periods, they also accelerate age changes in the kidneys and in the collagen fibers of tail tendon. Nephrotic lesions occur in the kidneys of these rats and more protein is excreted in the urine. When a rat is made to live in a cold environment its thyroid gland secretes more thyroxine which accelerates metabolic processes to increase heat production. Consequently its food intake increases and premature pathological lesions develop in kidneys and other organs which shorten the life of the animal. Similarly, the life-span of the fruit fly *Drosophila* is shortened when its metabolic rate is elevated by continuous exposure to a high environmental temperature. Evidently, above an energy threshold, the excessive energy turnover accelerates the intrinsic process of aging. Increased aging is a by-product of increased metabolism.

INTRODUCTION

Old age has many features in common with hypothyroidism, such as low metabolic rate, cold intolerance, skin dryness, hair sparseness, hypercholesterolemia and increased atherosclerosis (Lorand, 1904; Korenchevsky, 1961; Pittman, 1962). Consequently Lorand (1904) suggested that aging is caused by the failure of the thyroid gland to secrete sufficient thyroid hormone to maintain the youthful level of body function.

This chapter describes the relationship of thyroid function and metabolic rate with aging and longevity. Tsuji and Ogura (1969) and Everitt (1972) have previously reviewed this subject.

AGING OF THE THYROID

The thyroid ages just like other organs of the body. Many investigators have observed atrophic changes in thyroid morphology during old age in man and animals (Andrew and Andrew, 1942; Charipper, *et al.*, 1961; Korenchevsky, 1961; Bourne, 1967; Frolkis, *et al.*, 1973). In old age the thyroid gland decreases in weight. The follicles are atrophic, the epithelium is low or flat, the colloid is hard and the amount of connective tissue increases.

In Man

Age changes in thyroid function are discussed in detail by Hales, *et al.* in Chapter 27. There is clearly a decline in thyroid function with increasing age in man, and this probably reflects a decreased hormone requirement by the aged tissues. Reduced thyroid function is associated with the gradual decrease in basal metabolic rate with age in man.

In Rat

A decline in thyroid function with increasing age has not been conclusively established in the rat. Estimates of thyroid hormone secretion rate (TSR) based on the release of iodine¹³¹ from the thyroid indicate a decreased secretion in the old rat (Verzár and Freyberg, 1956; Wilansky, *et al.*, 1957; Johnson, *et al.*, 1964; De Gasperi, 1965; Oeriu, 1965; Kumaresan and Turner, 1967). However, Grad (1969) claims that the old rat is not hypothyroid because thyroidectomy produces the same reduction in basal metabolic rate in both young and old animals. Furthermore, there is no age difference in the plasma protein bound iodine (Wilansky, *et al.*, 1957). The target tissues of the old rat were found by Grad (1969) and Frolkis, *et al.* (1973) to be more responsive to thyroxine on the basis of increased O₂ consumption, food intake and heart rate. These data were interpreted by Grad to mean that in the old rat thyroid hormone secretion declines but there is not a hypothyroid state because of diminished excretion and/or inactivation of thyroxine. This is essentially the human situation in old age as assessed by Gregerman, *et al.* (1962). However in the rat, the thyroxine turnover study of Gregerman and Crowder (1963) clearly showed a 50 percent increase in thyroxine degradation rate in the old rat of similar age and of the same sex (female) as that used by Grad (1969). The data of Gregerman and Crowder indicate that the thyroxine secretion rate is increased in the old rat. See Denckla (Appendix p. 702).

It is interesting that this observation is in accord with the finding of raised metabolic rates in old rats by many observers (Benedict and MacLeod, 1929; Kleiber, *et al.*, 1956; Ring, *et al.*, 1964). These anomalies may be due to age changes in the rate of conversion, or the proportion of thyroxine converted to triiodothyronine (T₃) in the rat. T₃ is more potent, acts faster and has a shorter half life as compared with thyroxine (Larsen, 1972). More investigation into the metabolism of thyroxine and T₃ in the old rat is required to clarify this point. Frolkis, *et al.* (1973) have observed an intensification of deiodination processes in heart, muscles and liver of old rats compared with young adults.

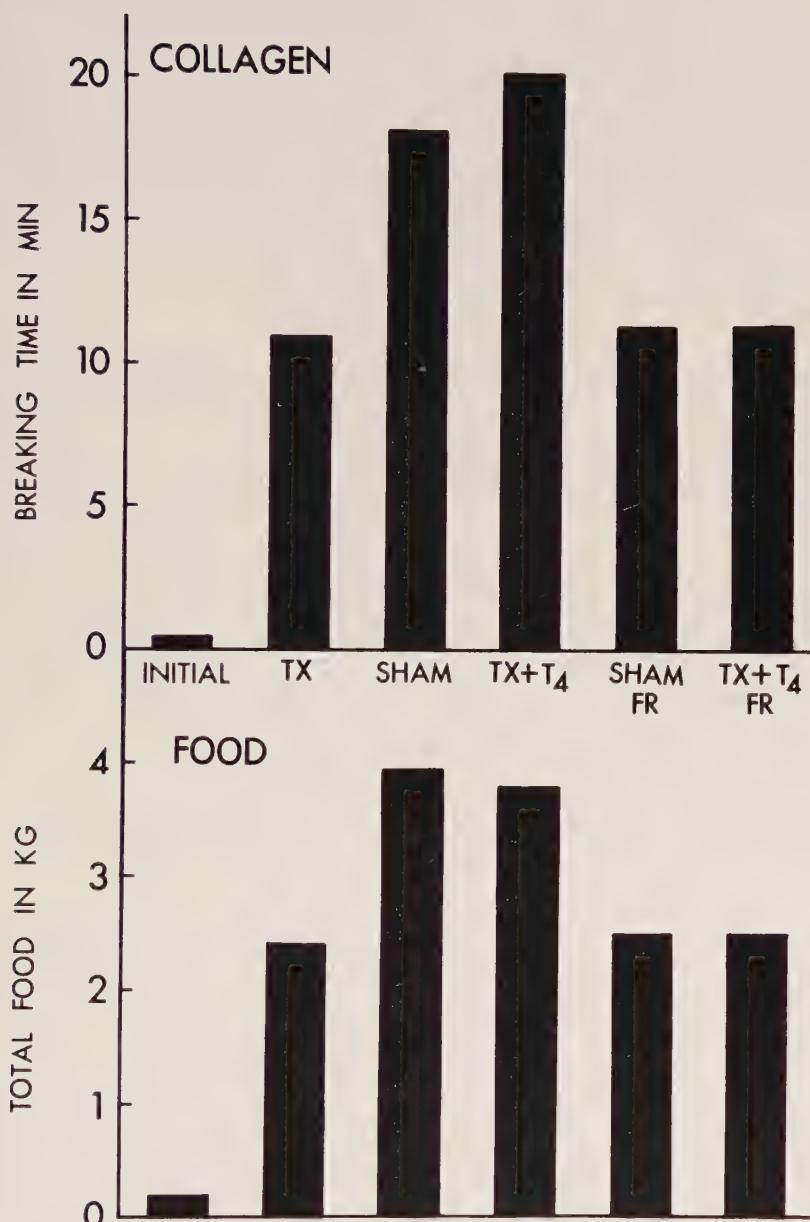


Figure 26-1. The effects of thyroidectomy (TX), sham operation (SHAM), thyroxine (T₄) and food restriction (FR) over a period of 6 months on the biological age of collagen fibers in rat tail tendon. Both thyroidectomy (TX) and food restriction (FR) significantly retarded the aging of collagen. The aging effect of thyroxine is mediated through the food intake. Reproduced from Giles and Everitt (1967) by permission of Gerontologia (S. Karger, Basel).

THYROID HORMONE AND AGING

The role of the thyroid gland in growth and maturation is well known, but the part it plays in the process of aging has not been fully established.

A number of aging processes appear to be accelerated in the hyperthyroid animal. In these animals the vast majority of metabolic processes are turning over at a faster rate than in the euthyroid animal. If age changes have a metabolic basis then aging processes would also be expected to proceed at a faster rate in the hyperthyroid animal.

Collagen Aging

The role of the thyroid gland in the aging of collagen is discussed by Everitt in Chapter 11. The long-term treatment of rats with thyroxine significantly increases the aging of collagen fibers in tail tendon as measured by breaking time in urea (Everitt, *et al.*, 1969). Conversely in Figure 26-1 it can be seen that the aging of collagen is retarded by thyroidectomy (TX) over a period of 6 months and restored to normal by replacement therapy with thyroxine (TX + T4) (Giles and Everitt, 1967). In rats eating the same amount of food neither the absence of thyroxine (TX) nor the presence of thyroxine (SHAM.RF) nor replacement of thyroxine (TX T4 + FR) had any effect on collagen aging. Thus thyroxine does not increase collagen aging directly, but indirectly by mediation of a rise in food intake. Milch (1965) has shown that certain metabolites from the oxidation of foodstuffs are able to cross-link collagen *in vitro*. Whether thyroxine increases collagen aging via increased production of cross-linking metabolites, or some other mechanism, is unknown.

Skeletal Aging

Thyroid hormones increase skeletal growth, maturation (Asling, *et al.*, 1954) and aging (Silberberg, Chap. 12).

Renal Aging

The excretion of protein in urine increases with age in the rat (Everitt, 1958; Berg, 1965; Beauchene, *et al.*, 1970) and is used as an index of renal aging (Everitt, *et al.*, 1966). The quantity of protein excreted corresponds with the frequency and severity of renal lesions in male rats (Berg, 1965).

Renal aging, like collagen aging, is increased by prolonged thyroxine treatment. Berg (1966 and Chap. 3) found that the continuous administration of thyroxine to rats in their drinking water increased the severity of spontaneous nephrotic lesions and greatly augmented the secretion of protein in urine. Thyroxine-treated rats had greater thickening of the basement membranes in the glomerulus. Kidney weight was also higher. Food intake was increased by 50 percent in the thyroxine treated rats.

PROTEINURIA

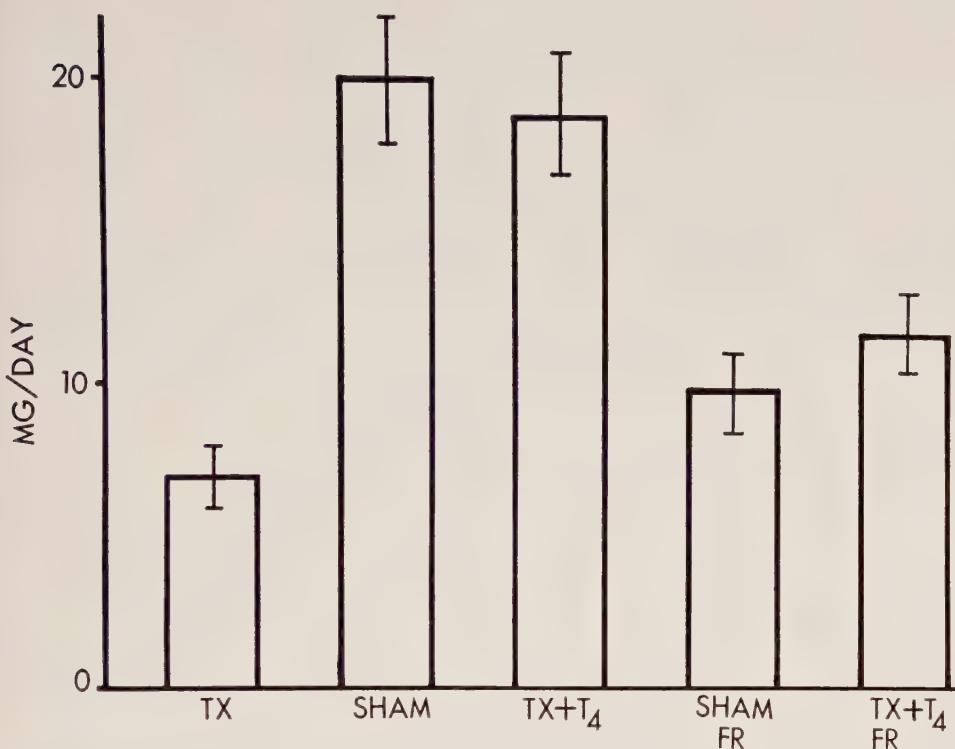


Figure 26-2. The effects over a 6-month period of thyroid function and food intake on renal aging as measured by protein excretion in male rats. The daily excretion of protein was measured in rats which were thyroidectomized (TX), sham operated (SHAM), thyroxine treated (T_4) and food restricted (FR).

In a factorial study Everitt, *et al.* (1966) related the aging effect of thyroxine on the kidney to the quantity of food eaten by the rat. Proteinuria was significantly reduced 6 months after thyroidectomy (TX) in the male rat (Fig. 26-2). Replacement therapy with thyroxine ($\text{TX} + \text{T}_4$) restored the proteinuria to normal. However, thyroxine did not increase the proteinuria significantly when the food intake was held constant ($\text{TX} + \text{T}_4 + \text{FR}$). Significantly increased aging occurred only when the food intake was permitted to rise ($\text{TX} + \text{T}_4$). Thus the aging effect of thyroxine on the kidney is related to food intake.

Cardiovascular Aging

The study of Berg (1966) on rats treated with thyroxine for long periods revealed a significant increase in heart weight, due to left ventricular hypertrophy. This confirmed the earlier work of Korenchevsky, *et al.*

(1950). Thyroxine can induce cardiac hypertrophy also in the mouse (Florini, *et al.*, 1973).

In Berg's experiment (1966) the serum cholesterol level was raised in thyroxine-treated rats. Hypothyroidism, rather than hyperthyroidism (as in Berg's study) is usually associated with hypercholesterolemia and atherosclerosis (Fisher, 1967; Miettinen, 1968; Wren, 1968). Thyroid therapy has been used with some success on human patients with coronary atherosclerosis (Wren, 1968). However, there are quite a number of animal studies in which atherosclerotic changes have been augmented by thyroid hormone and diminished by hypothyroidism (Harman, 1966; review Fisher, 1967). Further, in hyperthyroidism there is an increased sensitivity to adrenaline, which induces arteriosclerosis in rabbits (Kobayashi, 1969; Langner and Fuller, 1973). Thus the relationship between the thyroid and atherosclerosis still needs further investigation.

Cancer

The possible role of the thyroid in neoplastic growth has been discussed by Fisher and Fisher (1966). A considerable number of studies suggest an association between hypothyroidism and mammary cancer in women and rodents, although the literature is confusing. Loeser (1954) employed thyroid hormone for prophylactic treatment of mammary cancer after operation. It was proposed by Pelner (1957) that thyroid hormone may act as an anticarcinogen by inhibiting the pituitary gland. Prolactin is the pituitary hormone most closely linked to breast cancer (Palmer and Maurer, 1972; Skutsch, 1972; Friesen and Hwang, 1973). The secretion of prolactin is increased by thyrotropin releasing hormone (L'Hermite, *et al.*, 1972) and probably inhibited by thyroid hormone (Edwards, *et al.*, 1971; Black and Guillemin, 1973). Thus thyroid hormone could conceivably inhibit neoplastic growth in the breast by reducing the secretion of prolactin.

Histopathology

The pathological effects of prolonged thyroid administration in nontoxic doses to the rat were investigated after 9 months (Lhotka, *et al.*, 1959) and 10 months (McArthur, *et al.*, 1957) of treatment. In neither study from the same institute were there any significant changes, apart from an increase in intimal acid polysaccharides in the large blood vessels (Lhotka, *et al.*, 1959).

Clinical Aging in Hypothyroidism and Hyperthyroidism

If most animal studies suggest that thyroid hormones accelerate aging, how can we account for the premature aging of patients with myxedema as described by Lorand (1904), Taylor (1956), Boyd (1958), Korencheyevsky (1961), Zellman (1968), Tsuji and Ogura (1969). It is observed that

the signs of "aging" disappear after treatment with thyroid hormone even in patients who have been hypothyroid for many years (Boyd, 1958). Clearly the signs of aging in myxedema are not true age changes, which by definition (Strehler, 1959) are irreversible. See appendix p. 702.

Curshman (1929) emphasized that hyperthyroidism may accelerate aging. He drew attention to patients with Graves' disease who became prematurely senescent at the age of 40. This observation is in accord with the rat data which show increased aging in hyperthyroidism.

THYROID HORMONE AND LIFE DURATION

Because thyroxine treatment increases collagen, skeletal and renal aging in the rat it may have a life shortening action.

Rat and Mouse Experiments

Probably the earliest longevity study was that of Robertson (1928) who found that mice fed desiccated thyroid throughout their life were shorter lived than control mice (Fig. 26-3). Thyroid tissue was not toxic because

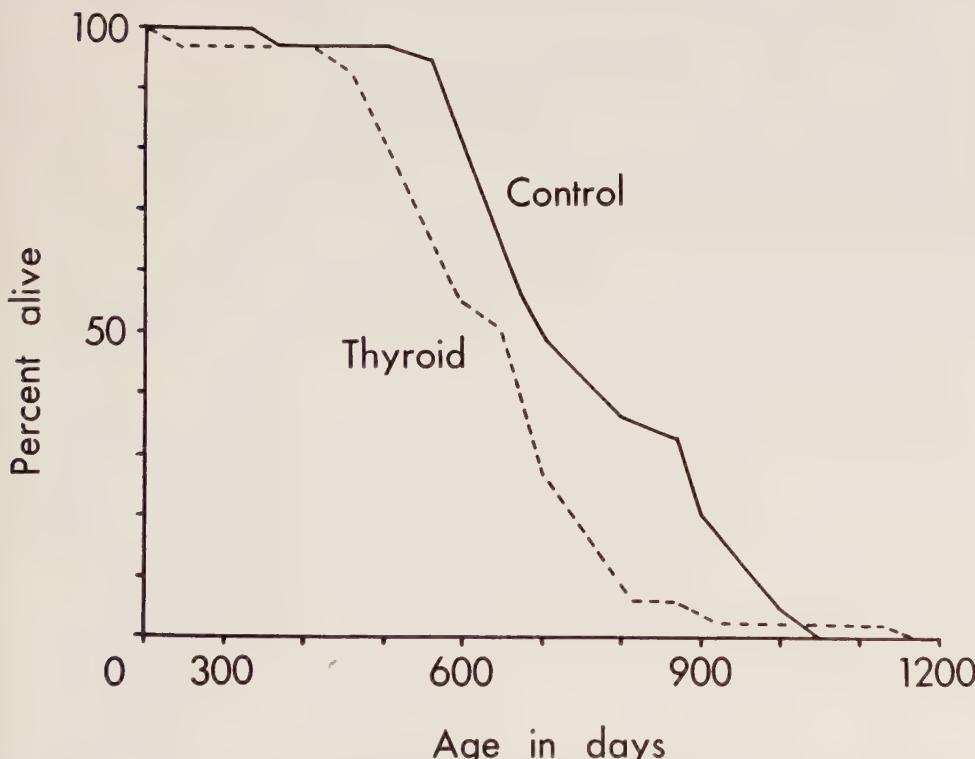


Figure 26-3. The life-shortening effect of thyroid feeding in the male mouse from weaning until death (Reproduced from Robertson (1928) by permission of the Australian Journal of Experimental Biology and Medical Science).

young mice which received thyroid, grew more rapidly than the untreated animals. Our own studies (Everitt, 1959) showed that middle-aged rats treated with thyroxine for 200 days had a reduced life duration, although the difference was not statistically significant. The observed life shortening of 50 days was consistent with the theoretical decrease of 72 days as calculated on the basis of a 36 percent increase in metabolic rate over a 200-day period. Thyroxine-treated rats were calculated to have done 272 days of metabolic work in 200 days, and thus would have their life shortened by 72 days. The period of treatment was too short to demonstrate a significant effect on life shortening. More studies are required to confirm the life-shortening action of nontoxic doses of thyroid hormone.

Longevity Data in Man

Currently available data are not in agreement. Tsuji and Ogura (1969) found that mild hypothyroidism increases life expectancy in a Japanese population. Whereas Ciucă and Jäcovsk (1964) reported that in a goiterous area in Romania, patients with goiter had a mean life duration of 51 years, compared with 61 for those without goiter.

Metabolic Drugs and Longevity

Metabolic rate can be elevated by 2:4 dinitrophenol (Terada and Tainter, 1935) which acts by increasing the secretion of thyroxine (England, *et al.*, 1973). Tainter (1938) studied the effect of long continued administration of various doses of dinitrophenol on growth, food intake and survival of the rat. In a group of 6 rats receiving 0.12 percent dinitrophenol in the diet, food intake was increased, growth inhibited and mean life duration reduced to 391 days as compared with 608 days in the controls. This action is similar to that of thyroxine (Everitt, 1959). Unfortunately group sizes were too small for conclusive results.

Heart rate is an index of metabolic rate (Morhardt and Morhardt, 1971). Both parameters are increased by thyroxine and diminished in hypothyroidism. When the heart rate of mice was halved by digoxin treatment throughout life, males lived 30 percent longer and females 13 percent longer than untreated controls (Coburn, *et al.*, 1971). These studies suggest there is a relationship between metabolic rate and life duration.

METABOLIC RATE AND LIFE DURATION

Pearl (1922) claimed that life duration is inversely proportional to the metabolic rate. Rubner (1908) was probably the first to relate life duration to energy turnover. He showed that small mammals are shorter lived and expend their energy at a faster rate than large mammals which are longer lived. Rubner calculated that the total energy expenditure by mammals during adult life is approximately 200 K calories per gram of body

weight. In other words each gram of tissue can do only a limited amount of work in a life time. In a small mammal like the rat, the 200 K calories per gram are expended quickly and so the program of life processes is completed rapidly resulting in a shorter duration of life. When the metabolic rate of the rat or mouse is increased further by thyroid hormone, the 200 K calories per gram are utilized even more rapidly and the life duration is shortened (Robertson, 1928; Everitt, 1959).

Temperature Experiments on Rats

Thyroxine is essential for the survival of rats exposed to cold (Bauman and Turner, 1967), but if the exposure is prolonged the life-span is shortened (Johnson, *et al.*, 1963). At low temperature more thyroxine is secreted in rats (Gregerman and Crowder, 1963; Johnson, *et al.*, 1964; Bauman and Turner, 1967), and the secretion rate does not decrease with age as in rats housed in a warm environment (Johnson, *et al.*, 1964). Rats maintained in a cool room (9°C) from early life until death in old age have also a higher oxygen consumption, an increased food intake (Kibler, *et al.*, 1963) and a significantly shorter life span (Johnson, *et al.*, 1963) as shown in Figure 26-4. The life-shortening effect of prolonged exposure to

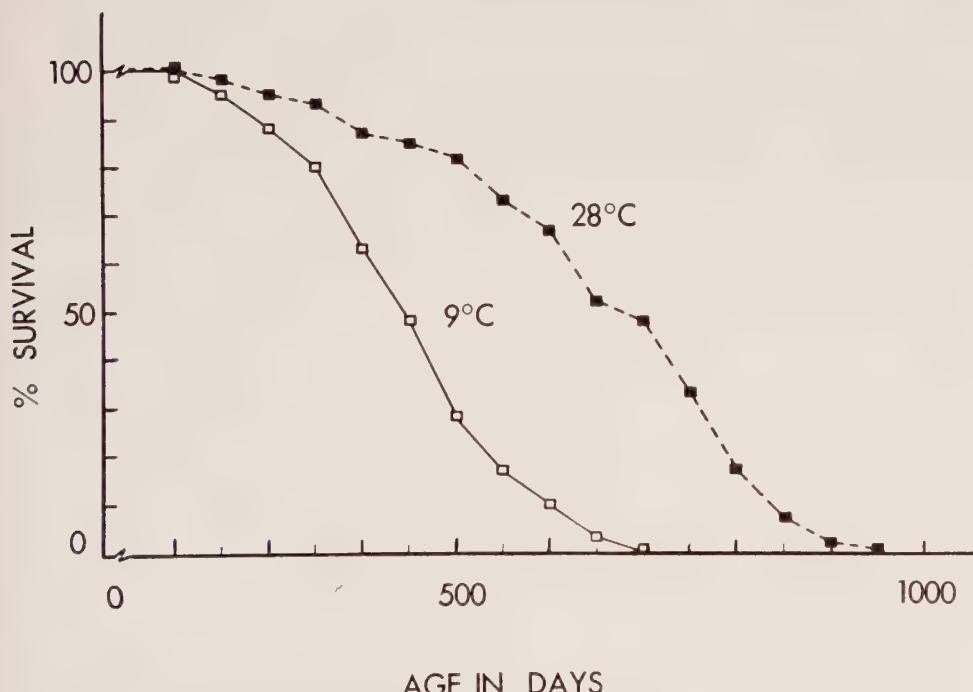


Figure 26-4. The life-shortening effect of exposure to cold (9°C) in the male rat. Control rats were housed at 28°C (Reproduced from Johnson, *et al.* (1963) by permission of the Journal of Gerontology).

cold was also observed by Heroux and Campbell (1960). In these longevity studies certain diseases of old age, such as nephritis and periarteritis, appeared earlier in rats acclimated to low temperature. It is tempting to suggest that the shortening of life reflects an increased "rate of living" as discussed by Kibler and Johnson (1961). Low temperature increases the secretion of many hormones apart from thyroxine. There are increases in the secretion of hypothalamic hormones (e.g. TRH), pituitary hormones (e.g. TSH), corticosterone, catecholamines, etc., and there is a decrease in growth hormone secretion (Eisenberg, *et al.*, 1972). Thus increased thyroxine secretion is only one of the many factors involved.

Prolonged exposure to high environmental temperatures was reported by Mills (1945) to increase life duration in the mouse. An extensive investigation of the effects of high temperature (34°C) on rat longevity was undertaken by Kibler and Johnson (1966). They found that rectal temperature was increased in rats housed at 34°C . However, rats which maintained a low body temperature in the hot environment and reduced their food intake, lived significantly longer than those that did not. On the same food intake, rats at 34°C had a significantly shorter life duration than those maintained at 28°C . Since metabolic rate rises as temperature increases, life duration is reduced as the metabolic rate rises. This confirms the work of Pearl (1922) on *Drosophila*.

A direct effect of temperature on the skin and other exposed organs has been investigated by Hruza and Hlaváčková (1969). These workers showed that the aging of collagen fibers in rat tail tendon increased as the environmental temperature rose.

Temperature and Human Longevity

In man there is no evidence of a detrimental effect of low temperature on longevity. On the contrary the highest life expectancy is actually found in Scandinavian countries which experience relatively severe winters. Sweden has the highest life expectancy for both males and females (United Nations, 1971). These people however, are only minimally exposed to the cold and cannot be compared with rats acclimated to low temperatures. In addition the high standard of public health in Scandinavian countries increases longevity. Reports of "premature senility" in Eskimos (Brown, *et al.*, 1948) are not supported by actuarial data.

Temperature Studies on Bats

Bats are hemipoikilothermic mammals and therefore have poor thermoregulation. In winter the body temperature of bats may fall to 5°C and as a result metabolic processes occur at a slower rate than in summer. Bourl-

TABLE 26-I

SURVIVAL TIMES OF HYBRID ADULT DROSOPHILA MELANOGASTER
REARED AT 25°C AND AGED AT 15°C, 20°C, 25°C, 30°C

Temperature	Survival Time (days \pm S.E.)	
	Male	Female
15°C	161.6 \pm 3.0	170.7 \pm 3.2
20°C	101.6 \pm 1.9	112.0 \pm 2.8
25°C	40.4 \pm 0.9	58.5 \pm 1.3
30°C	21.6 \pm 0.3	29.2 \pm 0.7

Reproduced from Burcombe and Hollingsworth (1970) by permission of Gerontologia (S. Karger, Basel) and the authors.

ière (1958) drew attention to the much greater longevity of bats (7 to 20 years) as compared with rats (3 years) whose body temperature remains high throughout the year and whose body size is similar to bats.

Temperature Experiments in *Drosophila*

Drosophila has been widely used to study the relationship between metabolic rate and life duration. In these poikilothermic animals, in contrast to the mammals, the rate of metabolism rises as the environmental temperature is increased. The classic studies of Loeb and Northrop (1916 and 1917) clearly showed that at high temperatures the life-span of *Drosophila* declined. The effect of temperature on survival is shown in Table 26-I. Pearl (1928) extended these studies and explained the effect of temperature on life duration by means of the "rate of living" theory. Pearl likened the "rate of living" to the rate of a chemical reaction, which increases as the temperature rises. Alternatively, the accelerating effect of temperature may be due to changes in the rates of secretion of hormones controlling growth and development (Burcombe and Hollingsworth, 1970). There is evidence that at high temperature (e.g. 30°C) the "rate of living" theory may fail (Clarke and Maynard Smith, 1961; Lamb, 1968; Hollingsworth, 1970), probably because these temperatures are close to the upper viable limit of the species (Hollingsworth, 1970).

EXERCISE AND LONGEVITY

Physical exercise increases energy consumption and, in the absence of compensatory energy adjustments, should shorten life according to the rate of living theory. Curtis (1968) writes "it is well known that exceedingly hard work, such as that of a Chinese rickshaw runner, is associated with a short life-span." The increased energy consumption during exercise is associated with a raised free thyroxine level in blood and increased thyroxine turnover (Winder and Heninger, 1973).

Rat and Mouse Experiments

The pioneering studies of Slonaker (1912) showed that rats given access to exercise wheels were shorter lived than those not allowed to exercise. Similarly Benedict and Sherman (1937) found that middle aged male rats, not previously exercised, died prematurely when subjected to vigorous daily exercise. A number of these animals could not adjust to the exercise, lost weight and died. From a series of exercise experiments, McCay (1941) concluded that exercise is probably disadvantageous to the sick rat, but may be advantageous to the healthy animal.

There are several studies which suggest that light exercise performed throughout life may be beneficial (Ordy, *et al.*, 1966; Retzlaff, *et al.*, 1966). However, the study of Retzlaff has been criticized because of the unusually short life-spans (controls 476 and 475 days, exercised 605 and 696 days) of the Sprague Dawley rats (Cohen, 1968). Other workers report life durations of 660 to 750 days for nonexercised Sprague Dawley rats (Cohen, 1968).

Edington, *et al.* (1972) have postulated the existence of a "threshold age" above which physical exercise is not beneficial to rats. These workers found that daily exercise in young rats increased their survival but in old rats shortened their life.

Human Longevity

Many investigators have studied the longevity of athletes (review Polednak and Damon, 1970). Studies reporting greater longevity of former sportsmen lack adequate controls. When former college athletes are compared with their less athletic classmates there are no statistically significant differences in life expectancy. Skinner (1968) points out that because "most athletes train for only a short time during their youth, it seems unlikely that there would be any carry-over value 20 to 40 years later with regard to their health or longevity." In these longevity studies it is difficult to compare the total energy consumption of high and low exercise groups over their life time.

There are a number of studies (review Skinner, 1968) which show that occupational physical activity is associated with a reduced incidence of coronary heart disease which is a major life shortening factor. Exercise may reduce cardiac infarction by increasing collateral circulation and the size of coronary arteries. In this respect continued light to moderate physical exercise in middle age must be beneficial in man. On the other hand prolonged heavy exercise is detrimental in middle and old age.

ENERGY THRESHOLD AND AGING

Our studies on rats (Everitt, 1971) indicate that the intrinsic aging process is accelerated when the metabolic rate (or food intake) rises above

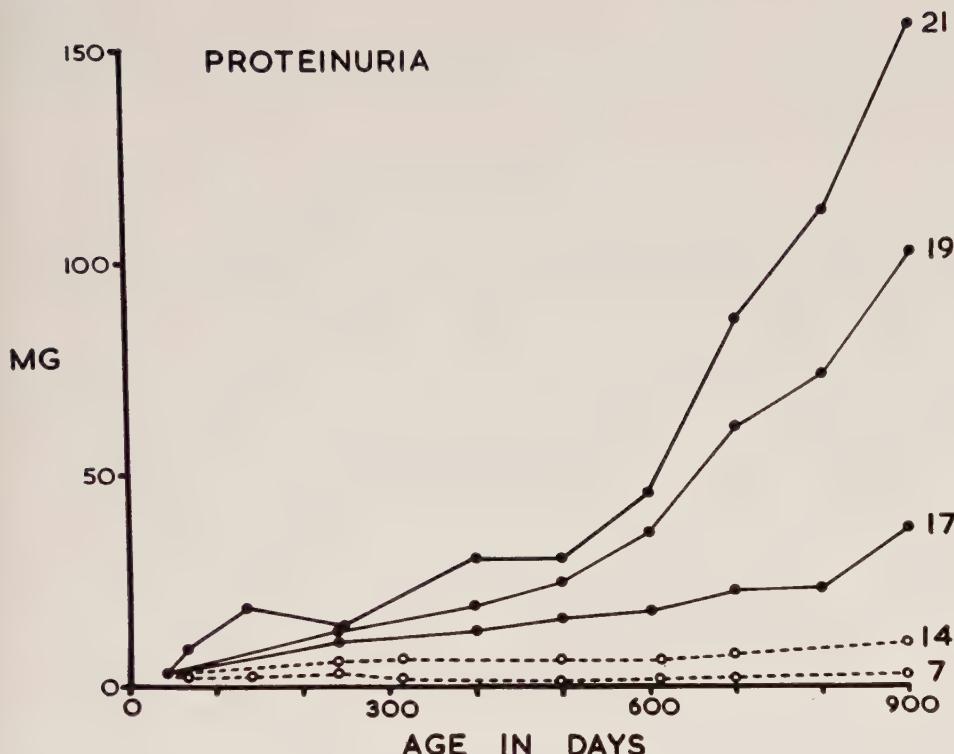


Figure 26-5. The effect of overeating (energy intake above 14 g food per day) in accelerating the aging of the kidney, as measured by protein excretion in urine (Reproduced from Everitt (1970) by permission of Proceedings of Australian Association of Gerontology). Plasma protein levels were not affected by food intake.

a threshold. This threshold corresponds to the food intake of the thyroidectomized rat, which is 14 g per day. A rat fed *ad libitum* normally eats 21 g of food per day. We have shown that the rate of ageing is relatively independent of food intake below this threshold for collagen in tail tendon (Everitt, 1971) and for the renal excretion of protein (Everitt, 1970). It can be seen in Figure 26-5 that proteinuria is affected very little by food intake up to 14 g per day, but above this threshold there is a large increase in proteinuria for a small rise in food intake. Because the food intake of the thyroidectomized rat is 14 g per day (Giles and Everitt, 1967), it is therefore proposed that by increasing food intake and metabolic rate, thyroxine accelerates the intrinsic rate of aging as controlled by the genetic constitution of the animal.

CONCLUSION

The thyroid appears to accelerate the program of life from conception through growth and maturity to senescence and death, just as it stimulates metabolic processes. Increased aging is a by-product of increased metabo-

lism. Harman (1972) believes that the higher the rate of oxygen utilization the greater is the damage to mitochondria. Other cellular components would also be subject to the metabolic damage, which contributes to aging phenomena.

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CHAPTER 27

THE THYROID AND AGE IN MAN

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SUMMARY

THYROID HORMONE is essential for normal mental and physical development. Thyroid disease may be severe enough to prejudice life expectancy. The evidence that so called physiological variation in thyroid function alters life expectancy is based on isolated reports in endemic goiter areas and is not conclusive.

Thyroid function significantly changes with age. Most current laboratory measurements reveal a reduction in all phases of thyroid metabolism. The reason for this reduction is not clear but could, at least in part, be due to a decrease in demands for thyroid hormone by the body. This assumes that the thyroid in the older subject continues to be equally capable of responding normally to TSH. If, however, this does not hold, then the reduced peripheral metabolism may be due to reduced response of either the pituitary or the thyroid.

The clinical manifestations of thyroid disease are unchanged in the elderly but may be overlooked because of other diseases presenting with similar symptoms and signs. The laboratory function tests are still valid in the elderly, but the changes in normal and abnormal values with age may affect the diagnostic interpretation of the test results.

INTRODUCTION

Although there are considerable data relating age and thyroid function in animals (Bellamy, 1967; Giles and Everitt, 1967; Kumaresan and Turner, 1967; Panda and Turner, 1967; Grad, 1969), it is by no means certain that this data can be usefully extrapolated to this problem in man. Therefore this chapter is confined to a discussion of data obtained in man.

The subject falls into three divisions:

1. The effect of thyroid function, normal or abnormal, on the aging process.
2. The correlation between age and thyroid physiology.
3. The effect of age on the type and clinical presentation of thyroid disease.

THE EFFECT OF THYROID FUNCTION ON THE AGING PROCESS

Thyroid hormone is necessary for the normal mental and physical development of man. Probably of most importance is its role in the normal development of the brain and central nervous system (Eayrs, 1960). Thyroid deficiency during pregnancy results in mental and developmental defects. The wide range and severity of such defects is probably dependent on the degree of deficiency and the stage of development of the thyroid in the fetus (McCullagh, 1968; Costa, *et al.*, 1964; Chouffoer, *et al.*, 1965). Apart from the central nervous system the most obvious effect of thyroid hormone is on bone growth, deficiency of thyroid hormone leading to growth retardation in children (Ingbar and Woeber, 1968). The importance of an adequate thyroid hormone level in adults is also stressed by Ingbar and Woeber (1968). Normal sexual maturity and reproduction are dependent on adequate circulating thyroid hormone levels, deficiency frequently resulting in infertility or abortion in females and loss of libido in males. Cardiac failure, central nervous system depression and hypercholesterolemia may occur as a result of thyroid deficiency and may decrease life expectancy. Other pathophysiological disturbances of the thyroid which may affect expectation of life are thyrotoxicosis, with complicating cardiac failure or thyroid crisis and thyroid cancer.

Less information is available about physiological fluctuation and life expectancy and longevity. Reports from Rumania (Cuica and Jucovski, 1964 and 1966) suggest there may be a correlation between minor degrees of thyroid dysfunction and life expectancy. These workers reported that in an endemic area patients with goiter had a mean age at death of 51 years, compared with 61 years for those without goiter.

Conclusions

There can be no doubt that the presence of normal amounts of thyroid hormone is necessary for the normal development and maturation of man. Also there is little doubt that diseases of the thyroid if untreated alter the life expectancy significantly. It would also appear that minor disturbances in thyroid function such as simple goiter may alter life expectancy.

CORRELATION OF THYROID PHYSIOLOGY AND AGE

The physiology of thyroid function is readily studied under the following headings: Its relationship to thyroid stimulating hormone (TSH), iodine kinetics, and circulating hormone and peripheral metabolism.

Thyroid Stimulating Hormone (TSH)**Plasma TSH Levels**

Although bioassays for TSH have been available for some time their use in physiological studies has been largely precluded because of the inability

to measure low normal values (Kirkham, 1966). Koshiyama (1962) reported in Japanese subjects lower TSH levels in older subjects than in younger ones. The plasma TSH level for subjects between 50 and 79 years of age was 0.12 ± 0.03 mU/ml compared with 0.20 ± 0.03 mU/ml for those aged between 10 and 49 years of age. On the other hand Lemarchand-Beraud, *et al.* (1966) using radioimmunoassay reported normal values of 0.21 ± 0.07 mU/ml in children, 0.19 ± 0.03 mU/ml in adults and 0.50 ± 0.13 mU/ml in old age. The increased levels in old age being significantly different from the other two groups ($p < .001$). Mayberry, *et al.* (1971) using radioimmunoassay describe in 307 euthyroid subjects a fall in circulating TSH levels with age with a rise after 60. Lemarchand-Beraud, *et al.* explain the rise in TSH levels with age as due to reduction in cellular metabolism. Mayberry, *et al.* suggest the late rise might be due to incipient myxedema. In the Japanese series protein bound iodine (PBI) estimations were lower in the older age group 4.65 ± 0.26 $\mu\text{g}/100$ ml compared with 5.95 ± 0.22 $\mu\text{g}/100$ ml. These workers also reported slightly lower thyroidal clearance rates in the aged 13.7 ± 3.8 ml/min compared with 14.3 ± 0.7 ml/min. It appears from this data that there is an associated reduction in turnover of radioiodine and of thyroxine production with age.

Although no data on iodine kinetics was included in the paper by Lemarchand-Beraud, *et al.*, it is tempting to explain the difference in TSH blood levels on the amount of iodine available in the diet. The Japanese live on an iodine excess diet whereas the Swiss rely largely on iodized salt for their iodine. It also may be that in older age groups in Switzerland with the advent of various forms of cardiac disease that salt restriction is common. With this introduction of relative iodine deficiency in the elderly the subsequent increased demand for hormone would result in an increase in TSH secretion.

The final answer to these conflicting results awaits the continued improvement of assay techniques and studies of patients at various levels of iodine repletion.

Pituitary TSH Reserve

The ability of the pituitary gland to secrete TSH has in the past been measured indirectly by the anti-thyroid drug rebound test (Friis, 1967; Jensen, 1969). This test depends on the reduction of secretion of thyroid hormone by the drugs, resulting in an appropriate increase in TSH secretion. Various problems have arisen in using this test, it has been suggested (Schneeburg and Kansal, 1966) that this test is largely dependent on the presence or absence of iodine deficiency. Snyder and Utiger (1972a, b) have studied the response of the pituitary to injected Thyrotropin Releasing Hormone (TRH) in normal males and females. They show reducing

maximal secretion of TSH with age in males: 20 to 39 years 14.3 ± 1.7 , 40 to 59 years 9.1 ± 2.1 and 60 to 79 years $6.1 \pm 1.2 \mu\text{U}/\text{ml}$. In females however no such change was shown: 20 to 39 years 16.8 ± 1.6 , 40 to 59 years 16.0 ± 2.2 , 60 to 69 years $16.1 \pm 2.0 \mu\text{U}/\text{ml}$. From the data it would be suggested that TSH reserves fall with age in males but remain constant in females. This is a surprising finding and a final decision on the significance of changes in TSH reserve must await more detailed studies on larger series.

Ability of the Thyroid Gland to Respond to TSH

It has been shown (Einhorn, 1958; McGavack and Seegers, 1959; Baker, *et al.*, 1959), that the thyroid glands of older subjects are as capable of responding to large doses of TSH as are those of younger subjects. The doses of TSH used in these experiments, however, were greater than would

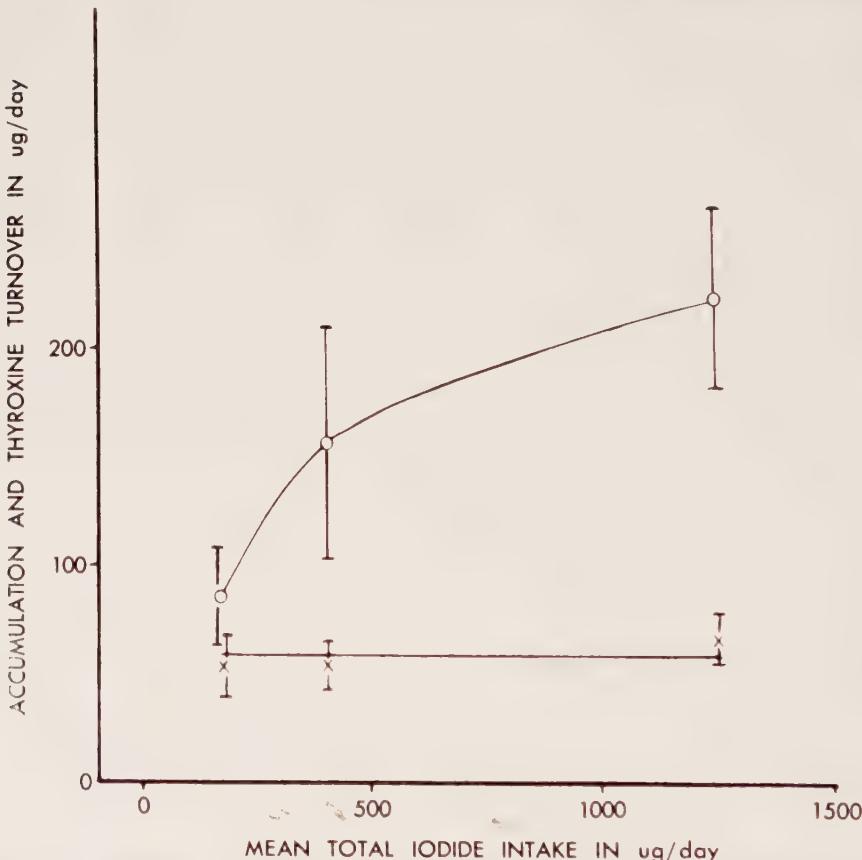


Figure 27-1. The effect of increasing load of iodide on young Sydney residents. a) Lower line - the thyroxine iodine turnover per day is unaltered. b) Top line ° the absolute thyroidal uptake of iodine increases with increasing iodide in the diet (Reprinted from Myhill, *et al.*, 1969, by permission of Acta Endocrinologica [Kbh]).

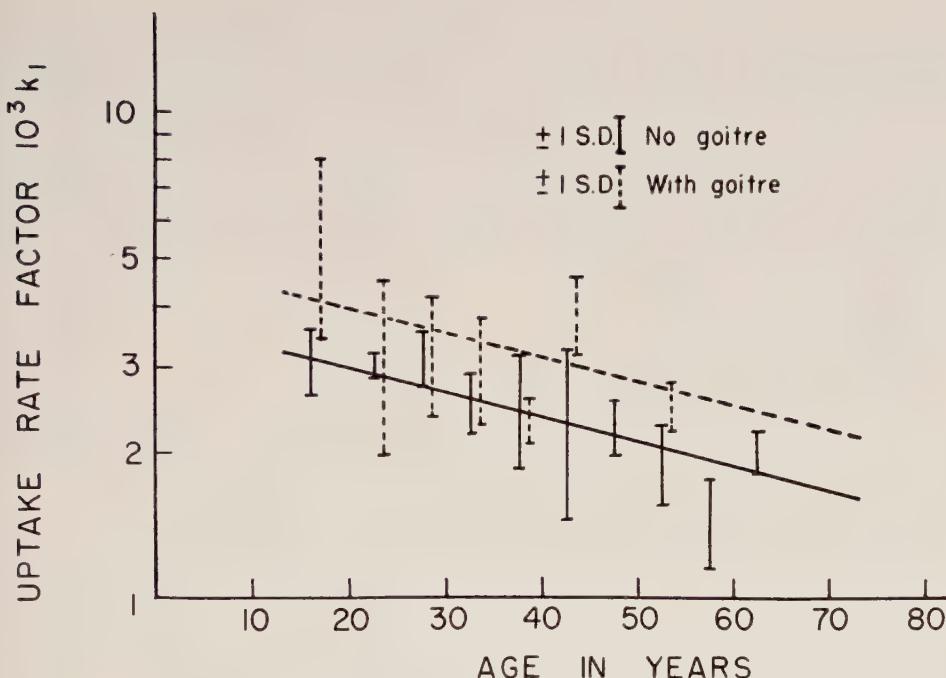


Figure 27-2. Mean thyroid I^{131} clearance rates in euthyroid males unaffected by medication (Reprinted from Oddie, *et al.* 1960, by permission of the Journal of Clinical Endocrinology).

elicit a maximal response and the data cannot necessarily be used to indicate identical response at physiological levels.

Iodine Kinetics

Iodine kinetics have been studied using radioisotope tracer techniques. The measurements which most directly reflect thyroid function are:

1. Secretion of thyroid hormone
2. Thyroidal radioiodine clearance rate
3. Absolute iodine uptake
4. Percent radioiodine uptake

The diagnostic significance of these tests may need modification in view of the effect of age.

Thyroxine Secretion Rates

Koshiyama (1962) shows a significantly lower thyroxine secretion rate in older subjects, $40.8 \pm 6.3 \mu\text{g}/\text{day}$ for those aged between 50 and 79 years of age compared with $112 \pm 10.9 \mu\text{g}/\text{day}$ for those aged between 10 and 49 years. The value in young adult Japanese is higher than those reported by

Fisher and Oddie (1964), who show mean values of $53 \pm 19 \mu\text{g}/\text{day}$ for young American adults and these values did not increase even when iodine intake was increased up to $1,500 \mu\text{g}$ of iodine per day. This effect of iodine is also borne out in an Australian population where it was shown that thyroxine turnover rates were unaffected by supplementary dose of up to $1,250 \mu\text{g}$ of iodide per day (Myhill, *et al.*, 1969; Fig. 27-1). Consequently iodide intake difference cannot explain the difference in thyroxine secretion rates between the young Japanese adults and those of the young American and Australian population.

Thyroidal Clearance Rates

During childhood the thyroidal clearance rate showed no significant change in an Arkansas population (Fisher, *et al.*, 1964). During adult life in both males and females there was a consistent decrease in thyroidal clearance rate with age (Oddie, *et al.*, 1960 and 1968; Fig. 27-2). These results are in general agreement with the data of Gaffney, *et al.* (1962).

While measuring thyroid clearance rate, renal clearance is also measured. The renal clearance of radioiodine falls with age whether measured as a clearance rate (Oddie, *et al.*, 1966) or as simple excretion of a fraction of the dose (MacGregor and Wagner, 1958). Possible age dependent changes in both renal and thyroid blood flow need to be considered in assessing these results.

Absolute $^{127}\text{Iodide Uptake}$

The absolute iodide uptake will depend on the clearance rate of the thyroid and the amount of iodide available. In normal man, as the dietary iodide is increased (Fig. 27-1; Myhill, *et al.*, 1969), the absolute iodide uptake increases from $86 \mu\text{g}/\text{day}$ on a diet of $170 \mu\text{g}/\text{day}$ to $220 \mu\text{g}/\text{day}$ on $1,250 \mu\text{g}/\text{day}$ intake. Similar results were also found in Arkansas (Fisher, *et al.*, 1965). Therefore in populations of equal iodide repletion the absolute iodide uptake would be expected to be related to age exactly as in thyroidal clearance rate. Fisher and Oddie (1964) were however unable to demonstrate a significant age effect in the Arkansas population. But it can be inferred from the absolute iodide content of the thyroid in Koshiyama's series (1962) that in Japan there is less uptake of iodide in older subjects.

Percent Radioiodine Uptakes

Quimby, *et al.* (1950), reported a higher 24 hour uptake of I^{131} by women than men and also a small step-wise reduction in the 24 hour uptake with age. Einhorn (1958) showed a small difference in 24 hour uptake between subjects 19 to 59 years of age of $43.4\% \pm 0.8\%$, compared with

$39.8\% \pm 0.9\%$ for those aged 60 to 82 years. Tubiana, *et al.* (1958) report 24 hour uptake figures of 50 percent for subjects 10 years of age and of 40 percent at 80 years of age, these results are in general agreement with Quimby's results. Koshiyama (1962) however shows no difference between old and young subjects ($17.3\% \pm 1.5\%$ and $18.5\% \pm 3.6\%$). The inability to show marked decrease in the 24 hour percent uptake of radioiodine is largely explained on the basis of reduced renal clearance of radioiodine with age. The reduced renal clearance results in a greater retained dose of radioiodine in the body. This results in a smaller fall in percent uptake by the thyroid compared with thyroid clearance rate, which is not affected by renal clearance. Any small difference of 24 hour percent uptake is further obscured by increasing iodide intake. These results further emphasize the relative insensitiveness of the 24 hour radioiodine uptake test.

Circulating Hormone

The level of thyroid hormone in plasma will depend on its secretion by the thyroid, its distribution and its utilization.

Thyroid hormone and its analogues are iodine containing compounds, consequently their identification and measurement has been largely dependent on identifying and measuring the iodine content of plasma fractions. It is therefore possible to measure hormonal iodine, to determine its distribution and transport and to study peripheral metabolism.

Protein Bound Iodine

This is a measure mainly of thyroxine iodine and although there have been continuing attempts to improve techniques of measurement to specifically measure thyroxine, in the absence of contamination by organic and inorganic compounds, the PBI remains valid as a measure of circulating thyroid hormone.

In childhood there is a very definite fall in PBI levels from birth to adolescence. Oddie and Fisher (1967), using a PBI measurement have computed thyroxine iodine values which fall from $6.15 \mu\text{g}$ per 100 ml at one month to two years of age, to $5.00 \mu\text{g}$ per 100 ml between 10 and 12 years of age and $5.04 \mu\text{g}$ per 100 ml between 18 and 20 years. Most authors have failed to show significant falls in PBI with advancing age (Gregerman, 1967). However, Koshiyama (1962) shows a significant difference in PBI between subjects aged 10 to 49 years ($5.95 \pm 0.22 \mu\text{g}$ per 100 ml) and those aged 50 to 70 years ($4.65 \pm 0.26 \mu\text{g}$ per 100 ml). These data are further supported by the work of Radcliff, *et al.* (1964) who showed a small but significant difference in PBI between subjects aged 20 to 39 ($5.34 \pm 0.96 \mu\text{g}$ per 100 ml) and those aged over 50 years ($5.09 \pm 0.93 \mu\text{g}$ per 100 ml). The failure of some authors to demonstrate fall of PBI with

age may merely reflect that present techniques for measurement of hormone levels are inadequate.

Composition of Circulating Compounds

It is generally accepted that the majority of the circulating thyroid hormone in health is in the form of thyroxine. However, following the description of triiodothyronine by Gross and Pitt-Rivers (1952-1953), many attempts were made to implicate this as the metabolically active hormone. Further interest in other compounds arose following the work of Block, *et al.* (1960), who described a circulating iodinated compound resembling diiodotyrosine in relatively large amounts, final identification was not made. However, some support for the presence of circulating iodotyrosine in normal subjects came from Dimitriadou, *et al.* (1964) and Row, *et al.* (1966) while others (Farren, *et al.*, 1957; Wellby and Hetzel, 1962) were only able to demonstrate circulating iodotyrosine either after TSH injection or in hyperthyroidism.

It appeared that thyroxine was the major thyroid hormonal factor in the circulation (Pillegi, *et al.*, 1964). However, since specific methods for the measurement of triiodothyronine (T_3) in human serum were developed by Bellabarba, *et al.* (1968) the role of this latter hormone in the circulation has to be re-examined. It is possible that the presence of T_3 in plasma may explain why some patients with low PBI values are not clinically hypothyroid. Snyder and Utiger (1972a) showed in males that the mean total triiodothyronine level in serum fell with age but could not demonstrate a significant difference between females aged 40 to 59 and those aged 60 to 79 (Snyder and Utiger, 1972b). Rubenstein, *et al.* (1973) however showed an overall fall in circulating levels in both males and females with age. Such a fall in circulating levels may indicate a reduction in relative hormone synthesis affecting triiodothyronine more readily than thyroxine or it could represent a reduction in deiodination of thyroxine. It is estimated that 42 percent of circulating triiodothyronine is derived from this source (Singer and Nicoloff, 1972) or it could represent an increase in the utilization rate of triiodothyronine compared with thyroxine with increasing age.

Hormone Transport

It is now accepted that thyroid hormone is carried in the blood on three protein moieties: Thyroxine binding globulin (TBG), pre albumin (TBPA) and albumin. Hollander, *et al.* (1962) showed that normal TBG carried 60 percent, TBPA 30 percent and albumin 10 percent of circulating thyroxine. Lutz and Gregerman (1969) confirm these results at pH 8.6 but find lower values of TBPA at pH 7.4; the reason for the discrepancy

is not known. TBPA seems to play an important role in regulating the levels of free thyroxine, this is supported by the work of Surks and Oppenheimer (1964) showing decreases in TBPA associated with reciprocal rise in plasma free thyroxine. Lutz and Gregerman (1969) further support this by showing a decrease of TBPA and rise in free thyroxine as a result of acute infection. Bellabarba, *et al.* (1968) while studying non-thyroidal illness showed significant decreases in both TBG and TBPA in severe debilitating illnesses (mainly malignant disease). With the fall in these proteins there was a significant rise in free thyroxine levels with an associated decrease in thyroxine half life. However, the effect of age on the thyroid hormone transport system is not well established.

Stabilini, *et al.* (1968) have shown a significantly higher level of pre-albumin in men than in pre-menopausal women, children and newborns. The TBPA levels in postmenopausal women were not significantly different from either premenopausal or pregnant women. The data presented in debilitating disease would certainly make it reasonable to investigate TBPA levels in older age groups.

The level of thyroxine binding proteins can be measured indirectly by

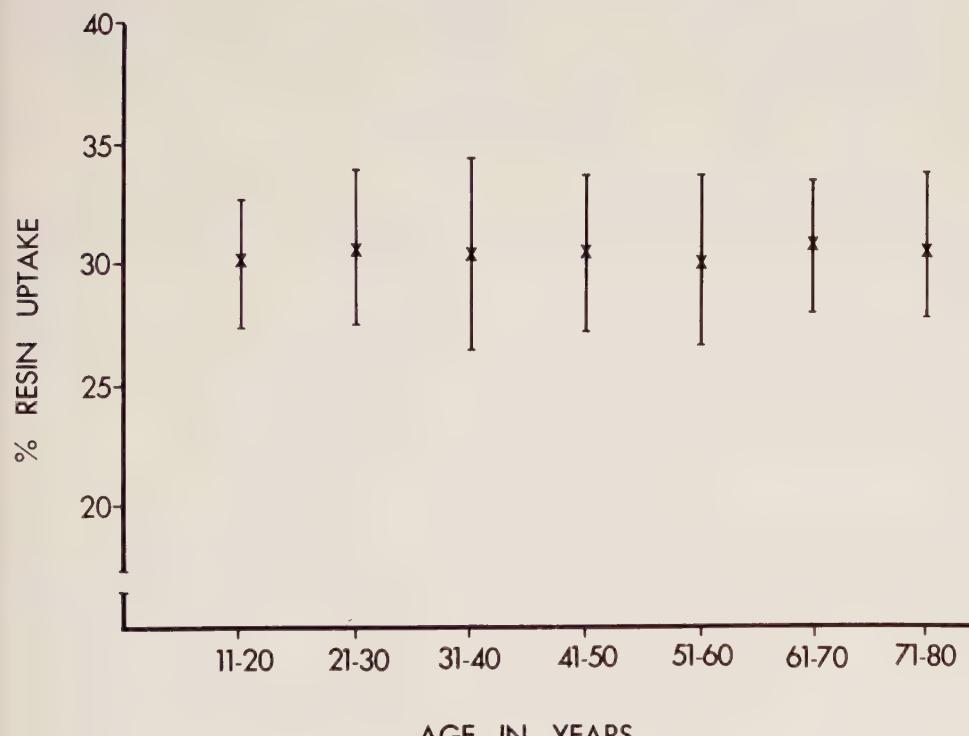


Figure 27-3. T_3 Resin Sponge results using Abbott Triosorb— I^{131} kits. Results show the mean and S.D. of mean for 374 euthyroid females on no affecting medication.

the T₃ resin uptake technique (Mitchell, *et al.*, 1960). As it is well known that testosterone (Dickinson, *et al.*, 1969) and estrogens (Musa, *et al.*, 1967), affect these proteins and as these hormones change with age, measurement of T₃ resin uptake at different ages may show age dependent changes in thyroxine binding proteins. No such change was demonstrated in a series of 374 euthyroid females on no medication (Fig. 27-3).

Metabolism

Basal metabolic rate of oxygen consumption decreases with age. In his review Gregerman (1967) shows that the basal oxygen consumption decreases with body water and that if basal oxygen consumption per liter of body water is calculated, there is in fact no change with age. This may mean, as he says, that there is no actual reduction per cell, but there is still a considerable reduction in total oxygen consumption.

Gregerman, *et al.* (1962) reported an increasing half life of thyroxine with age up to the seventh decade; this was associated with a decrease in distribution space of thyroxine. Inada, *et al.* (1964) showed, in a Japanese population, a thyroxine degradation rate in 70-year-old males of 28.2 ± 3.9 μg per day compared with 60.6 ± 16.7 μg per day for males ranging from 19 to 49 years of age. The same trend is reported by Gregerman, *et al.* (1962), but their absolute values ($88.65 \mu\text{g}$ per day for 20-year-olds and $53.65 \mu\text{g}$ per day for 74-year-olds) are higher than the Japanese data.

Conclusions

It appears that there is a reduction in all phases of thyroid metabolism with increasing age as shown by the usual thyroid function studies. The failure of some reports to confirm this observation may be due to technical problems differentiating small but consistent changes. This decrease in thyroid function with age may merely reflect decreased hormone requirements. Such a conclusion is in agreement with Pittman (1962).

RELATION OF THYROID DISEASE AND AGE

The Thyroid Gland and Goiter

The maximum size of the normal thyroid is probably reached at about 20 years of age, from 50 years on the size decreases (Rice, 1938). In contrast nodular goiter may continue to enlarge into the sixth or seventh decade (Hollis, 1968). Nodular goiters appear to be relatively rare in the first, second and third decades, however, the incidence increases thereafter (Table 27-1). Further evidence for this continuing development of nodular goiter with age is shown by the age of presentation of substernal extension of goiter, particularly those found by mass X-ray surveys (Reeve,

TABLE 27-I
GOITER TYPE ACCORDING TO AGE

Age	Diffuse	Goiter Type		All Goiters
		Multi Nodular	Single Nodules	
0-29	251	23	83	357
30-49	336	101	117	554
50+	49	84	61	194
Total	636	208	261	1,105

Derived from data on a series of 1,105 consecutive proven euthyroid subjects attending the Thyroid Investigation Clinic, The Royal North Shore Hospital. Supported by Research Contract 135/RB, International Atomic Energy Agency.

et al., 1962). The clinical importance of substernal extension with tracheal compression cannot be overstressed, and the very dramatic improvement in respiratory function that results from the removal of these goiters is most rewarding.

Hyperthyroidism

Ngu and Paley (1968) state that the difficulty in diagnosing hyperthyroidism in the elderly is well known. This thought has been taught to generations of students and it is essential to ascertain why the diagnosis should be difficult. Difficulty may arise because:

1. The clinical manifestations of the disease are different in older patients,
2. The clinical picture is masked by associated disease in the elderly or,
3. The usual tests of thyroid function are not appropriate in older patients.

Ngu and Paley (1968) present a series of twelve cases over the age of 65 to indicate the clinical problems involved. One third of their patients presented with cardiovascular symptoms and another third with nervous system symptoms. Eleven of these patients had I^{131} uptakes measured and all were elevated. This small series fails to clarify the reason for difficulty in diagnosing hyperthyroidism in the elderly.

In an attempt to clarify the situation, a series of 136 elderly thyrotoxic patients attending the Thyroid Investigation Clinic at the Royal North Shore Hospital of Sydney, have been studied. Weight loss was the most frequent reason for the patients to seek attention (22%), the next most common reasons were related to symptoms of the cardiovascular system (20%), and those due to the presence of a goiter (15%). These are listed in detail in Table 27-II. It is of interest to see that five patients (4%) had no symptoms attributable to thyroid disease and presented to their physi-

TABLE 27-II
PRESENTING SYMPTOMS^a IN OLDER THYROTOXIC SUBJECTS

<i>Symptoms</i>	60-64	65-69	Age 70-74	75-79	80-84	Total
Weight loss	9	12	7	4	1	33
Goiter ± pressure	8	5	3	5	1	22
SOB ± CCF	2	7	4	2	1	16
palp ± fib	7	3	3	2	—	15
Nerves	7	3	1	2	—	13
Shakes	3	2	1	1	—	7
Fatigue	3	3	—	1	—	7
Eyes	4	1	—	—	—	5
Diarrhoea	1	2	—	1	—	4
Weakness	1	1	2	—	—	4
Headache	2	1	—	—	—	3
Turns-fainting	1	—	—	1	—	2
Heat intolerance	1	—	—	—	—	1
Coincidental	—	2	2	1	—	5

^a The principal reason for the 136 older thyrotoxic patients seeking medical attention. The table shows the number of patients in each group with each symptom.

cian because of unrelated complaints such as upper respiratory tract infection and ulceration of the leg. When all symptoms are analyzed (Table 27-III) weight loss is still the most prominent (65%), followed by those related to the cardiovascular system (49%) and emotional disturbances (40%). Neurological symptoms were the least common group (5%).

Auricular fibrillation was found in 17 patients (12%), the diastolic blood pressure was greater than 90 mm Hg in 11 percent of patients and lower than 70 mm Hg in 15 percent of patients. No goiter was palpable in 18 percent while diffuse and nodular goiter was present in equal numbers

TABLE 27-III
MAIN SYMPTOMS^a IN OLDER THYROTOXIC SUBJECTS

<i>Symptoms</i>	60-64	65-69	Age 70-74	75-79	80+	Total
No. patients	55	42	23	13	3	136
Weight loss	32	30	16	7	1	86
Cardio-vascular	26	22	11	6	1	66
Emotional	27	15	9	3	1	55
Heat intolerance	15	9	8	4	—	36
Lack of energy	12	10	2	1	1	26
Diarrhoea	8	4	2	0	—	14
Neurological	2	2	3	—	—	7

^a The symptomatology as elicited on questioning of the 136 older thyrotoxic patients on presentation to the clinic have been classified under the above headings. The number of patients in each age group with each group of symptoms is shown.

TABLE 27-IV
TYPE OF GOITER IN OLDER THYROTOXIC SUBJECTS

Goiter Type	60-64	65-69	Age		80+	All
			70-74	75-79		
Nil	6	11	5	2	1	25
Diffuse	22	16	11	6	1	56
Nodular	25	15	7	5	—	52
ITG ^a	2	—	—	—	1	3
All	55	42	23	13	3	136

The table shows the number of subjects in age groups with each classification of goiter.

^a Intrathoracic extension of the goiter.

(Table 27-IV) showing a distinct difference from thyrotoxicosis in the young for a Sydney population (Hales, *et al.*, 1969). Eye signs were present in 49 (36%) of whom 13 (9%) had exophthalmos or ophthalmoplegia.

The mean values of I^{131} clearance rate, PBI and T_3 resin uptake were all elevated in this group of patients (Table 27-V).

The frequency with which laboratory tests in elderly patients fall within the generally accepted normal range for patients of all ages is shown in Table 27-VI. It is seen that between 13 and 26 percent of patients in whom tests were performed had results that could be considered normal. Only 38 patients had all three tests performed and of these five had two results that could be classified as normal.

The problem in diagnosing hyperthyroidism in the elderly is therefore due to both clinical and laboratory problems. The clinical manifestations of thyrotoxicosis are not very different in the elderly, however, the main clinical features can often be due to other causes in this age group. Inter-

TABLE 27-V
TESTS OF THYROID FUNCTION IN OLDER THYROTOXIC SUBJECTS

Test	60-64	65-69	Age		80+	All
			70-74	75-79		
$^{131}I^a$ clearance $k_1 \times 10^{-3}$	14.45 ± ^b	13.54 ±	11.06 ±	8.19 ±	16.41 ±	13.01 ±
PBI $\mu\text{g}/100 \text{ ml}$	9.6 ±	14.0 ±	7.4 ±	4.6 ±	12.4 —	10.7 ±
T_3 ^c % resin uptake	12.1 ±	13.0 ±	13.3 ±	11.9 ±	9.3 —	12.6 ±
	3.7 ±	4.0 ±	3.3 ±	3.6 ±	—	3.6 ±
	34.8 ±	43.9 ±	43.7 ±	35.7 ±	—	39.9 ±
	4.7 ±	7.7 ±	8.8 ±	3.2 ±	—	8.0 ±

^a Measured as described by Oddie, *et al.*, 1955.

^b Standard deviation of mean.

^c Abbott sponge.

TABLE 27-VI
FREQUENCY OF NORMAL LABORATORY DATA IN
OLDER THYROTOXIC SUBJECTS

Test	Age					Total	
	60-64	65-69	70-74	75-79	80+		
$k_1 \times 10^{-3}$	No. tests . . .	54	42	23	13	3	135
	No. normal . . .	10	9	4	5	0	28
	% normal . . .	18.5	21.4	17.4	38.5	—	20.7
PBI ^b	No. tests . . .	27	28	18	11	1	85
	No. Normal . . .	5	4	2	0	0	11
	% normal . . .	18.5	14.3	11.1	—	—	12.9
T_3 resin ^c	No. tests . . .	11	11	10	6	—	38
	No. normal . . .	5	—	2	3	—	10
	% normal . . .	45.5	—	20	50	—	26.3

^a Normal range for thyroidal clearance rate $k_1 \cdot 10^{-3}$ in Sydney has been published (Oddie, *et al.*, 1960).

^b Normal range for PBI using alkaline ash technique for Sydney has been published (Radcliff, *et al.*, 1964). Using the autoanalyser the range has been calculated to be from 4.16 to 9.63.

^c T_3 resin uptake was performed using the Abbott Triosorb-131 kit. Normal range calculated on 374 euthyroid females not on any medication was 24—36%.

interpretation of laboratory data is made more difficult by the general trend to have lower values in the elderly and is often further affected by not having available a suppression test (Hales, *et al.*, 1961) to confirm the diagnosis. Suppression tests are frequently excluded in the elderly because of the risk of precipitating cardiac complications in the presence of co-existing coronary artery disease.

Hypothyroidism

The elderly frequently appear hypothyroid because they often have dry faces, loss of hair, slow speech, decreased tolerance to cold, constipation and delayed reflexes. True hypothyroidism, however, appears to be seen more frequently in elderly patients (Ngu and Paley, 1968; Plotz and Friedlander, 1967; Hollis, 1968).

Primary hypothyroidism has been assumed to be due in many cases to Hashimoto's disease (Doniach and Roitt, 1958). Buchanan and Harden (1965) and Hollis (1968) report increasing lymphocytic infiltration of the thyroid with age; one may suspect that this may explain the relative high incidence of hypothyroidism in older patients, however no increasing incidence of thyroid antibodies was shown with age (Hollis, 1968).

Thyroid Cancer

Thyroid cancer is reported in all age groups.

Differentiated carcinoma is seen with a peak incidence in the third and

fourth decades (Burn and Taylor, 1962), but significant instances are seen even in the eighth decade. Follicular and papillary carcinoma have similar distributions, anaplastic carcinoma however, occurs more often in the sixth, seventh and eighth decades. It can, therefore, be said that cancer of older age groups is largely undifferentiated and that anaplastic thyroid cancer is a disease of the elderly (Marchetta and Seki, 1968).

Conclusions

Nodular goiter with its possible complications of tracheal compression or hypothyroidism is more commonly seen in the older age group. Clinical features of both hypothyroidism and hyperthyroidism do not appear to change significantly with increasing age, however, in the older age group these symptoms and signs are often due to other diseases and therefore the diagnosis is overlooked more readily.

The usual laboratory investigations for thyroid states are valid in the elderly providing it is remembered that there is a reduction in gland function with age. Thyroid carcinoma, although not specifically a disease of the aged, is more often of anaplastic type in this age group.

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CHAPTER 28

AGING AND ADRENOCORTICAL FUNCTION

GAIL D. RIEGLE

SUMMARY

AGE-RELATED ALTERATIONS in the body's primary homeostatic control systems, the nervous and endocrine regulators, have for a long time been implicated in the aging syndrome. Studies on the biological aging process, including the role of endocrine factors, have been limited despite the recent surge in basic biological research. At this juncture the role of the endocrines in the aging process is still open for speculation due to the limited research in this area.

Studies concerning changes in adrenocortical function with age have indicated that although there is some evidence for anatomical degenerative changes with increasing age, aging mammals are capable of maintaining normal levels of blood corticosteroids. Our own studies have shown decreased reserve adrenocortical secretory capacity following ACTH or stress stimulation. The most important age-related alteration in adrenocortical function from our work is the decreased sensitivity of the hypothalamic-pituitary adrenocorticotropic control mechanism in the aged rat.

INTRODUCTION

A well-known characteristic of biological aging is a gradual decline in the organism's capacity to maintain homeostasis. The adrenal cortex, an important endocrine component in the control of homeostasis, has been quite logically considered to play a role in the aging process particularly because it is in turn under the regulatory control of the central nervous system. Whether the involvement of the adrenal cortex with age-related changes in structure and function of mammals is the cause or the result of aging phenomena has been the subject of several investigations.

MORPHOLOGIC CHANGES IN THE ADRENAL CORTEX

The concept of age-related changes in adrenocortical function originated with studies by Jackson (1919) on postnatal development of the parenchymal cells of the rat adrenal. Anatomical changes in the adrenal cortex with aging have been studied by several other investigators. Blumenthal (1945) reported a decrease in the number of mitoses in aging guinea pig adrenals. Other studies (Dribben and Wolf, 1947; and Jayne, 1953)

showed changes in the connective tissue structure of the adrenal glands of aged rats.

Das and Magilton (1971) have reported sex differences in adrenal size in aging beagle dogs. Although the adrenal glands of male beagle dogs were smaller after 9.4 years of age, the size of the adrenals of female beagles continued to increase linearly with age and surpassed male dog adrenal weights during senescence.

Several investigators (Reichel, 1968; Balogh, *et al.*, 1970; and Szabo, *et al.*, 1970) have reported marked increases in the accumulation of age pigment apparently of lysosomal origin in the adrenal cortices and anterior pituitaries of aged rats. Jayne (1957) suggested that adrenocortical cellular degeneration might impair adrenal steroidogenesis.

A high incidence of adrenocortical nodules with increasing age has been reported in humans by Dobbie (1969). Dobbie found a high correlation between adrenocortical nodules and hypertension and postulated that arteriopathy was the cause of nodule formation. Dobbie suggested that the decreased steroidogenesis associated with adrenocortical nodules could be due to decreased ACTH availability to adrenocortical tissue. This observation is in agreement with our report of a high incidence of adrenocortical nodules associated with low adrenocortical responsiveness to ACTH stimulation in the aged bull (Riegler and Nellor, 1965).

CHANGES IN ADRENOCORTICAL HORMONES

Several groups have studied patterns of adrenocortical secretions during aging. Early studies indicated that 17-ketosteroid excretion decreases with increasing age (Hamilton and Hamilton, 1948; Pincus, 1955; Romanoff, *et al.*, 1957). Tyler, *et al.* (1955) found that although corticosterone distribution volume and plasma turnover rates were decreased, ACTH responsiveness in terms of plasma 17-hydroxycorticosteroid level and corticosteroid metabolism was not different in geriatric patients compared to young adults. In 1958, Romanoff, *et al.*, reported lower excretory levels of 17-hydroxycorticosteroid metabolites in the urine of young and old women and in old men than that found in the urine of young men. In a subsequent study Romanoff, *et al.* (1961) found that the 24 hr secretion rate of corticosteroids in old men was only 75 percent of that of young men. However, the age-related differences in both of these studies disappeared if the urinary metabolite secretion or cortisol secretion rate was expressed as mg/g creatinine excretion/24 hr.

Samuels (1956) demonstrated an increased biological half-life and decreased apparent distribution volume of cortisol in aged human subjects which would require only about 50 percent as great a cortisol secretory rate to maintain plasma cortisol levels in the aged subjects. West, *et al.* (1961)

found a progressive decrease in the rate of cortisol removal from the circulation but no change in the distribution volume with increasing age in humans which is in agreement with the report of Hess and Riegle (1972) concerning biological half-life and distribution volume of corticosterone in the aged rat.

Moncloa, *et al.* (1963) showed that both 17-hydroxycorticosteroid excretion and adrenocortical responsiveness to multiple levels of ACTH decreased progressively in healthy men ranging from 20 to 85 years of age. In a series of experiments we have shown that although the resting plasma corticosteroid levels are not affected by increasing age in cattle, goats and rats (Riegle and Nellor, 1967; Riegle, *et al.*, 1968; Hess and Riegle, 1970), adrenocortical responsiveness in terms of increased levels of plasma corticosteroids following ACTH or stress stimulation was reduced in aged subjects. In studies in aged dogs, Breznock and McQueen (1970) found only an insignificant decrease in plasma corticosteroids following ACTH stimulation in older dogs and in a recent study on serum corticotropin in aging human subjects, Blichert-Toft (1971) found an insignificant decrease in circulating ACTH and normal diurnal serum ACTH patterns in aged subjects.

Although there is evidence of functional impairment of adrenocortical secretory capacity, biological half-lives and distribution volumes in aged subjects in a variety of species, it is apparent that most aged mammals retain normal resting plasma adrenocortical hormone concentrations, considerable reserve adrenocortical secretory capacity and there is little conclusive evidence that aged mammals are subjected to a chronic deficiency in plasma concentrations of adrenal corticosteroids.

AGED TISSUE RESPONSIVENESS TO ADRENOCORTICAL HORMONES

Recent consideration has been given to age-related differences in target tissue responsiveness to adrenocortical hormones. Singhal (1967) reported almost a three-fold increase in glucose-6-phosphatase and fructose 1,6-di-phosphatase activity following three days of dexamethasone stimulation in one mo compared to 15 mo rats. Adelman, *et al.* (1972) found that although the time required for glucocorticoid initiation of glucokinase, tyrosine aminotransferase and cytochrome c reductase was increased three-fold in 24 mo rats, the capacity of response was not limited in the aged animals. Although Rahman and Peraino (1973) agree with Adelman, *et al.* (1972) that there is a delayed but eventually a maximal response to an enzyme inducer in aged tissue, their work suggests a decrease in the overall amount of enzymes and the degree to which an enzyme can respond to a hormone stimulus in the tissues of aged subjects. Although these studies do not yet provide molecularly definitive bases for aged-related alterations

in tissue responsiveness to adrenocortical hormones, they certainly suggest significant changes with aging in the kinetics of certain enzyme systems regulated in part by adrenocortical hormones.

CHANGES IN ADRENOCORTICAL CONTROL MECHANISM

Although our earlier work suggests some age-related decrease in reserve adrenocortical secretory capacity, these studies did not consider changes in adrenocortical control mechanisms to inhibition by corticosteroid feedback or chronic stimulation such as that which would occur with physical or psychological stress. Our initial experiments regarding changes in adrenocortical secretions with aging considered the effect of chronic ACTH stimulation of the adrenal cortex on adrenocortical responsiveness to stress or acute ACTH stimulation in young and aged rats (Hess and Riegle, 1972). Daily subcutaneous depot injections of 2 U of ACTH 100 g day were made for six weeks. Plasma corticosterone concentration following acute ACTH injection or ether vapor stress stimulation was the index of adrenocortical responsiveness. Adrenocortical response to the direct ACTH stimulation was elevated in all groups receiving the depot-ACTH stimulation. The response was greater in young than in the old rats of each sex. Although chronic depot-ACTH injections resulted in decreased responsiveness to ether vapor stress in young male and female rats, the responsiveness of the aged groups was not reduced. The decreased responsiveness of the young rats to stress suggested that the hypothalamic-pituitary corticotropin control mechanism of the young rats was responding differently to the elevated blood corticoid levels following prolonged adrenocortical activation than that of the old rats.

The sensitivity of the hypothalamic-pituitary corticotropin control mechanism to direct corticosteroid feedback was next studied (Riegle and Hess, 1972). The responsiveness of the adrenocortical control mechanism to stress was measured in young and aged rats following both acute and chronic dexamethasone treatments. Both chronic and acute dexamethasone treatments produced greater adrenocortical inhibition in young than in aged rats. These data again supported the concept of a decrease in the sensitivity of the adrenocortical control mechanism to feedback inhibition in the aged rats.

In another experiment the effect of chronic stress on adrenocortical function was tested in young and aged male and female rats (Riegle, 1973). The rats were subjected twice daily to two hr restraint stress for 20 days. Adrenocortical responsiveness to the restraint or to an ether vapor stress was decreased in all stressed groups. The decrease in responsiveness was greater in the young than in the aged groups. This study suggested that corticosteroid feedback from chronic stress activation of the adrenal cor-

tex results in incomplete inhibition of the adrenocortical control mechanism. The increased inhibition of the adrenals of the young groups supports the concept of decreased adrenocortical control system sensitivity in the aged animal.

CONCLUSIONS

Although there is considerable evidence for anatomical degeneration of the adrenal glands of aged mammals, decreased adrenocortical reserve secretory capacity with age, decreases in distribution volume and increases in biological half-lives of corticosteroids in the body, the adrenocortical system seems capable of maintaining normal levels of blood hormones in the aged. The most important age-related alteration in adrenocortical function is the evidence for decreased sensitivity of the hypothalamic-pituitary corticotropin control mechanism in the aged rat.

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CHAPTER 29

STRESS IN RELATION TO AGING AND DISEASE

HANS SELYE and BEATRIZ TUCHWEBER

SUMMARY

A PARALLEL HAS BEEN DRAWN between aging and the General Adaptation Syndrome (G.A.S.) developing during stress. The entire life-span can be compared to a protracted G.A.S., occurring in response to the ordinary stresses and strains of daily life. This concept is supported by some experiments showing that continuous exposure of animals to stressors (e.g. cold, irradiation) can shorten the life-span and accelerate the onset of many chronic diseases. There are also data suggesting that stress initiates premature physiological aging. However, in comparing the effects of various stressors, it was found that some (e.g. psychological stress, irradiation) influenced longevity while others (toxic substances) were apparently ineffective in this respect. Moreover, not all stressors which shorten life can initiate certain changes considered to be characteristic of physiological aging. Thus, the tensile properties of collagen fibers are not affected by irradiation, although this is brought about by treatment with hormones (e.g. ACTH, cortisol) and by exposure of the animals to psychological stressors or cold. It is difficult at present to explain these discrepancies.

In animals, administration of the hormones produced during stress (ACTH, cortisol, catecholamines) can elicit certain diseases (e.g. hypertension, nephrosclerosis, arteriosclerosis) similar to those encountered in old age. Some of these experimental diseases are more easily produced in old than in young animals and they are frequently enhanced by concurrent exposure to stress.

INTRODUCTION

Throughout life we do not maintain the same level of resistance to stress, injury and disease (Comfort, 1956).

Although life proceeds according to a definite program, which is undoubtedly coded in the genes of the individual somatic cells, the machinery of the body deteriorates at an ever-increasing rate. However, the pathological conditions accompanying old age are not clearly separable from the

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normal process of senescence, but it is certain that people do not die of old age as such, but rather of the diseases incident to it.

The pattern and rate of aging may be modified by altering endocrine balance or environment (Paré, 1965; Everitt, 1966; Bellamy, 1967). In experimental animals, retardation of growth by restriction of dietary intake prolongs the life-span and decreases the incidence of some chronic diseases (McCay, 1952). On the other hand, the rate of aging is increased by many environmental stresses, especially ionizing radiation (Upton, 1960; Curtis, 1967).

The observation that neonatal and senescent organisms do not readily adapt to stress led us (Selye and Pioreschi, 1960) to emphasize the resemblance of the aging process to the adaptation syndrome which develops during stress. Assuming that individuals are born with a fixed quantity of adaptive energy, we theorized that the onset of aging is a function of the rate of adaptive-energy expenditure. This view has been supported by several investigators (Bortz, 1955; Comfort, 1956; Platner, 1961) and has received some experimental verification (Grad and Kral, 1957; Paré, 1965). However, Curtis (1963) showed that at least some stressors do not accelerate the aging process.

Before discussing the relationship between stress, disease and aging, it will be advisable first to briefly review the stress concept.

The Concept of Stress

In 1936, a series of animal experiments showed that the organism responds in a stereotypical manner to such diverse factors as infections, intoxication, trauma, nervous strain, heat, cold, muscular fatigue, and X-ray irradiation. These agents have in common that they place the body in a state of systemic stress but are quite different in their specific actions. It was concluded that the stereotypical response, which is superimposed upon all specific effects, represents the somatic indications of nonspecific stress as such (Selye, 1936). The most outstanding manifestations of the stress response were adrenocortical enlargement with histologic signs of hyperactivity; thymicolumphatic involution (with certain concomitant changes in the blood count such as eosinopenia, lymphopenia, leucocytosis) and gastrointestinal ulcers, often accompanied by other signs of damage or shock.

However, while most of the organs of the body showed involutorial or degenerative changes, the adrenal cortex actually seemed to flourish under stress. This combined response was thought to be a useful part of the systemic, nonspecific adaptive reaction. Its role was visualized as a "call to arms" of the body's defense forces and was named the alarm reaction. Later studies showed that the alarm reaction is but the first stage of a much more prolonged general adaptation syndrome.

The General Adaptation Syndrome

The General Adaptation Syndrome (G.A.S.) follows a triphasic course: alarm reaction, stage of resistance and stage of exhaustion (Selye, 1950).

In animals exposed to a certain stressor (e.g. cold), the alarm reaction develops first and is characterized by loss of weight, adrenal enlargement with diminished lipid storage, gastric ulceration, thymicolympathic involution, etc. Later, the animals seem to adjust to the stressor, in this case, low temperature. During this second stage (stage of resistance), body weight increases, and all physiologic functions seem similar to those of animals living at a normal temperature. If one performs an autopsy at this time, no striking lesions are found, except occasional remnants of thymus involution and adrenal enlargement but the cortex is now rich in lipids. At this point, the observer would have no reason to believe that life expectancy is shortened but, quite surprisingly, after a period of time depending on the intensity of the stressor, the animal again manifests all the signs of the alarm reaction, begins to lose weight and eventually dies.

It became evident quite early in the course of these experiments that when the stressor is strong enough, the stage of resistance is followed by this third phase, that is, the stage of exhaustion, in which the acquired adaptation is lost.

The discovery of this triphasic course led to the development of the concept of some "adaptive energy" required to perform adaptive work and manifested by the resistance of the organism to chronic stress. The amount of adaptation energy was believed to be finite and subject to exhaustion under the influence of stress. Without assuming that the store of this energy is depleted by the adaptive work, it is difficult to explain why an animal that acquires resistance to daily treatment with a certain stimulus loses this resistance after prolonged treatment and eventually reacts just as a nonadapted animal does (Selye, 1950; Selye and Prioreschi, 1960).

The Mechanism of the G.A.S.

Ever since the first description of the G.A.S., special attention has been given to the pituitary-adrenal axis. When adrenalectomized rats were exposed to stressor agents, they no longer exhibited thymicolympathic involution or characteristic blood count changes. On the other hand, gastrointestinal ulcers and other manifestations of pure damage or shock were more severe in the absence of the adrenals and could be lessened by treatment with cortical hormones. It was concluded that these lesions are not mediated through the adrenal but are combated by an adequate adrenocortical response to stressor agents (Selye, 1946, 1950). Normally an adrenalectomized rat exhibits very little resistance to drugs or cold; yet, if the animal

has been previously adapted to these stimuli, it does not lose its resistance following adrenalectomy. Apparently, the corticoids are required primarily for the acquisition of adaptation; they are much less essential for the maintenance of previously acquired adaptive behavior. Hypophysectomy prevents the adrenal response during the alarm reaction; stress stimulates the cortex through the release of ACTH (Selye, 1950).

It is well known that impulses arrive at the anterior pituitary through the discharge of "releasing factors" by the hypothalamus. The pituitary then increases its output of ACTH so that the adrenal cortex is stimulated to secrete more corticoids (Harris and Fortier, 1954; Guillemin, 1968). The adrenal can produce the following classes of hormones: the mineralocorticoids, the "adrenal sex hormones," and the glucocorticoids.

The secretion rates of corticoids and catecholamines during stress have not been dealt with in this chapter because of lack of space. The reader is therefore referred to several monographs and articles on these topics (Selye, 1951; Selye and Horava, 1952, 1953; Selye and Heuser, 1954, 1956; Csalay and Frankl, 1962; Elmadjian, 1962; Cope, 1964; Elmadjian and Forchielli, 1965; Le Quesne, 1967; Wolstenholme and Porter, 1967; Fröberg, *et al.*, 1971).

Conditioning by Hormone Actions

It has long been known that biologic reactions depend not only upon the nature of the evocative stimulus but also upon other factors (e.g. heredity, age, hibernation, drugs or disease) which condition reactivity. This modifying effect may take many forms, variously described in pharmacology as permissive action, blocking action, sensitization, desensitization, resistance, etc. As a generic term for all of these types of interactions, the designation "biochemical conditioning" has been suggested (Selye, 1950). The activity of stress hormones depends largely upon a variety of conditioning factors such as previous exposure to stress, age and the nutritional state. Production of ACTH by the pituitary, for example, is enhanced by a high-protein diet (Henriques, *et al.*, 1949). Conversely stress hormones can act as conditioning factors for various agents, including nonspecific stressors.

In the production of morbid lesions by DOC, it was observed that neither a mineralocorticoid with a sodium-free diet, nor NaCl in the absence of corticoid treatment produced any striking pathologic changes. Yet, when DOC and NaCl were given conjointly, fatal nephrosclerosis, periarteritis nodosa, and myocarditis developed. Subsequent work revealed that such conditioning actions play a particularly important role during the G.A.S. and especially in the pathogenesis of the "diseases of adaptation" (Selye, 1950).

Stress itself is a most effective factor in conditioning for the actions of adaptive hormones. Systemic stress increases the anti-inflammatory, lympholytic, catabolic and hyperglycemic actions of glucocorticoids, but the adaptive hormones cannot act in modifying the cause of inflammation unless some topical stressor elicits an inflammatory response (Selye, 1950). A somewhat related concept was developed by Ingle (1951), who refers to this type of effect as a "permissive" or "supportive" action. He claims that the adrenal hormones do not affect the targets of stress, but merely permit a stressor to act upon them. Furthermore, the presence or absence of a permissive factor can only allow or prevent a reaction, but cannot vary its intensity. However, this concept is hardly applicable to such phenomena as the dose-dependent production of thymic atrophy or cardiac necroses by corticoids during stress.

Relationship Between Stress and Aging

The G.A.S. is in many ways similar to the life cycle of the individual in that constant adjustments are necessary to the ever-changing requirements of existence. In infancy, the organism is not yet adapted to life and is less resistant to variations in muscular work, infection, starvation, etc. In an adult organism resistance reaches its peak; thereafter it slowly decreases until death. Thus, the life-span of an individual can be described as a protracted G.A.S. that develops in response to the stresses and strains of daily life (Selye, 1950; Selye and Prioreschi, 1960). The term "adaptation energy" was coined to differentiate between energy involved in continued adaptive work and the caloric energy we receive from food, although, at present, we have no precise concept of what this adaptive energy might be (Selye, 1950).

Among the most reliable parameters utilized to assess aging are the life-span, changes in the physical and biochemical properties of connective tissue and the capacity for adaptation to change. Associated with these is an increased susceptibility to chronic and malignant diseases. It is not possible to distinguish between the changes produced by age as such and those caused by previous diseases. According to the stress theory of aging, there is no clear distinction between the two groups of phenomena, because every stressful episode is a fundamental component of the aging process.

One of the implications of the stress theory of aging is that exposure to stressful stimuli accelerates the process. For technical reasons it is difficult to prove that in animals every transient exposure to stress shortens the life-span. However, several experiments have shown that this is so when stressors are continuously applied (Grad and Kral, 1957; Upton, 1960; Timiras, 1962; Johnson, *et al.*, 1963; Paré, 1964; Curtis, 1967).

According to Johnson, *et al.* (1963), exposure to cold significantly shortened the life-span of rats; longevity depended upon the incidence and severity of lesions, particularly nephritis and periarteritis nodosa. Heroux and Campbell (1960) did not consider the difference in longevity significant between animals living in a cold environment (6°C) and those raised at 30°C , but reported an increase in the incidence of the above mentioned lesions. Wexler (1964) reported that arteriosclerotic lesions are greatly accelerated in rats subjected to the stress of repeated breeding.

The mechanism whereby cold accelerates the appearance of nephritis and periarteritis nodosa is not fully understood. It is supposed that these diseases may result from a derangement of the G.A.S. Overdosage with DOC alone or in combination with unilateral nephrectomy plus high salt diet is highly effective in inducing both nephrosclerosis and periarteritis nodosa (Selye, 1950). Similar changes are produced by exposure to cold and other damaging agents in uninephrectomized rats (Selye, 1950). Increased adrenocortical activity is noted in all these animals. Therefore, it seems that the renal and vascular lesions elicited by DOC or exposure to cold are caused by a similar pathogenic mechanism. Many investigators have observed an increased secretion of corticosteroids during acute exposure to cold, but it remains to be demonstrated in rats subjected to long-term exposure to cold. Some authors believe that the adrenal cortex is involved in the initial response to cold and that it is not essential for maintenance of cold acclimation (Heroux, 1960; Schönbaum, 1960).

It is known that restriction of caloric intake increases life-span and delays the onset of many chronic diseases (Saxton and Kimball, 1941; McCay, 1952; Berg and Simms, 1960). Since rats exposed to cold eat more than control ones (Grad and Kral, 1957; Kibler and Johnson, 1961), their shortened life-span could be due to the greater quantity of calories consumed.

The effect of exposure to high altitude has been extensively investigated by Timiras (1962). Rats subjected to this stress exhibited retardation of growth, cardiovascular alterations and metabolic impairments, all of which contribute to a shortened life-span. As noted with other stressors, adrenal hypertrophy and thymus atrophy occurred during the first days of exposure; thereafter, the organ weights were similar to those of control rats.

Contrary to these findings, Curtis (1963) reported that mice subjected to chemical stressors (e.g. injection of turpentine) had as good a life expectancy as the controls, while under identical conditions radiation exerted a life shortening effect. He concluded that these experiments failed to support the view that nonspecific stress is a factor of aging. These apparent discrepancies in the action of different types of stress could be ex-

plained on the basis of differences in their intensities as well as in the distribution of the chemical stressor agents (e.g. turpentine) throughout the body.

There is some evidence to support the view that exposure to chronic stress can initiate premature physiological aging. In animals subjected to psychological stress, Paré (1964, 1965) observed changes (in basal metabolic rate, prostate size, swimming ability, thermic contraction of collagen) similar to those which occur during aging. Likewise, repeated pregnancies (Árvay, *et al.*, 1963) and the stress of inanition induced an aging-like behavior of collagen from tail tendon (Steinetz, *et al.*, 1966). Treatment of rats with ACTH and cortisol caused an alteration of tendon contractility; thus, increased secretion of adrenal steroids during stress might account for the collagen changes (Árvay and Takács, 1965). However, these investigators concluded that besides cortisol, increased sex hormone production could explain the effect on collagen. Allegedly a cold environment does not affect fiber contractility (Rassaert and Steinetz, 1968). Exposure to ionizing radiation results in numerous lesions, such as nephrosclerosis, fibrosis of heart muscle, anemia, tumors, etc., which all contribute to shorten the life-span (Upton, 1960). There is, however, one exception to the radiation-induced senescence: the tensile properties of collagen fibers are not appreciably altered (Verzár, 1964). On the other hand, as previously noted, application of psychological stress, pregnancies, etc., do induce aging of collagen. It is difficult to reconcile this discrepancy between the results of irradiation and those obtained after exposure to other types of stressors. Based on these and other inconsistencies, some workers (Alexander, 1967) have concluded that irradiation does not age the organism uniformly. While certain tissues are specific target sites for irradiation, others, such as collagen fibers, possess a relatively low radio-sensitivity.

Another implication of the stress theory of aging is that adaptability to changed environmental conditions declines with progressing age. There is considerable experimental evidence to support this view.

Age influences the adaptability of rats to mechanical trauma (Fabry and Hruza, 1960). Although no differences in mortality rate were observed between young and old animals exposed to trauma for the first time, following an adaptation period (during which animals were exposed to increasing trauma) young animals proved to be significantly more resistant. These findings accord well with those of Grad and Kral (1957) who reported a greater mortality rate of old mice upon exposure to cold but only following a period of adaptation to the stressor. The mechanism responsible for the decreased adaptability of old rats is not yet fully understood, but it is known that neurohumoral mechanisms, especially the pituitary-adrenocor-

tical system, play a role in the adaptation both to cold and to trauma. Moreover, the observed modifications in intermediary metabolism, circulation, etc., during adaptation have been thought to be important in the process (Chytil and Hruza, 1956; Hruza and Chytil, 1959).

Generally, aged people are not very resistant to infections and are highly subject to the development of psychoses under acute physical or mental stress (Grad and Kral, 1957). However, a number of physiologic and biochemical tests revealed no differences in the stress reactions of normal young, and healthy old people (Bortz, 1955; Pincus, 1956; Grad and Kral, 1957; Carlson, *et al.*, 1970). Although there are more so-called poor-risk patients in the older group, not every individual of a given age is necessarily deficient in his reaction to a given stress.

Stress and Cardiovascular Disease

Here we shall discuss the principal cardiovascular lesions produced by exposure to stress or by the stress hormones which regulate the body's response during the G.A.S. The role of corticoids in cardiovascular disease is substantiated by many experimental and clinical findings that showed the participation of these steroids in hypertension, various forms of arteriosclerosis, and myocardial necrosis. Here, the reader is referred to two monographs (Selye, 1970b; Raab, 1971) for data on catecholamines and cardiovascular diseases.

Hypertensive Disease Caused by Mineralocorticoids

The first evidence showing that a chemically pure mineralocorticoid can cause hypertensive cardiovascular disease and that its pathogenicity depends upon the availability of sodium, came from experiments on chicks and rats treated with desoxycorticosterone (DOC). In these animals, DOC alone or excess NaCl alone, caused hypertension; however, especially severe hypertensive disease with cardiovascular and renal manifestations (of acute arteriolonecrosis with "hyalinosis," edema and hypertensive encephalopathy) was readily produced only by conjoint treatment with DOC + NaCl. Chicks proved to be far more sensitive in this respect than rats but the latter could be further conditioned by removing one kidney (Selye, 1942; Selye and Pentz, 1943).

The technique of unilateral nephrectomy combined with excess NaCl-administration (in the diet or drinking water) has been found a highly effective conditioning procedure for the induction of experimental hypertension in various animal species. It increases sensitivity in this respect not only to DOC but also to other mineralocorticoids, to methylandrostenediol (an anabolic testoid) and to somatotrophic hormone (STH). Among the other steroids capable of eliciting the same syndrome, aldosterone is par-

ticularly noteworthy because it is the chief mineralocorticoid produced by the human adrenal. The relationship between adrenocortical and renal hypertension, although still not fully understood, appears to be a very close one. Even these first experiments showed that unilateral nephrectomy is a potent conditioning agent for mineralocorticoid hypertension and subsequent investigations taught us that renin stimulates aldosterone production.

Under ordinary conditions, cortisone and cortisol, unlike DOC or aldosterone, failed to elicit hypertensive cardiovascular disease, even in rats maximally conditioned by unilateral nephrectomy and NaCl.

Fifteen years later, several clinical investigators were able to demonstrate increased urinary aldosterone excretion in patients with certain types of hypertension, nephrosis, cirrhosis of the liver, cardiac failure or toxemia of pregnancy (Gaunt, *et al.*, 1955; Laragh, 1956; Genest, *et al.*, 1965) and the responsible factor was isolated and identified as aldosterone (Lutschner, *et al.*, 1955, 1956).

Thus, both experimental and clinical observations revealed some connection between mineralocorticoids and cardiovascular disease. The increased aldosterone secretion in man could have been secondary and without pathogenic significance, but the fact that mineralocorticoids produce cardiovascular lesions with edema in animals strongly suggested a causal relationship. In 1959, Kagawa, *et al.*, discovered spironolactone (Aldactone®), a hormonally inactive steroid which, according to current opinion, antagonizes both aldosterone and DOC by a selective competitive inhibition of the mineralocorticoid effect at the level of the renal tubule. This may well be the mechanism (or at least one of the mechanisms) through which spironolactone exerts its beneficial effect in rats with DOC-induced hyalinosis (Ducommun, *et al.*, 1960). However, as we shall see, recent experiments have shown that spironolactone also possesses extrarenal actions.

Stress and Arteriosclerosis

Although degenerative arterial diseases, particularly arteriosclerosis, may occur in young individuals, these lesions are considered characteristic of advancing age and often represent the immediate cause of death.

A considerable amount of work suggests the participation of stress in the development of human atherosclerosis (Enos, *et al.*, 1953; Hirsch, 1955; Carlson, *et al.*, 1968; Heyden, 1969). However, more direct evidence is furnished by animal experiments (Sobel, 1962; Jayle, 1963; Jelinek, 1967).

In rats kept on an atherogenic diet, exposure to periodic electrical discharges increases coronary atherosclerosis (Uhley and Friedman, 1959). Atherosclerosis and even thrombosis are produced in female breeder rats

subjected to uninephrectomy and ACTH (Wexler and Miller, 1959; Wexler, *et al.*, 1960). Indeed, breeder rats show arteriosclerosis even without additional treatment. Since the disease is accompanied by hypertrophy and hyperplasia of the adrenal cortex (Wexler, 1964), it was suggested that a correlation may exist between abnormal adrenal function due to repeated breeding and the development of arteriosclerosis. Stress with liberation of ACTH and catecholamines is thought to play an important role here. Shimamoto (1968) reported that the "edematous reaction of the arterial wall" occurring under the influence of various stressors (e.g. trauma, endotoxin) can be interpreted as one aspect of the G.A.S. which may represent an early stage of atherosclerosis.

Experimental Metabolic Myocardial Necrosis

The observations discussed up to now have shown that mineralocorticoids can produce hypertensive disease, often with widespread vascular lesions in various organs including the heart and brain. However, occlusive thromboses with myocardial infarction were exceptional, irrespective of the technique used. Ever since the first description of the G.A.S. we were anxious to develop a conditioning technique in which stress would be the immediate cause of cardiac necrosis. An experimental model of this type seemed especially desirable since, in man, physical or mental exertion has long been suspected of provoking myocardial infarction. Moreover, cardiac infarction is generally considered to be related to aging, although it may occur in young individuals.

At first all our efforts along these lines were unsuccessful; even when exposed to fatal stress (severe traumatic injuries, intoxications, prolonged immobilization on a board), normal laboratory animals failed to develop myocardial infarction. However, when rats were simultaneously pretreated with glucocorticoids and mineralocorticoids (or with certain synthetic halogenated corticoids possessing both mineralo- and glucocorticoid activity in the same molecule), in conjunction with certain electrolytes (e.g. Na_2HPO_4) they became highly susceptible to the production of myocardial necroses by subsequent exposure to stress (forced exercise, cold bath, restraint, etc.). This experimental disease model came to be called "Electrolyte Steroid Cardiopathy with Necrosis" (ESCN) (Selye, 1961). Since obstructive coronary lesions were not observed, the large necrotic areas were referred to as "infarctoid." Through this term we wanted to indicate their similarity to true cardiac infarcts and to emphasize (by the suffix "-oid" = like) that they are not necessarily identical to the typical myocardial infarcts as they occur in man. Still, on the basis of statistical data available at the time, we suggested that the considerable number of clinical myocardial infarcts in which no recent occlusive coronary thrombi could be

found within the coronary vessels might be due to a biochemical mechanism similar to that operative in the ESCN.

Acute stress greatly sensitizes for the cardiotoxic effect of electrolytes and steroids. On the other hand, gradual adaptation to stressors (e.g. forced muscular exercise, cold) protects against the induction of cardiac necrosis by subsequent humoral conditioning and renewed exposure to stress. Indeed, inurement to one stressor protects the trained animals against the production of myocardial lesions, not only by the same, but even by unrelated stressors. Hence, the question not only involves specific adaptation to one agent (which would then lose its stressor effect) but also the induction of a kind of tolerance to stress itself, no matter how produced (Selye, 1961).

In rats, susceptibility to various cardiotoxic agents (e.g. electrolytes and steroids, isoproterenol, and cardiac glycosides) increases with age (Selye and Bajusz, 1959; Rona, *et al.*, 1963). Similarly, aged dogs placed on an atherogenic regimen are highly sensitive to the production of cardiac infarcts by exposure to stress (Sobel, *et al.*, 1962).

The production of an ESCN by gluco-mineralocorticoids plus sodium salts is greatly facilitated by concurrent oral administration of lipids. In this respect, both triglycerides of animal or vegetable origin and a great variety of pure fatty acids have been found to be active (Selye, 1961). This finding is of interest in view of the well known increase in the serum free fatty acid level during stress and the relationship that seems to exist between hyperlipemia and predisposition to cardiovascular disease in man.

The ESCN is associated with a marked drop in myocardial and serum potassium (Prioreschi, 1962). Moreover, this experimental cardiopathy can be prevented by the administrations of KCl or MgCl₂ (Selye, 1958) and potassium-sparing agents such as amiloride (Selye, 1968).

In our animal experiments, we also looked for more convenient and lasting ways to provide the myocardium with the necessary amount of potassium. Using spironolactone, we could inhibit the myocardial necroses induced by corticoids (Selye, 1960). It was first assumed that the effect was exerted through its mineralocorticoid blocking action, but it was later shown that necrosis caused by treatment with digitoxin and Na₂HPO₄ could likewise be prevented (Selye, *et al.*, 1969). Spironolactone prevents not only the cardiac but also the extracardiac manifestations (e.g. convulsions) of digitoxin poisoning. This prophylactic effect was observed also after bilateral nephrectomy; therefore, the effect of the steroid against cardiac necrosis cannot be ascribed to its classic effect: the blockade of mineralocorticoid-induced sodium retention and potassium elimination at the level of the renal tubule (Selye, *et al.*, 1969).

More recently, attention has been called to hitherto unsuspected mech-

anisms that may be just as important as changes in electrolyte metabolism or specific antimineralcorticoid effects in determining predisposition for various types of infarctoid myocardial necroses. It was found that spironolactone, as well as other "catatoxic steroids"^{*} (e.g. norbolethone, ethyles-trenol), protect the rat not only against the ESCN-type of myocardial necrosis (Selye, 1969a, 1970a, 1971), but also against a great variety of quite unrelated intoxications. Thus, compounds of this group inhibit the anesthetic and sedative effects of pentobarbital, methyprylon and steroids, the soft-tissue calcification elicited by heavy overdosage with dihydrotachysterol (DHT), the motor disturbances characteristic of digitoxin poisoning, the adrenal necrosis induced by 7,12-dimethylbenz(a)anthracene (DMBA), the production of multiple intestinal ulcers and peritonitis by indomethacin, etc. (Booth and Gillette, 1962; Conney, 1967; Kovacs and Somogyi, 1969; Solymoss, *et al.*, 1969; Selye, 1970; Selye, *et al.*, 1970). As suggested by several experiments, the catatoxic effects of steroids may partly be due to hepatic microsomal enzyme induction and this mechanism is presently being investigated. It is conceivable that derangements in catatoxic hormone activity may contribute to the development of organ changes characteristic of aging.

CONCLUSION

The triphasic course of the G.A.S., as far as resistance to stress is concerned, seems to resemble the life course of the individual. The three stages are reminiscent of responses in the infant, the adult, and the senile individual. The child manifests a marked reaction to stress, but he adapts readily. The adult's reaction to stressors is less severe, but he adapts less easily, and adaptability reaches the lowest level in senility. It has been hypothesized that some form of energy is necessary for adaptation and that the onset of senescence is related to the rate of its expenditure.

Several experiments have demonstrated that exposure of animals to protracted environmental stressors (e.g. high altitude, irradiation, cold) shortens the life-span by causing pathologic changes prematurely. These lesions resemble those which normally occur in man during old age and are caused by some of the hormones released during stress. Furthermore, some of these lesions (e.g. arteriosclerosis, myocardial infarction) are induced more easily in old than in young animals, and are frequently aggravated by stress. Obviously, these investigations do not deal with the process of aging itself; yet they do provide us with data for the analysis of the degenerative diseases which accompany senility.

* The term "catatoxic" (from the Greek kata = down, against) has been proposed to designate this effect, since "antitoxic," which would also be appropriate, is already in current use for a class of specific antibodies (Selye, 1969).

Although the intensity of experimental stressors often greatly exceeds that of agents to which organisms are normally exposed, it seems possible that the cumulative effect of the stress of life may also become pathogenic and gradually conducive to senility.

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CHAPTER 30

NUTRITION AND AGING

A. V. EVERITT and BARBARA PORTER

SUMMARY

LONG-TERM FOOD RESTRICTION (by up to 50%) retards the aging of collagen, delays the onset of terminal diseases and prolongs the life of many animal species. Caloric restriction inhibits many endocrine functions of the anterior pituitary, the thyroid and the gonads, as well as depressing metabolic, cardiovascular and renal functions. The reduced level of organ function may prolong the life of individual organs thereby increasing the survival of the whole body. Increasing the work load of an organ (as when one kidney does the work of two) shortens its life.

Elevation of the food intake for a long period accelerates the aging of collagen, leads to the premature appearance of the diseases of old age and consequently shortens the life duration. Rats maintained at low temperatures have very high food intakes, develop terminal diseases early and die prematurely. Under these conditions the secretion of thyroid hormone is increased.

At low intakes of food the aging of collagen and the development of renal disease is independent of food intake. When the food intake rises above a critical level the aging process is accelerated. It is postulated that the hypothalamic-pituitary-thyroid axis regulates the aging process, via its action on food intake. The aging process is accelerated when excessive quantities of food are eaten. Food intake is increased physiologically during recovery from fasting, in growth and lactation, during exposure to cold and by physical work. Pathological rises in food intake occur in hyperthyroidism, diabetes mellitus, hypothalamic disease and obesity. Under conditions of high food intake the animal ages more quickly and dies prematurely.

INTRODUCTION

The existence of a relationship between diet and longevity has been suspected for a long time. A warning about the ill-effects of overeating is given in the Apocrypha (*Ecclesiasticus 37:31*): "Many have died of gluttony, but he who is careful to avoid it prolongs his life." The belief that food restriction would prolong life did not receive scientific confirmation until the 20th century. In most investigations the rat has been used as the

experimental model, because of the difficulties of long-term studies in man. The work of McCay and his colleagues clearly showed that reducing the intake of food extended the life-span of the rat (McCay, *et al.*, 1935).

NUTRITION AND AGING

The literature on nutrition in relation to aging and longevity has been reviewed recently by a number of authors (Barrows and Beauchene, 1970; Kahn, 1972; Ross, 1972).

Undernutrition—Caloric Restriction

In the absence of specific nutrient deficiencies a general lowering of nutrient intake is beneficial; reducing the intake of calories has been shown to increase life duration, to delay the onset of certain diseases of old age and to retard a number of aging processes.

Life Duration

Osborne, Mendel and Ferry (1917) were probably the first to demonstrate that food restriction could increase the life duration of the rat. Their observations were confirmed and extended in the well-known studies of McCay and his collaborators (McCay, Crowell and Maynard, 1935; McCay, Maynard, Sperling and Barnes, 1939; McCay, Sperling and Barnes, 1943). In these experiments it was found that the mean life duration of underfed rats allowed to grow by 10 g every 2 to 3 months, was more than 50 percent greater than that of fully fed controls (Fig. 30-1). The diets of underfed rats were adequate in all constituents excepting calories. The increased life-span was apparently due to the lowered incidence of chronic diseases in the old rat (Saxton, 1945). Marked growth retardation is not essential for the increased life-span (Carlson and Hoelzel, 1946; Berg and Simms, 1960).

Other workers have successfully prolonged life by food restriction in the rat (Riesen, *et al.*, 1947; Ross, 1959 and 1961; Berg, 1960; Berg and Simms, 1961; Nolen, 1972) and the mouse (Saxton, *et al.*, 1944; Lee, *et al.*, 1956; Lane and Dickie, 1958). However, severe restriction of food intake to 16 percent of the *ad libitum* level markedly reduces life duration in the rat (Everitt, 1971). It is likely in this case that there are severe nutrient deficiencies and that the concomitant gross restriction in energy to a level below the critical level is detrimental.

The life-span of many invertebrates is increased when food intake is diminished (Comfort, 1960). Successful experiments have been carried out using the protozoan *Tokophyra* (Rudzinska, 1962), the rotifer (Fanestil and Barrows, 1965), the crustacean *Daphnia* (Ingle, *et al.*, 1937), the silk worm (Kellogg and Bell, 1903), the fruit fly *Drosophila* (Northrop, 1917;

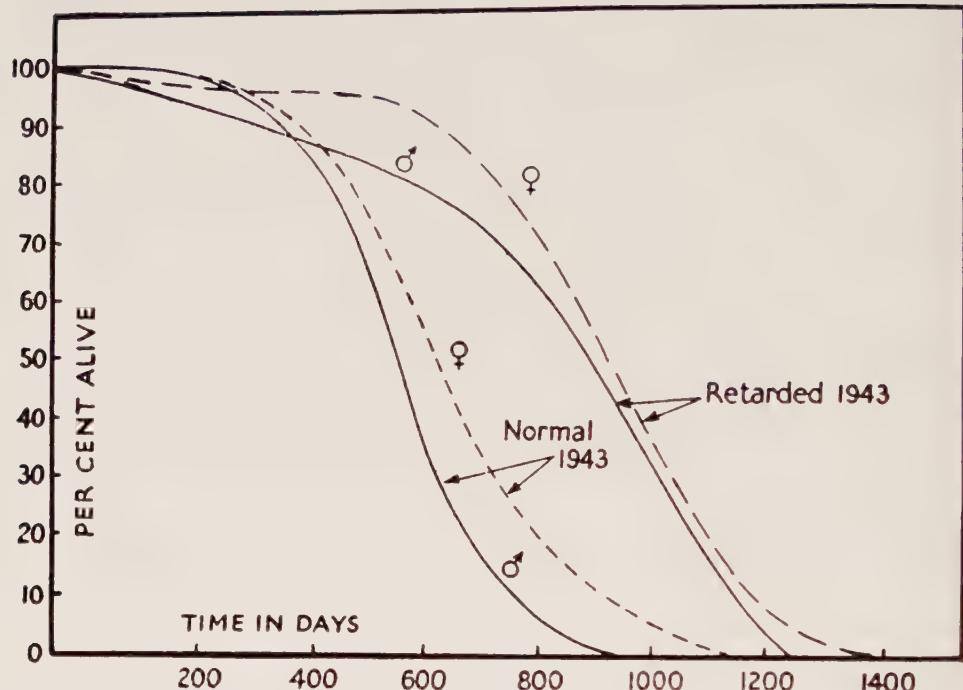


Figure 30-1. The survival curves of normal and retarded (food-restricted) male and female rats (From McCay, Sperling and Barnes, 1943, reproduced by permission of Academic Press Inc., New York).

Greiff, 1940) and certain other species. It is quite clear that underfeeding increases the life-span in a number of different animal species.

The nutritional requirement depends on the phase of the life cycle, being different in the mammal for suckling, growth, maturity and old age. Consequently food restriction in different phases of the life cycle will have different effects on aging and longevity (Barrows and Beauchene, 1970; Ross, 1972). The life-prolonging effect of underfeeding is greatest when begun in the young growing rat soon after weaning. Food restriction for 49 days between ages 21 and 70 days, followed by *ad libitum* feeding, will prolong the life of the rat (Ross, 1972). Underfeeding of the middle-aged rat is reported to have only a slightly beneficial effect on life duration (McCay, *et al.*, 1941; Ross, 1964) or even a detrimental effect (Barrows and Roeder, 1965; Everitt, *et al.*, 1969). Reduction of food intake, before weaning, by increasing the number of young rats fed by the mother, decreases body size and shortens the life-span of the male (Widdowson and Kennedy, 1962). In the mouse, underfeeding at an early age increases juvenile mortality and so decreases the mean life duration (Silberberg, *et al.*, 1961). Undernourishment in the young animal may have an adverse

effect on the course of chronic infections and the development of immunity (Silberberg and Silberberg, 1955).

Invertebrate studies also show that the effects of food restriction vary with the stage of the life cycle. In the rotifer, Fanestil and Barrows (1965) found that food restriction prolonged the second phase of the life cycle (the period of egg production), but had no effect on the length of other phases. In holometabolous insects, dietary restriction prolongs the larval stage, but in the adult longevity is determined by stored reserves (Comfort, 1960). For example, Rockstein (1959) found that the adult life of *Musca domestica* was prolonged by food supplements or by prevention of egg laying.

The effect of nutrition on human longevity has been studied by Watana-be, *et al.* (1968). On a world-wide basis, the life duration increases as the intake of nutrients rises. Thus undernutrition in poorly developed countries has an unfavorable effect on longevity, due to the lack of essential nutrients. The undernourished populations of the world are very different from McCay's underfed rats, which were well supplied with all nutrients, but deficient in calories.

Disease

Autopsies performed on the food-restricted rats of McCay revealed a delayed onset of certain diseases such as chronic pneumonia, chronic nephrosis and tumors (Saxton and Kimball, 1941; McCay, *et al.*, 1943; Saxton, 1945). At about the same time Tannenbaum (1940) and Visscher, *et al.* (1942) reported a greatly diminished incidence of tumors in underfed mice. Many years earlier Moreschi (1909) had found that transplanted tumors would not grow in ill-nourished mice.

The increased resistance to disease in the food-restricted rat was confirmed in later studies by Ross (1959 and 1964) and Berg and Simms (1960 and 1961). The latter group compared the life-long effects of a 33 and a 46 percent reduction in food intake (see Berg, Chap. 3). They found that the frequency of lesions in 800-day-old male rats increased in proportion to the quantity of food consumed (Berg and Simms, 1960). In the *ad libitum* fed group, 100 percent had one or more lesions (glomerulonephritis, periarthritis and myocardial degeneration). In the 33 percent food-restricted group, 64 percent had lesions, while in the 46 percent food-restricted group only 24 percent had lesions. The effect of food restriction on the incidence of glomerulonephritis at different ages is shown in Figure 30-2. Food restricted mice have superior immune function in old age (Wal-ford, R. L. 1975, *10th Int Congress Gerontol*, Abst Vol I, p. 87, 1975).

In man the reduced supply of both calories and fat during World War II appears to have been responsible for the temporary reduction in coronary heart disease in several Scandinavian countries (Strøm and Jensen,

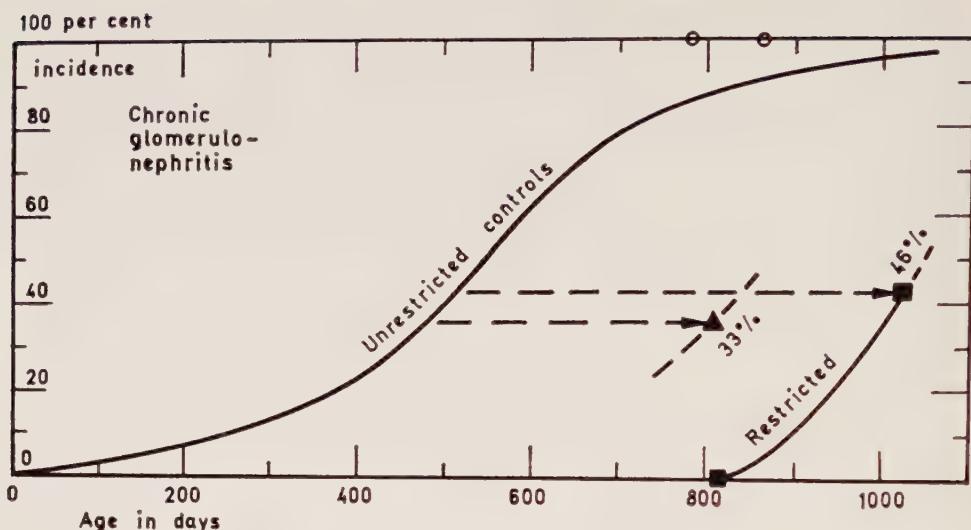


Figure 30-2. Effect of 33 and 46 percent food restriction on the incidence of chronic glomerulonephritis in the male rat (From Simms and Berg, 1962, reproduced with permission of the authors from *Geriatrics*, volume 17, copyright The New York Times Media Company Inc.).

1951), although this has been questioned by Yudkin (1957). Undernutrition in primitive or underdeveloped populations has been associated with a very low incidence of hypertension, hypercholesterolemia and atherosclerosis (de Wolf and Whyte, 1958; Walker, 1968; Williams, 1969; Black et al., 1970).

Physiological Aging

In 1955, Verzár developed a physiological test of collagen aging using the rat tail tendon fibers. With a similar test Chvapil and Hruza (1959) showed that food restriction retarded the aging of collagen. This was later confirmed by Giles and Everitt (1967) and Hruza and Hlaváčková (1969).

The relationship of food intake to the biological age of collagen is shown in Figure 30-3. The aging of collagen was at its lowest level in rats eating 14 g food per day (33% food restriction); an excess of food above this level appeared to accelerate the aging process. At very low food intakes the aging effect of "starvation" stress counteracts the anti-aging action of reduced food intake.

Hruza and Hlaváčková (1969) found that tail temperature falls in the food restricted rat and suggest that this is the reason for the retarded aging of collagen. Deyl, et al. (1971) noticed that food restriction also delayed the deposition of collagen in kidney, lung and liver.

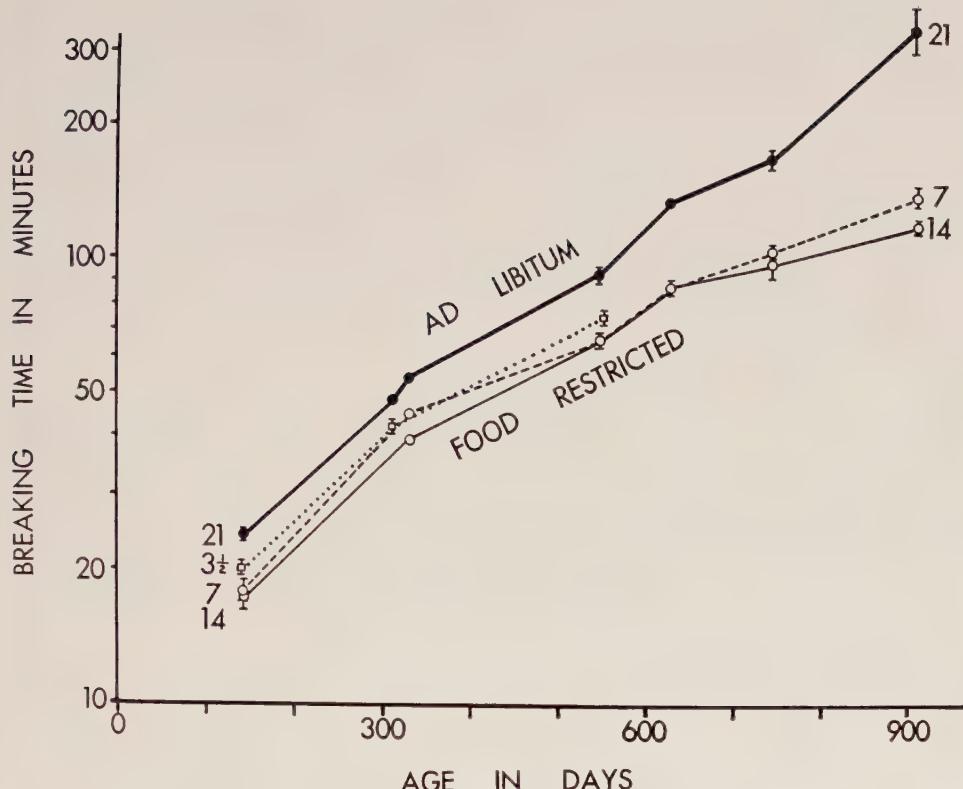


Figure 30-3. The effect of increasing the food intake from $3\frac{1}{2}$ to 21 g/day, on the aging of tail tendon collagen fibers in the rat. Collagen age was measured in individual fibers by determining the breaking time in min in 7 M urea at 40°C under a load of 2 g (From Everitt, 1971, reproduced by permission of S. Karger, Basel, Switzerland).

Holečková and Chvapil (1965) studied the physiological effects of intermittent feeding and fasting for long periods. In all tests intermittently starved animals appeared to be younger than *ad libitum* fed controls of the same age. Collagen aging was retarded in intermittently starved rats. The emigration time of the first cells in tissue culture of liver from intermittently fasted animals was very short, just as in cultures of young tissue. Rats adapted previously (for 41 weeks) to intermittent feeding and fasting were better able to adapt to low temperature (5°C) than fully fed controls. Adaptability to adverse conditions decreases with age (Fabry and Hruza, 1960). The function of the central nervous system, as measured by the nonspecific excitability level deteriorated at a slower rate in intermittently starved rats (Lát and Holečková, 1971).

The reproductive life of the female rat is prolonged by food restriction or intermittent fasting. Berg (1960) obtained litters from 16 out of

24 food-restricted rats aged 730 to 790 days, at an age when fully-fed rats are sterile. Holečková and Chvapil (1965) reported that 80 percent of intermittently fasted rats of age 524 days were fertile, whereas all of the controls were sterile. Calorie restriction also delayed the senescent onset of infertility in mice (Carr, *et al.*, 1949; Visscher, *et al.*, 1952). The onset of puberty is delayed by underfeeding in man (Butler, *et al.*, 1945) and the rat (Kennedy and Mitra, 1963).

The age change in the pattern of hepatic enzyme activity is retarded by long-term caloric restriction (Ross, 1959 and 1969), regardless of whether the enzyme activity increases or decreases with age.

Overnutrition—Excess Calories

In man overeating leads to overweight, which is known to shorten life (Armstrong, *et al.*, 1951; Marks, 1957 and 1960; Olsen, 1959; Walker, 1968). The shortened life-span is due to the increased mortality from degenerative diseases of the cardiovascular system and kidneys, and from diabetes mellitus, liver and biliary tract disorders (Dublin and Marks, 1952;

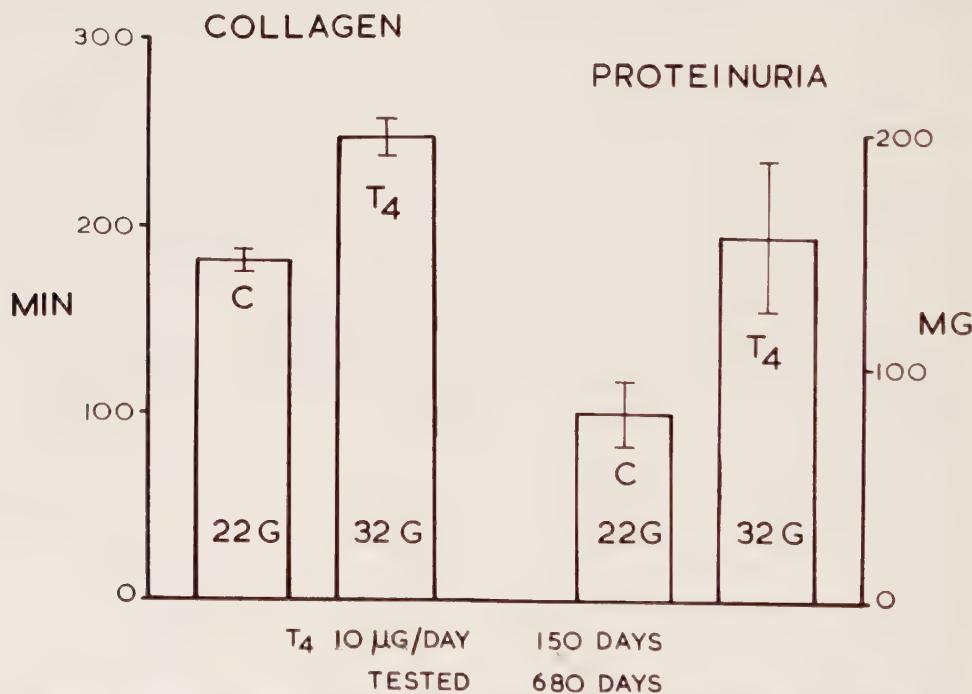


Figure 30-4. The effect of elevated food intake (due to injections of thyroxine) over a period of 5 months on the aging of collagen and the development of renal disease as indicated by the excretion of protein in urine (From Everitt, 1970, reproduced by permission of the Australian Association of Gerontology). Controls (C) ate 22g food per day and thyroxine (T₄) treated rats 32g.

Marks, 1957). Overeating favors the development of hypercholesterolemia, hyperbetaipoproteinemia and coronary heart disease (Blacket, 1970). The work of Watanabe, *et al.* (1968) indicates that energy uptakes in excess of 3,000 kcal per day shorten the life-span.

There is also evidence of accelerated physiological aging in people who overeat. The progressive fall in the age of puberty during the last century has been attributed in part to better nutrition and increased calorie intake (Donovan and Werf ten Bosch, 1965). Puberty usually occurs earlier in obese children (Bruch, 1941; Wolff, 1955). The adrenal cortex is reported to age prematurely in obese subjects (Abbo and Meyer, 1966).

In the rat, hypothalamic lesions in the satiety center increase food intake and accelerate the onset of renal disease (Kennedy, 1957). Thyroxine raises the food intake, increases the excretion of protein (Everitt, 1958; Berg, 1966), produces an early onset of renal lesions (Simms, 1967) and accelerates the aging of collagen (Everitt, *et al.*, 1969). Figure 30-4 shows the effect of thyroxine in raising food intake, increasing the urinary excretion of protein and accelerating the aging of collagen.

Rats maintained at low temperature (9°C) were found to increase food intake by 60 percent (Kibler and Johnson, 1961). At low temperatures rats develop pathological lesions early and die prematurely (Heroux and Campbell, 1960; Johnson, *et al.*, 1963).

Mice which overeat and become obese have a shortened life-span (Lane and Dickie, 1958). Obesity accelerates the development of degenerative joint disease in the mouse (Silberberg, *et al.*, 1956), and increases the incidence of tumors (Heston and Vlalakis, 1962).

Specific Nutrients

Apart from the specific effects of nutrients on aging and longevity, the dietary content of fat, protein and carbohydrate influences the energy requirement of the body (Kaunitz, *et al.*, 1956). For example, there is an increased efficiency of utilization of nutrients in high fat diets (Forbes, *et al.*, 1946; Seidler, *et al.*, 1962) thereby lowering the energy requirement of the animal. Also dietary fat increases the absorption of fat soluble vitamins (Abrams, 1961).

In this laboratory we investigated the specific effects of fat, carbohydrate and protein on renal and collagen aging (see Fig. 30-5). Diets rich in fat, carbohydrate and protein, respectively, together with a control diet (33% of calories from each of these nutrients) were fed isocalorically at a restricted level (50%) and after 5 months (at age 250 days) the effects on aging were assessed. Renal aging was specifically increased by dietary protein, but collagen aging was not affected by specific nutrients.

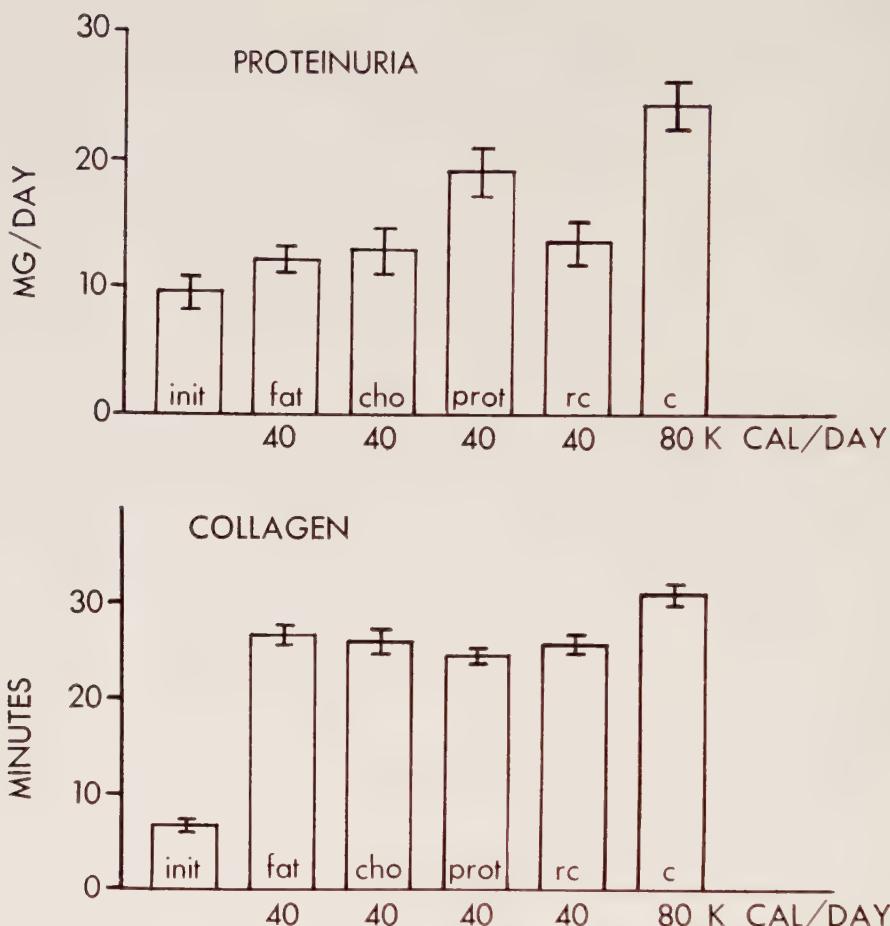


Figure 30-5. Specific nutrients and aging. The effects of diets rich (60% of calories) in fat, carbohydrate and protein respectively, fed isocalorically for 5 months at a restricted level (40 Kcal/day) on the aging of collagen (breaking time in 7 M urea) and kidney (protein excretion/day). The *ad libitum* control (c-80 Kcal/day) and restricted control (rc-40 Kcal/day) received 33 percent of calories from each of these nutrients. Rats were 250 days old when tested.

Fat

Requirements for fat are only for traces of the essential fatty acids, yet ample evidence suggests that additional fat is desirable for growth and reproduction (Deuel, 1955).

LIFE DURATION. In laboratory animals, fat enriched diets are generally associated with a decreased life expectancy (Silberberg and Silberberg, 1955). Male rats maintained on a high fat diet (20% corn oil) were found to have a significantly shorter life duration than animals living on a low-fat stock diet (French, *et al.*, 1953). Rats eating the fat-enriched diet con-

sumed fewer calories. Therefore the life-shortening effect is specifically attributable to fat. Harman (1969) found that rats eating a diet containing 20 percent unsaturated fat (safflower oil) were shorter lived than those eating 20 percent saturated fat (lard).

The life-span of most strains of mice is shortened by high fat diets containing 25 percent lard. The effect depends on the strain and sex of the mouse, the amount of fat in the diet, the duration of consumption and the age at which the fatty diet is fed (Silberberg and Silberberg, 1955).

ATHEROSCLEROSIS AND CORONARY DISEASE. There is much literature on the relationship of fat intake to the development of atherosclerosis and coronary heart disease in man. It is generally believed that the high dietary intake of fat, especially saturated fats, is a major factor in the high mortality from coronary heart disease in many affluent Western Countries.

The international studies of Watanabe, *et al.* (1968) suggest that fat intakes in excess of 50 g per day are deleterious. Polyunsaturated fatty acids are reported to lower serum cholesterol and to retard the development of atherosclerotic disease (Keys and Parlin, 1966), but to increase the incidence of cancer (Pearce and Dayton, 1971). A number of recent studies consider the role of nutrition in the lowering of serum cholesterol and the prevention of heart disease (Food and Nutrition Board, 1972; Nelson, 1972; Blacket, 1973; Woodhill and Bernstein, 1973; Gotto, *et al.*, 1974).

COLLAGEN AGING. Atherogenic diets rich in fat and cholesterol accelerate the aging of tail tendon collagen (Hruza and Chvapil, 1962). In our laboratory, the addition to the diet of either saturated fat (21% coconut oil) or unsaturated fat (21% safflower oil) was found to increase the aging of collagen in rat tail tendon, even though the intake of calories was kept constant (Everitt, 1970). However, when the energy content of the diet was reduced to 50 percent of the *ad libitum* intake, the proportion of fat did not affect collagen aging (Fig. 30-5).

PATHOLOGY. In the study just described (Fig. 30-5) the fat content of a restricted diet did not significantly affect urinary protein excretion of the male rat.

In C57BL mice the addition of 25 percent lard to the diet hastened the development of senile osteoarthritis and articular aging changes. These effects were partly reversed by a dietary supplement of 30% linolenic acid (Silberberg, *et al.*, 1965).

In women, a positive correlation between neoplasms of the breast, ovary and rectum and the consumption of dietary fat has been reported (Lea, 1966), and confirmed by Carroll, *et al.* (1968) for breast cancer. Likewise, high fat diets increase the growth of experimental mammary cancers in rats (Gammal, *et al.*, 1967; Carroll and Khor, 1970).

Carbohydrates

In man the majority of calories consumed are derived from carbohydrates, because foods rich in carbohydrate are cheap, readily prepared and easily digested. Sugar (sucrose) is being consumed in progressively greater amounts in Western Countries. Yudkin (1972) has linked the intake of sucrose with a number of diseases, including coronary heart disease. Other workers (Keys, 1971; Walker, 1971) have discussed the relationship between sugar intake and heart disease.

In rats extra dietary sucrose leads to the early development of glomerulonephritis (Dalderup and Visser, 1969) and diffuse glomerulosclerosis (Cohen and Rosenmann, 1971). Both of these changes are age-related. As in man, sucrose has been shown to have an atherogenic effect in the rat (Markelova and Ljapkov, 1971). In view of its pathological effects on the kidneys and arteries, sucrose addition to the diet would be expected to shorten life in the rat. Dalderup and Visser (1969) have demonstrated a significant shortening of the life-span of the male rat when 15 calorie percent of starch in the diet was replaced by sucrose. The intake of calories, protein, fat and carbohydrate was the same in both high and low sucrose groups. The earlier work of Whittier, *et al.* (1935) had shown that young rats fed a diet containing 30 percent sucrose were shorter lived than those receiving 30 percent lactose. However the study of French, *et al.* (1953) failed to demonstrate any effect of adding 20 percent sucrose to a stock diet.

An unexpected life prolonging effect of a high-sucrose diet has been reported by Ross (1961). He found that rats eating a diet containing 83 percent sucrose and 8 percent casein lived significantly longer than those groups eating less sucrose (34-61%) and more casein (21-51%). The low protein diet depressed the appetite and so lengthened the life-span.

When isocaloric rations were fed at a 50 percent restricted level in this laboratory the sucrose enriched (60% of calories) diet did not affect the aging of either collagen or kidney (Fig. 30-5).

Protein

Currently available data indicate that high protein diets promote the development of certain age-related diseases, but so far a life-shortening action has not been clearly established.

Longevity studies are complicated by age changes in protein requirement (Hartsook and Mitchell, 1956), a linkage between calcium and protein requirements (Campbell, *et al.*, 1943), and a reduced efficiency of utilization with high protein diets (Forbes, *et al.*, 1944). It was clearly demonstrated by Forbes and his co-workers that increasing the protein content (between

isocaloric rations for maintenance) causes a decrease in the efficiency of the ration by increasing heat production and decreasing the metabolizable energy.

The relationship of the protein content of the diet to longevity was first studied by Slonaker (1931). He found that the life duration of rats was greatest when fed a diet containing 18 percent protein. One complication in protein feeding studies is that the calcium requirement of the rat rises with the protein intake (Campbell, *et al.*, 1943). A study performed by McCay, *et al.* (1943) took into consideration this calcium-protein interrelationship and showed that the life-span of the adult rat is independent of the protein level of the diet between 8 and 30 percent. Similarly, Nakagawa and Mansana (1971) found that raising the protein level from 10 to 27 percent had no significant effect on life-span in the rat. Silberberg and Silberberg (1952) reported that the life-span of C57BL mice was not adversely affected by a 52 percent casein diet.

The protein requirement of the rat decreases with age. Hartsook and Mitchell (1956) found that the rat requires 28 percent protein in the diet for maximal growth at 30 days, 14 percent at 40 days and 7 percent at 80 days. Consequently low protein intakes in adult life may be desirable. Miller and Payne (1968) reported that the feeding of a low protein diet to a small group of adult female rats (from 120 days onwards) significantly increased their life-span.

It has been known for many years that high-protein diets favor the development of renal disease in the rat (Newburgh and Curtis, 1928). However, the work of Bras and Ross (1964) suggests that the intake of calories is more important than protein in the development of glomerulonephritis. Our own study (Fig. 30-5) showed that both calorie and protein intakes are important.

The incidence of tumors in rats increases as the protein and caloric content of the diet rises (Ross and Bras, 1965; Ross, *et al.*, 1970; Ross and Bras, 1973). These workers showed that tumor incidence is directly related to protein intake, when rats are fed isocaloric diets.

Jones and Huffman (1956) reported that a 40 percent casein diet raised the serum cholesterol level and accelerated the development of atherosclerotic lesions in old rats.

From a study of human mortality in 30 different countries, Watanabe, *et al.* (1968) concluded that the life duration increased as the protein content of the diet was raised from an inadequate level; however, mortality from cardiovascular disease became higher. In advanced countries the mortality rate of males in the fifties increases as the protein content of the diet rises (Watanabe, *et al.*, 1968).

Amino Acids

Since the provision of an appropriate pattern of amino acids is the crux of optimal protein nutrition, it is desirable to obtain dietary protein from more than one source to avoid amino acid deficiencies of individual proteins. Thus the addition of amino acids to the diet which is reputed to affect longevity may simply reflect the correction of deficiencies. Methionine, and lysine to a lesser degree, increase the life duration of the rat (Lang, 1957).

Harman (1957) found that cysteine hydrochloride and two other reducing sulphides significantly increased the life duration in AKR mice. According to Oeriu (1964) cysteine reduces the disulphide accumulation in tissues, and therefore counteracts the natural aging change.

Vitamins

Vitamin supplements have been found to increase life duration, but the effects observed may have been due to the correction of deficiencies. Sherman and Trupp (1940) increased life-span by adding vitamin A to the diet of the rat. Pelton and Williams (1958) prolonged the life of mice with supplements of pantothenic acid.

Vitamin E is a biological antioxidant which may counteract biochemical degenerative processes of aging which result from the formation of free radicals (Tappel, 1968). The role of free radicals in aging has been extensively investigated by Harman (1969). Although increases in mean life duration of mice have been observed after the administration of free radical inhibitors (Harman, 1969), there has been no increase in the maximum life duration. Therefore, Kohn (1971) concluded that antioxidants inhibit not aging, but a harmful factor in the diet or environment. Studies in this laboratory failed to establish any protective effect of vitamin E against processes of aging in collagen and kidney.

Conclusions

It has been clearly demonstrated that moderate reductions in the intake of food will retard the aging of collagen, delay the development of certain age-related diseases and prolong the life of the laboratory rat. However, in food restriction studies it is difficult to separate the effects due to caloric restriction from those due to the reduced intake of fat, protein and carbohydrate.

Severe food restriction or the consumption of diets deficient in essential nutrients will shorten the life-span.

High intakes of energy, fat, protein or sucrose accelerate the development of diseases which shorten the life. According to Watanabe, *et al.*

(1968) human daily intakes in excess of 3,000 K calories, 90 g of protein and 50 g of fat are detrimental. It appears that nutritional factors may program the development of diseases later in life.

NUTRITION AND PITUITARY FUNCTION

The retarded aging which results from food restriction may be mediated through the pituitary gland (Comfort, 1964). Undernutrition depresses pituitary function (Mulinos and Pomerantz, 1940), and hypophysectomy retards the aging of collagen and delays the development of certain diseases of old age (Everitt, 1966). Thus retarded aging may be due to the diminished secretion of pituitary hormones in the food restricted animal.

The relationship of nutrition to the function of the pituitary and other endocrine glands was fully reviewed by Ershoff in 1952 and some recent developments by Sims and Horton in 1968. The effects of hormones on nutrition have been reviewed by Hamwi and Tzagournis (1970).

Effects of Undernutrition

In 1908 Lucien reported that malnutrition diminished the weight of the pituitary gland. However, it was probably Jackson (1925) who first suggested that changes in the pituitary may be responsible for the general phenomena of undernutrition.

A number of observers (Boenheim, 1934; Escamilla and Lisser, 1942; Perloff, *et al.*, 1954; Danowski, *et al.*, 1972) have pointed out that the clinical picture of malnutrition closely resembles that of Simmonds' disease (hypopituitarism). Mulinos and Pomerantz (1940) emphasized the depression of pituitary function in the underfed rat, a condition which they called pseudo-hypophysectomy. In such animals there were decreases in the weight of the liver, spleen, thymus, hypophysis, adrenal, thyroid, ovary, uterus and vagina. These changes were similar to, but less marked than, those occurring after hypophysectomy. The major differences are: 1) the food restricted rat possesses a pituitary gland, which is functional even though depressed, and 2) the changes caused by food restriction are reversed by refeeding.

Growth Hormone

To early workers the decreases in body growth and skeletal length in the starved animal indicated a diminished secretion of growth hormone. It was found that the administration of GH was able to induce growth in the underfed rat (Lee, 1938; Li, *et al.*, 1949; Huzra and Fabry, 1957). Only recently, due to the development of the radioimmunoassay technique, has it been possible to measure plasma GH levels satisfactorily, although the levels are very labile. A further problem with these studies is that some

factors which stimulate GH secretion in man (such as insulin-induced hypoglycemia) inhibit GH secretion in the rat (Takahashi, *et al.*, 1971).

The effects of food restriction on GH secretion in the rat depend on the duration of restriction. Starvation for 2 to 5 days in the rat elevates plasma GH level (Trenkle, 1970), which is similar to the human response to starvation or fasting (Roth, *et al.*, 1963; Marks and Howorth, 1965; Cahill, *et al.*, 1966; Hadden and Beif, 1967; Pimstone, *et al.*, 1968). However, when food restriction is continued for 7 days or longer there is depression of both pituitary and plasma GH levels (Dickerman, *et al.*, 1969; Trenkle, 1970; Sorrentino, *et al.*, 1971). The hyposecretion of GH is due to both protein and calorie malnutrition (Samuel and Deshpande, 1972).

GH synthesis by rat pituitary decreases progressively during starvation for 5 days (Akikusa, 1971). The earlier work of Meites and Fiel (1965) suggested that a lack of hypothalamic GRF may be responsible for the impaired synthesis and release of pituitary GH in the chronically starved rat.

ACTH

The secretion of ACTH, based on changes in adrenocortical function, appears to be increased by food restriction at least in the early stages. Most observers report hypertrophy of the adrenal cortex during caloric restriction in man and experimental animals (Lucien, 1908; Byrne, 1919; Selye, 1936; Cameron and Carmichael, 1946; D'Angelo, *et al.*, 1948; Ershoff, 1952; Chowers, *et al.*, 1969; Bouille and Assenmacher, 1970). The study of Mullinos and Pomerantz (1941) suggests that the initial hypertrophy may be succeeded by atrophy in long-continued undernutrition. Adreno-cortical atrophy has been observed in human starvation (Zubiran and Gomez-Mont, 1953; Perloff, *et al.*, 1954). The urinary excretion of 17-hydroxy-steroids is usually decreased in human starvation (Emanuel, 1956; Huseby, *et al.*, 1959; Castellanos and Arroyave, 1961), whereas plasma cortisol levels are either normal (Huseby, *et al.*, 1959; Leonard and D'Arbela, 1966) or elevated (Neuwirth, *et al.*, 1964; Alleyne and Young, 1967). In starved rats the plasma corticosterone level is usually elevated (Boulouard, 1963; Bellamy, *et al.*, 1968; Chowers, *et al.*, 1969), but Eisenstein (1967) observed a fall in the secretion of corticosterone into the adrenal vein after 2 days of starvation. Chowers, *et al.* (1969) found a significant rise in the corticotropin releasing factor (CRF) content of the median eminence of the starved rat. These findings suggest that starvation is usually associated with increased or normal adrenocortical function, but prolonged starvation may lead to adrenocortical depression (Bouille and Assenmacher, 1970).

TSH

A decline in the secretion of thyroid stimulating hormone during food restriction was suggested by the early observations of thyroid atrophy in

the starved experimental animal and in human malnutrition (review Ershoff, 1952). Normal thyroid structure may be restored in undernourished guinea pigs by the administration of a pituitary extract containing TSH (Stephens, 1940). Reduced blood TSH levels have been demonstrated in starved rats and mice (D'Angelo, 1951) and in malnourished infants with marasmus and kwashiorkor (Varga and Mess, 1968; Godard and Lemarchand-Béraud, 1973). The secretion of thyroxine by the thyroid is diminished by food restriction in rats (Grossie and Turner, 1962) and mice (Pipes, *et al.*, 1960).

The decline in thyroid function may be influenced by changes in the metabolism of thyroid hormone in starved rats. Nathanielsz (1970) found that starvation abolishes the biliary-fecal loss of thyroxine and increases the tissue utilization of thyroxine in rats.

Gonadotropins

A diminished secretion of gonadotropins during starvation in female rats is indicated by atrophy of the ovary and uterus and anestrous vaginal smears, and in male rats by atrophy of the testis, prostate and seminal vesicle (review Ershoff, 1952). Chronic malnutrition in human populations has a similar action in diminishing gonadal function and reducing fertility in both sexes (Butler, *et al.*, 1945; Jacobs, 1948; Keys, *et al.*, 1950; Zubiran and Gomez-Mont, 1953). The reduced fertility is associated with a decreased excretion of pituitary gonadotropins (Klinefelter, *et al.*, 1943; Zubiran and Gomez-Mont, 1953). Gonadotropin excretion is also reduced in anorexia nervosa (Emanuel, 1956; Russell, *et al.*, 1965; Danowski, *et al.*, 1972).

In starved animals normal ovarian and uterine weight can be restored by injection of hypophyseal or chorionic gonadotropins (Marrian and Parkes, 1929; Ershoff, 1952). The pituitary content of gonadotropins in starved rats is reported to be decreased (Leathem, 1958; Piacsek and Meites, 1967; Negro-Vilar, *et al.*, 1971) or unchanged (Maddock and Hellar, 1947; Howland, 1972). However, in the hamster, Printz and Greenwald (1970) reported that starvation increased the pituitary FSH content. The effects of starvation on pituitary gonadotropin content appear to depend on the gonadotropin (FSH or LH), sex and species.

Blood levels of gonadotropins also appear to be low in underfed rats (Howland, 1972), probably due to a decreased release rather than synthesis of gonadotropins (Ibrahim and Howland, 1972). Starvation and undernutrition probably act on the hypothalamus decreasing the synthesis and secretion of gonadotropin releasing factors (Piacsek and Meites, 1967; Negro-Vilar, *et al.*, 1971).

Prolactin

The prolactin (luteotropin) content of the pituitary in the underfed rat is reported to be either diminished (Meites and Reed, 1949) or unchanged (Akikusa, 1971). No change occurred in prolactin synthesis in the anterior pituitary of starved rats (Akikusa, 1971).

Conclusion

Food restriction appears to decrease the secretion of most anterior pituitary hormones. For some hormones the picture is confused due to insufficient data or to differences in species, sex and duration of food restriction.

Effects of Overnutrition

The effect of overnutrition on endocrine function has received considerably less attention than that of undernutrition. This subject was reviewed by Rabinowitz (1970). Obesity, or the accumulation of excess body fat, results from the ingestion of more calories than are expended (Wohl, 1968). Lesions of the medial hypothalamus will induce obesity in the rat (Brobeck, 1946), by increasing food intake. However, in human obesity hypothalamic lesions are rarely found (Wohl, 1968) although hypothalamic function may be abnormal. Obesity may be caused by environmental, psychological, familial or endocrine factors.

In Frohlich's syndrome, pituitary disease causes obesity and genital hypoplasia with decreased excretion of gonadotropins. Overeating in the mouse is associated with reduced testicular endocrine function and probably diminished gonadotropin secretion (Liddell and Hellman, 1966).

In Cushing's syndrome, adrenocortical hyperfunction is accompanied by obesity. An increased cortisol production rate in obesity has been reported by a number of investigators (Mlynaryk, *et al.*, 1962; Migeon, *et al.*, 1963; Schteingart, 1965; Eisenstein, 1967; Jackson and Mowat, 1970; O'Connell, *et al.*, 1973), although the increase was not significant when related to body weight. Starvation reduces cortisol secretion in obesity but not in Cushing's syndrome (Jackson and Mowat, 1970). Elevation of body weight in normal subjects by overeating increases cortisol production rate (O'Connell, *et al.*, 1973). Adrenocortical function is thus related to body size.

Less growth hormone is secreted by obese subjects in response to stimulation by hypoglycemia, fasting or arginine infusion than by nonobese (Sims and Horton, 1968; Rabinowitz, 1970).

Thyroid function appeared to be normal in a large series of obese children (Mossberg, 1948; Mayer, 1960). However, Scriba, *et al.* (1967) reported that clinically euthyroid obese patients had a significantly reduced PBI and increased binding of T₃. In the genetically obese rat, thyroid function

is reduced (Bray and York, 1971), but plasma and pituitary TSH are normal (York, *et al.*, 1972). An impairment of TRH formation or release from the hypothalamus is postulated in these rats (York, *et al.*, 1972).

Hyperinsulinism often occurs in spontaneous human obesity and may be responsible for the increased storage of fat (Albrink, 1968; Rabinowitz, 1970). Hyperinsulinemia is necessary for the development of obesity resulting from damage to the ventromedial nucleus in the hypothalamus of the rat (York and Bray, 1972). Obesity was prevented by destruction of the insulin secreting cells of the pancreas with streptozotocin (York and Bray, 1972).

Effects of Specific Dietary Constituents

Fat

The fat content of the diet may affect endocrine function. Fat-free diets fed to rats inhibited growth, caused atrophy of the male reproductive organs and led to a loss of FSH cells in the pituitary (Panos, *et al.*, 1959; Clausen, 1969). The pituitaries of female rats fed a fat-free diet contained a decreased number of acidophils and an increased number of basophils (Panos and Finerty, 1953). Low fat diets brought about atrophy of the adrenal cortex (Alfin-Slater and Bernick, 1958) and decreased the secretion of corticosterone in the rat (Skovsted, *et al.*, 1963). High fat diets are reported to increase the excretion of adrenocortical steroids in man (Glatzel and Hackenberg, 1967). Prolonged feeding of a diet containing 1 percent cholesterol and 5 percent cottonseed oil (Bernick and Patek, 1961) increased the number of pituitary thyrotrophs and caused hyperplasia of thyroid follicles. Corn oil plus heparin inhibits the secretion of GH induced by insulin or arginine in human subjects (Blackard, *et al.*, 1971). This treatment causes a physiological increase in free fatty acid in plasma, a factor believed to control GH secretion (Hertelendy and Kipnis, 1973). These studies indicate that the quantity of fat in the diet influences the level of anterior pituitary, thyroid, adrenocortical and gonadal function.

Carbohydrate

Glucose administration is known to suppress the secretion of growth hormone (Roth, *et al.*, 1963), while a fall in the blood sugar level stimulates GH secretion. Neither a deficiency nor an excess of carbohydrate exerts any deleterious effect on reproduction (Mason, 1949).

Protein

Rats placed on protein free or protein deficient diets show marked atrophy of the adenohypophysis, adrenal cortex, ovary and testis (Hand-

jiev and Dashev, 1971). Low-protein, protein-free or specific amino-acid-deficient diets depress reproductive function in the female rat (Mason, 1949; Ershoff, 1952; Srebnik, *et al.*, 1958). The secretion of pituitary gonadotropins is impaired in protein-deficient diets (Ershoff, 1952). The effect is specifically due to the low protein intake and not to caloric restriction (Samuels, 1950). Srebnik and Nelson (1962) showed that a protein-free diet diminished the secretion of both gonadotropic and growth hormones by the rat pituitary. However, the relationship between protein intake and GH secretion is controversial (Samuel and Deshpande, 1972). The secretion of GH is found to be increased by both high-protein (Pallosta and Kennedy, 1968) and low-protein diets (Pimstone, *et al.*, 1968; Samuel and Deshpande, 1972). Pituitary ACTH secretion declines in the protein-depleted rat (Munro, *et al.*, 1962) and is enhanced by high-protein diets (Henriques, *et al.*, 1949). The thyroid gland involutes in severe protein depletion in the rat (Aschkenasy, 1962; Srebnik, *et al.*, 1963). The thyroxine secretion rate is depressed when the protein content of the diet is less than 5 percent (Singh, *et al.*, 1971). In protein-depleted rats, Florschheim, *et al.* (1970) found that the renal clearance of iodide was reduced and that this raised the protein bound iodine level in serum.

Other Nutrients

The dietary content of iodine, calcium, ascorbic acid, pantothenic acid and other vitamins may influence the functions of the pituitary and other endocrine glands (Mason, 1949; Ershoff, 1952).

Conclusions

The functions of the anterior pituitary, thyroid, adrenal cortex and gonads can be influenced by the amount of fat, carbohydrate and protein in the diet. However, in many studies it is difficult to separate the effects of specific nutrient deficiency from the general effects of caloric restriction.

Nutrition and the Tissue Response to Pituitary Hormones

Nutrition affects not only the synthesis and secretion of hormones, but also the destruction of the hormone and the response of the target organ to the hormone (Samuels, 1950).

Growth hormone promotes little or no body weight increment in nutritionally deficient rats (Ershoff, 1952). The normal growth response is lacking when diets are deficient in vitamin A, vitamin B₁, essential fatty acids or protein. Gordan, *et al.* (1948) found that the amount of nitrogen retained with a given dose of GH increased as the protein intake was raised.

The response to gonadotropic stimulation is not appreciably impaired in the underfed animal (Ershoff, 1952; Leathem, 1961). Gonadal involu-

tion caused by undernutrition appears to be primarily of pituitary origin (Samuels, 1950). However, dietary protein, both in content and type, influences the response of the ovary to gonadotropins (Leathem, 1961). The response of the mouse to LH and HCG is increased by protein deficiency (Srebrik, *et al.*, 1958).

The adrenal response to ACTH is reported to be normal in rats whose diet is deficient in thiamine, riboflavin and pantothenic acid (Ershoff, 1952). The pituitary-adrenocortical system is maintained in the face of severe protein deficiency (Samuels, 1950).

Food Restriction, Pituitary and Aging

If food restriction alters pituitary function, then changes in the secretion of pituitary hormones may be responsible for the retarded aging and increased life-span of underfed rats. The role of the pituitary gland in aging and longevity may be assessed by comparing the aging process in hypophysectomized rats with that of intact rats eating the same quantity of food (see Everitt, Chap. 4, Table 4-I and Table 30-I in this Chapter).

Hypophysectomized rats consume approximately one third of the *ad libitum* food intake of intact control rats. The aging of collagen, as measured by fiber breaking time in 7 M urea, is retarded in both hypophysectomized and food restricted rats (Table 30-I), but the retardation is greater in hypophysectomized rats. In a similar manner renal disease, as monitored by protein excretion, is significantly retarded in both hypophysectomized and food-restricted rats (Table 30-I), the effect being greater in food restricted animals. Life duration is increased in food-restricted rats

TABLE 30-I
THE COMPARATIVE EFFECTS OF HYPOPHYSECTOMY AND FOOD RESTRICTION ON AGING AND LONGEVITY IN THE RAT

Parameter (Age)	<i>Hypophysect.</i> Rats (10) ^a	Food Restricted Rats (10) ^a	Intact Control Rats (10) ^a
Food intake (g/day) (600 days)	6.1 ±0.6 ^b	7	19.9 ±1.2
Collagen breaking time (min) (600 days)	60 ±3.3	82 ±2.6	139 ±2.3
Protein excretion (mg/day) (600 days)	12.8 ±1.2	6.8 ±1.4	60.6 ±11.1
Life duration (days)	(25) ^a 515 ±41	(25) ^a 895 ±38	(25) ^a 785 ±28

^a Number of rats.

^b Standard error of the mean.

but is greatly reduced in hypophysectomized rats eating the same quantity of food. Clearly pituitary hormones are essential for the life prolonging effect of food restriction.

Conclusions

Both experimental and clinical studies indicate that prolonged food restriction depresses anterior pituitary function. However, acute food restriction increases the secretion of ACTH and growth hormone. A number of studies suggest that these effects of food restriction may be due to the lack of specific dietary constituents such as protein, amino acids, fatty acids, vitamins and minerals, as distinct from calories.

The effect of food restriction in retarding aging is partly due to the depression of pituitary function. However, when pituitary function is completely eliminated by hypophysectomy in the rat, life-span is not prolonged by the low food intake, because the pituitary is essential for a normal life-span.

THE PHYSIOLOGY OF UNDER- AND OVERNUTRITION

The food restricted rat has been shown to live longer than its fully-fed control, because of its greater resistance to the diseases of old age (Saxton, 1945; Berg and Simms, 1960; Berg, Chap. 3). It is possible that other changes in the physiology of the underfed animal may contribute to its increased survival. The physiological adaptation to undernutrition has been reviewed by Grande (1964) and Young and Scrimshaw (1971). Most body functions operate at a reduced level in the underfed animal (Table 30-II).

Body Size

When the caloric intake is reduced, the body metabolizes its own energy stores and consequently body weight falls. In adult men 25 percent of the

TABLE 30-II
THE DEPRESSION OF BODY FUNCTIONS IN UNDERNUTRITION

Body weight	Decreased
Basal metabolic rate	Low
Physical activity	Decreased
Intestine	Small
Liver	Small
Plasma proteins	Decreased
Hemoglobin	Low
Heart rate	Low
Cardiac output	Low
Glomerular filtration rate	Low
Vital capacity	Decreased
Thyroid function	Diminished
Gonad function	Diminished

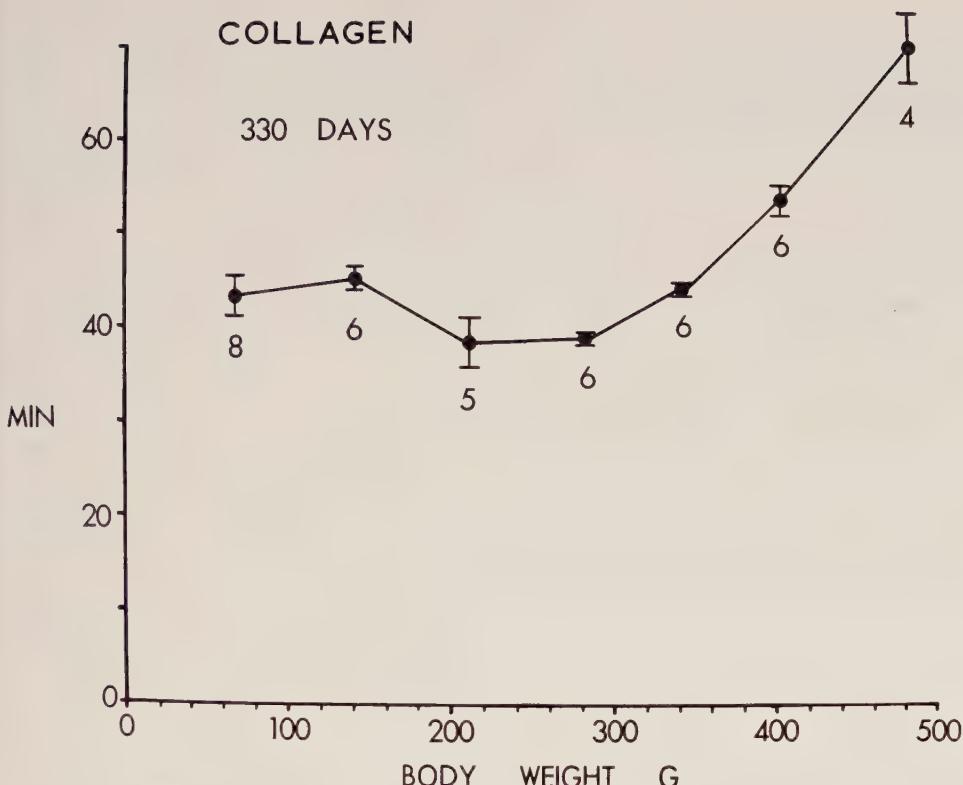


Figure 30-6. The effect of increasing body weight on the biological age of collagen fibers in rats aged 330 days. Collagen age was measured as breaking time in min in 7 M urea under a load of 2 g (From Everitt, 1971, reproduced by permission of S. Karger, Basel, Switzerland).

body weight may be lost in 40 days of starvation. There is a loss of weight in practically all organs of the body except the central nervous system. Chemically there is a loss, not only of fat but also of protein, water and minerals.

Does the reduced body size affect the rate of aging? In male Wistar rats fed a restricted diet from early life the equilibrium body weight in g was approximately 6 times the daily intake in K cal. The aging of collagen was found to be relatively independent of body size in animals weighing less than 300 g (Fig. 30-6). However, above this weight, the aging of collagen increased with body size. Collagen aging appeared to be accelerated by the excessive intake of food, which raised body weight above 300 g. Adult rats fed *ad libitum* weigh 450 g on the average and therefore have increased aging of their collagen fibers. Overweight is known to increase the mortality rate in man (Marks, 1957).

Energy Metabolism

When the food intake is reduced the metabolic rate falls. This adaptation enables the organism to live longer on its own energy reserves. Lee and Lucia (1961) observed a 40 percent reduction in the calorie requirement to maintain body weight in the food-restricted rat after 6 weeks. Starved prisoners of war in concentration camps often had BMR readings of -40. Taylor and Keys (1950) calculated that 65 percent of the decrease in BMR is due to the loss of body mass and 35 percent to the decrease of metabolic rate per unit weight of active tissue. Physical activity is also decreased. Olewine, *et al.* (1964) demonstrated a significant decline in the total daily random movement of the underfed rat.

The lowered metabolic rate may contribute to the increased life duration of calorie restricted rats. If the rate of aging is proportional to the metabolic rate, as suggested by the rate of living theory (Pearl, 1928), then food restricted rats would be expected to live longer. Other factors being equal, an organ working at a slow rate should last longer than one working at a fast rate.

Overeating increases the metabolic rate and heat production (Miller, *et al.*, 1967). In obesity, the oxygen consumption rises but the BMR per unit surface area is found to be almost the same as in the nonobese (Strouse, *et al.*, 1924; Wohl and Ettelson, 1936; White and Alexander, 1965). Thus accelerated aging in the obese subject may be associated with the increased consumption of oxygen by the whole body as well as the excessive intake of food.

Intermediary Metabolism

In the fasted animal, changes in intermediary metabolism enable it 1) to metabolize its energy reserves in adipose tissue, and 2) to supply the brain with glucose. The metabolic pattern shifts to one in which fat becomes the major substrate, resulting in the sparing of protein and carbohydrate (Golden, *et al.*, 1960; Masoro, 1965). There is a fall in the blood sugar level, depletion of liver glycogen, cessation of fatty acid synthesis, increased gluconeogenesis to supply glucose to the brain and decreased carbohydrate utilization by other tissues. The mobilization and oxidation of fat is the major metabolic feature of the fasting state. The plasma FFA level rises and ketosis may occur.

A similar metabolic pattern is found in the animal fed a high fat diet. In these animals the capacity of the tissues to oxidize fat increases, while the ability to utilize glucose is curtailed. Animals adapted to a high fat diet have a diabetic response to the glucose tolerance test and also produce excessive amounts of ketone bodies.

Apparently the metabolism of fat is not a major factor in the aging process. In both food-restricted and fat-fed animals the dominant pattern of metabolism is the utilization of fat as a fuel. Despite the similarity in metabolic pattern the rates of aging and longevity are vastly different. The factor which accelerates aging appears to be the excessive turnover of energy.

Digestive System

A characteristic effect of starvation is great loss of weight by the intestine and liver. Gastric emptying time is increased in semi-starvation (Keys, *et al.*, 1950). High intakes of food lead to enlargement of the digestive tract (Fabry, 1967).

Respiration

In human undernutrition there is a definite decrease in vital capacity, respiration rate and minute volume at rest (Keys, *et al.*, 1950). All these changes are reversed by refeeding.

Studies of pulmonary function in obesity (Barrera, *et al.*, 1967) reveal a tendency to low lung volumes in general, such as reduced vital capacity. The respiratory work is increased in the obese subject, probably due to the extra energy required to move the adipose tissues overlying the chest and abdominal walls (Fritts, *et al.*, 1959).

Cardiovascular Function

Many studies have shown that systolic blood pressure is directly correlated with body weight (Chiang, *et al.*, 1969). Both systolic and diastolic blood pressures are reduced in undernutrition and are restored to normal by refeeding (Keys, *et al.*, 1950). As body weight increases in obesity, the blood pressure rises (Chiang, *et al.*, 1969).

Heart rate is lowered by undernutrition in man (Keys, *et al.*, 1950) and rat (Doerr and Hokanson, 1968). This is to be expected in view of the direct relationship between heart rate and energy expenditure (Payne, *et al.*, 1971).

Cardiac output is reduced by starvation in the dog (Haxhe, 1967) and in man (Keys, *et al.*, 1947; Alleyne, 1966), and increased in human obesity (Whyte, 1959; Alexander, 1963). The circulating blood volume is also greater in obesity (Alexander and Dennis, 1959).

Renal Function

In caloric restriction, renal function is depressed. The urine of malnourished persons is large in volume (Mollison, 1946; Klahr, *et al.*, 1967), but this is probably due to the drinking of large volumes of water in order to feel full (Keys, *et al.*, 1950). The clearances of creatinine (Grande,

et al., 1955) and urea (Sargent and Johnson, 1956) are decreased in under-nutrition. Glomerular filtration is decreased by low protein diets (Pullman, *et al.*, 1954).

In obese persons without pathological findings, the glomerular filtration rate was raised significantly, but there was no change in renal plasma flow (Gabe and Irmscher, 1967). Prolonged fasting of obese patients usually leads to a reduction in the glomerular filtration rate (Gelman, *et al.*, 1972).

Muscular Work Capacity

The ability to perform exhausting work is markedly impaired in acute starvation (Henschel, *et al.*, 1954). Semistarvation over a long period decreases the work performance due to the loss of muscle mass, diminished cardiovascular and respiratory efficiency and increasing anemia, which limit the capacity to supply O₂ to the working muscles (Grande, 1964).

Obese subjects are often physically inactive. This results in reduced energy expenditure and overweight (Morris and Crawford, 1958; Tibblin, 1967). In a treadmill study, Dempsey (1964) found that performance decreased as the subject became more obese.

Endocrine Factors in Adaptation

The role of endocrine glands in the adaptation to starvation and obesity has not been adequately studied. The endocrine glands must play an important part in the adjustment of the body to different nutritional states, as they do in other adaptations.

The major changes in undernutrition are the decrease in energy expenditure and the utilization of body energy stores, principally fat. The lowered secretion of thyroxine in undernutrition probably contributes to the metabolic decline. The increased secretion of pituitary GH and ACTH would stimulate the mobilization of fat as a source of energy. It has been shown that the pituitary gland is necessary for the production of the urinary polypeptide which stimulates fat mobilization (Chalmers, *et al.*, 1960). However, some metabolic changes in starvation, such as the increased release of FFA also occur in the hypophysectomized animal (Goodman and Knobil, 1959).

In obesity the pattern of hormone secretion favors the acquisition and maintenance of fat stores (Sims and Horton, 1968). The decrease in GH secretion would promote storage of the excess food as adipose tissue. Oversecretion of insulin likewise would lead to increased storage of fat and also protein and glycogen. Increased plasma cortisol would promote gluconeogenesis, diminish the peripheral utilization of glucose and so would increase the amount of glucose available for the synthesis of fat.

Conclusion

Undernutrition results in a loss of weight and a decline in metabolic, cardiovascular and renal functions. The calorie-restricted animal uses its body stores of fat as a source of energy. These changes are probably regulated by the endocrine system, whose functions are modified by food restriction.

An increased intake of food reverses the effects of undernutrition, leading to stimulation of metabolic, cardiovascular and other functions. Over-nutrition in the obese subject further increases these functions, usually in proportion to the gain in weight.

HYPOTHALAMIC—PITUITARY—THYROID AXIS AND AGING

It has been shown that the level of food intake influences the aging of collagen, the metabolic rate, the function of the cardiovascular and other systems, the susceptibility to certain diseases of old age and the duration of life. Therefore factors which regulate the intake of food, might be expected to affect organ function, aging and longevity.

The Regulation of Food Intake

The quantity of food eaten is controlled principally by the hypothalamus (Brobeck, 1965; Mayer, 1968; Soulairac, 1969; Fig. 30-7), which receives and integrates information about blood chemistry, temperature, gastrointestinal activity and taste (Lepkovsky, 1973). Other brain structures participate in the control (Grossman, 1972). The intake of food is also influenced by social and cultural factors (Lepkovsky, 1973).

Brobeck (1948) postulated that "animals eat to keep warm and stop eating to prevent hyperthermia." On exposure to cold, large increases in food intake occur (Hamilton, 1967). Army troops living at -40°C consumed 5,000 k cal per day, compared with 3,000 k cal at $+38^{\circ}\text{C}$ (Johnson and Kark, 1947).

Mayer (1955) postulated that glucoreceptors in the ventromedial nucleus of the hypothalamus are sensitive to glucose in the blood. When the blood sugar level falls, due to the action of insulin, the food intake rises. Mayer believed that the animal eats in order to maintain a normal blood sugar level. Kennedy (1953) suggested that the hypothalamus may monitor the blood lipids in order to assess the size of the fat depots. This lipostatic theory shows how long-range regulation of energy exchange might be achieved. There is evidence of a set-point regulation of the adipose tissues, controlled by the hypothalamus (Lepkovsky, 1973).

The hypothalamus also regulates food intake via the pituitary and thyroid (Reichlin, 1967). Both the pituitary and the thyroid have profound

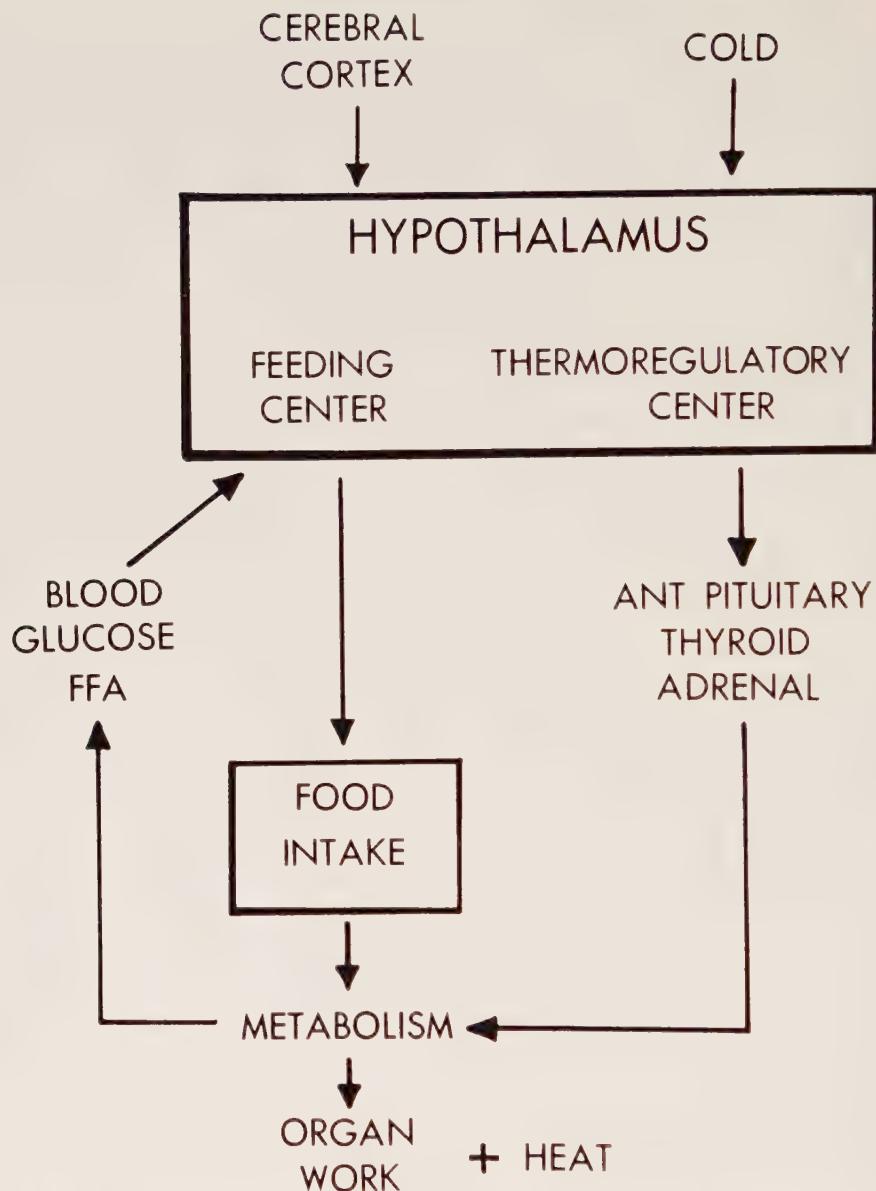


Figure 30-7. The interrelationship of hypothalamic, endocrine and metabolic factors in the control of food intake.

effects on food intake in the rat, probably due to their metabolic effects (Fig. 30-7). Food intake is markedly reduced by hypophysectomy (Ayres and Lee, 1936; Andik, 1957; Everitt and Cavanagh, 1965; Hahn, *et al.*, 1965; Jensen and Privett, 1969) and by thyroidectomy (Andik and Donhoffer, 1957; Grossie and Turner, 1965; Giles and Everitt, 1967). Treat-

ment of adult rats with thyroxine produces large increases in food intake (Andik and Donhoffer, 1957; Everitt, 1958; Hsieh and Ti, 1960; Grossie and Turner, 1961; Everitt, *et al.*, 1969). Increased appetite is a feature of human hyperthyroidism (McClintock, *et al.*, 1956). There is evidence that other hormones such as growth hormone, adrenocortical steroids and insulin increase food intake, as well as increasing the intestinal absorption of carbohydrates (Soulairac, 1969; York and Bray, 1972).

Food Intake and the Regulation of Aging

It is proposed that the aging process is regulated via the hypothalamic-pituitary-thyroid control over food intake (Fig. 30-8). An increased secretion of thyroid hormones raises food intake and metabolic rate, thus en-



Figure 30-8. Simplified scheme showing how activation of the hypothalamic-pituitary-thyroid axis increases food intake, metabolism, organ work and aging. As the work output of an organ rises it ages more rapidly and prematurely develops age-related pathology which leads to organ failure.

abling organ processes to function at a faster rate. The greater the amount of work done by an organ, the sooner it ages and develops pathological changes which cause functional breakdown of the organ.

Activation of the hypothalamic-pituitary-thyroid axis by an appropriate stimulus, such as exposure to low temperature in a mammal produces a large rise in food intake (Kennedy, 1953; Andik and Donhoffer, 1957; Hsieh and Ti, 1960; Kibler and Johnson, 1961). Kennedy (1953) found that the rat acclimated at 4°C ate three times as much as the one acclimated at 36°C. There is a rapid increase in the secretion of TSH and thyroxine or T₃ in experimental animals exposed to a cold environment (Brown-Grant, 1966; Hersham, *et al.*, 1970; Gale, 1973), but not in human subjects acutely exposed to cold (Hersham, *et al.*, 1970). The thyroid hormones are regarded as the hormones of slow adaptation to cold exposure (Heroux, 1969), the hormones of fast adaptation being the catecholamines and the corticosteroids.

Johnson, *et al.* (1963) maintained rats at low temperature (9°C) and found that the food intake increased by 60 percent, pathological lesions appeared early and the life-span was diminished significantly. Controls were kept at 28°C. Similar findings were earlier reported by Heroux and Campbell (1960). Whether the shortened life-span is due to the high food intake or to some other effect of low temperature has not been established. There is little data available on the effects of low temperature on human mortality. Populations experiencing severe winters, as in Scandinavia and Canada, do not have an increased mortality, possibly because the exposure to cold is minimized.

Paradoxically the aging of collagen in tail tendon is retarded by chronic exposure of rats to low temperature (Rassaert and Steinertz, 1968; Hruza and Hlaváčková, 1969). This appears to be a local effect of low temperature on the tail, which is independent of the compensatory rise in metabolic rate occurring elsewhere in the body. The accelerated metabolism of cold-exposed animals is due mainly to the increased secretion of thyroxine. When thyroxine is administered for long periods to rats maintained at normal animal house temperature (25°C) the aging of tail collagen is increased (Everitt, *et al.*, 1969).

The intake of food is raised by those activities of living whose performance demands additional energy. The energy requirement is increased during recovery from fasting, during growth, lactation, exposure to cold, and by physical exercise (Brobeck, 1965). Food intake is increased abnormally by hyperthyroidism, pancreatic diabetes, hypothalamic lesions and in obesity. If the food intake is maintained above the "aging threshold" the aging process will be accelerated, the diseases of old age will appear prematurely and the life-span will be shortened.

Food and the Mechanism of Aging

The mode of action of food in accelerating aging and reducing the resistance to age-associated disease is not clear. A physiological and a biochemical mechanism is suggested.

The physiological mechanism (Fig. 30-8) relates organ work to organ life-span. When the food intake rises, the increased supply of energy permits metabolic, cardiovascular, renal and other functions to work at a greater rate. As the work output of a given organ rises, then its life-span will be reduced. There will probably be an "aging threshold" of activity for each organ. When this threshold is exceeded the aging process will be accelerated. The relationship of work load to organ life-span has been demonstrated in long term studies on the effects of partial loss of function, as for example in unilateral nephrectomy (Kennedy, 1958; Striker, *et al.*, 1969; Elema, *et al.*, 1971). The remaining tissue or organ undergoes compensatory hypertrophy, but eventually shows signs of premature breakdown with the appearance of senile or pathological changes. Kennedy (1958) observed that in the unilaterally nephrectomized rat, lesions appeared prematurely in the remaining kidney. Similarly, after unilateral ovariectomy in the mouse (Table 30-III), the remaining ovary hypertrophies and reaches the end of its reproductive life prematurely (Jones and Krohn, 1960; Biggers, *et al.*, 1962). This is not seen in rats and rabbits (Adams, 1970), although the remaining ovary in women after hem ovariotomy usually becomes exhausted well before the normal age of menopause (Magendie, *et al.*, 1953). Further, liver regeneration following partial hepatectomy is accompanied by cytological changes characteristic of senile livers (Thung and Hollander, 1967). This agrees with the observations of Hayflick (1968) who found that cells divide for a finite number of generations and then die. Tumors frequently develop in overstimulated thyroid glands, following partial thyroidectomy (Doniach and Williams, 1962), sub-total destruction of thyroid tissue with radio iodine (Goldberg

TABLE 30-III

EFFECT OF UNILATERAL OVARIECTOMY ON THE REPRODUCTIVE PERFORMANCE OF LAC GREY MICE

	One-Ovary Mice	Two-Ovary Mice
Number of mice	10	10
Offspring/litter	4.9 ± 0.75	5.9 ± 0.35
Litters/mouse	4.1 ± 0.8	8.4 ± 1.1
Offspring/mouse	23.7 ± 5.3	50.1 ± 7.3
Age at birth of last litter	280 days	363 days

Jones and Krohn, 1960. Reproduced by permission of the authors and the Journal of Endocrinology.

and Chaikoff, 1951), or exposure to goiterogens (Purves and Griesbach, 1946) or low iodine diet (Axelrod and Leblond, 1955). Pituitary hormones are required for the compensatory growth (and presumably the accelerated aging) of the kidney (White, *et al.*, 1947; Kennedy, 1958), ovary and thyroid.

The biochemical mechanism relates metabolic activity to aging. Several workers (Bjorksten, 1968; Milch, 1965) have shown with *in vitro* studies that certain metabolites, derived from the intermediary metabolism of foodstuffs, cause cross-linkage and accelerated aging of collagen. The physiological and pathological changes may have their origin in this biochemical process of cross-linkage of macromolecules, or possibly some other biochemical age change. Metabolites may cause the cell damage, which we call aging.

CONCLUSIONS

An excessive intake of food increases the rate of aging in tail tendon collagen, hastens the development of the diseases of old age and shortens the life-span. Conversely a reduction in food intake retards aging and prolongs life. It is postulated that the hypothalamic-pituitary-thyroid axis regulates the aging process via its control over food intake. The long-continued elevation of food intake occurring in obesity, in a cold environment, and in hyperthyroidism accelerates the aging process, precipitates the early development of age-associated disease and so reduces the life duration.

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CHAPTER 31

THE HYPOTHALAMIC MECHANISMS OF AGING

V. V. FROLKIS

SUMMARY

THE AGE-INDUCED CHANGES in the hypothalamus are so essential and diverse that they determine a number of important mechanisms of aging as well as the changes in metabolism and function of cells and the decrease in the adaptive capacities of the organism as a whole.

It was noted that there was a certain reduction in the activity of the neurosecretory process in the hypothalamo-hypophyseal system of old animals. However, the changes undergone by the neurosecretory process in young adult and old animals differ under the influence of various stimuli. Thus, when adrenaline is introduced, the activation of the neurosecretory process is more pronounced in old animals, whereas pain stimulation of the skin and stimulation of the hypothalamus by other means (electric stimulation of the amygdaloid complex nuclei) cause more marked activation in young-adult animals.

The rhythms of the bioelectric potentials in parts of the hypothalamus become somewhat retarded with the advance of old age. With increasing age, the electro-excitability of some structures of the hypothalamus (anterior and posterior parts) rises, while in others (lateral part) it diminishes.

At the same time, the sensitivity of the hypothalamic structures to the direct effect of adrenaline and acetylcholine (intra-hypothalamic micro-injection) increases.

Stimulation of the hypothalamus of old animals results in a less pronounced activation of protein biosynthesis. The decrease in the thresholds of pressor reactions from anterior and posterior parts of the hypothalamus with the invariability of the depressor thresholds, the increase of their sensitivity to humoral factors, the alteration of correlation between the reactions of heart and vessels promotes the development of hypertensive shifts in the arterial blood pressure.

INTRODUCTION

An organism's aging is not merely the sum of aging in its individual cells. For this reason, to understand the essence of aging it is necessary to

know the sequence of emergence of age-related changes, as well as to define the primary and secondary shifts in the course of development of this biological process. The similarity between a number of manifestations of aging, on one hand, and clinical aspects of hypothalamic disease, on the other, has led to the suggestion that age changes in the hypothalamus influence the mechanism of functional and metabolic changes in aging (Groen, 1959; Aschheim, 1965; Borisov, 1966; Dilman, 1968 and 1971; Frolkis, 1970; Everitt, 1972; Frolkis, Bezrukova, Duplenko and Genis, 1972; Mankovsky and Mints, 1972). It should be emphasized that in the overwhelming majority of cases, conclusions about changes in the state of the hypothalamus with age have been drawn from the study of its structural changes and on the basis of clinical and physiological analogies between the manifestations of aging and disturbances in the function of the hypothalamus. Thus from comparisons of this kind, Aschheim (1965) and Dilman (1968 and 1971) arrive at a belief that the functional activity of the hypothalamus is increased in old age, Groen (1959) that it is reduced, Borisov (1966) that the general activity of the hypothalamus, and especially of its anterior section, is reduced and the activity of the posterior part of the hypothalamus is relatively increased. The contradictory character of these concepts is in many respects due to the fact that workers have not made full use of the repertoire of modern physiological techniques. These make it possible to characterize the state not only of the hypothalamus as a whole, but of its individual structures.

A direct study of the functional state of the hypothalamus would be of great importance for clarification of its age changes. We think that a comprehensive evaluation of the neurosecretory function of hypothalamic nuclei, including their electric activity, excitability, sensitivity to humoral factors, with an analysis of the influence of the hypothalamus on the cardiovascular and respiratory systems will make it possible to establish the true role and position of this structure in organismic aging. The experiments were performed jointly with Bezrukova, Duplenko and Genis.

NEUROSECRETORY ACTIVITY

The existence in the hypothalamus of cells showing a specific form of activity which may intrinsically lead to self-destruction of neurons with age led a number of investigators to attribute an important role to neurosecretory changes in the mechanism of aging.

Besides claims of a reduced functional activity of the hypothalamic neurosecretory elements with age (Nikitin and Tverskaya, 1951; Andrew, 1956; Sager, 1962), there are data which show no difference in hypothalamic neurosecretion between young adult and old animals (Rodeck, Lederis

and Heller, 1960), although this might be connected with difficulties of method. According to Khmelnitsky (1969), the determination of neurosecretory peculiarities in people of various ages is very difficult, since they depend much on the character or length of the agonial state.

Age Changes

In our approach to the problem of neurosecretory activity of the hypothalamus in aging, we have tried firstly to determine the peculiarities of neurosecretion in animals of various ages in the state of rest; secondly, to study specific features of neurosecretory nuclei of the hypothalamus under conditions of activation by afferent actions on the hypothalamus; and thirdly, to compare age changes of neurosecretory activity of the hypothalamus with age-related peculiarities in the development of stress.

The experiments were carried out on 64 healthy albino rats (males) of two age groups (9-10 and 24-26 months). Animals received water *ad libitum*. Unanaesthetized rats were killed by guillotining. The functional state of neurosecretory elements was determined by histochemical detection of the neurosecretory substance with paraldehydefuchsin by the method of Gomori (Gabe, 1953) and employing karyometry with subsequent calculation of the nuclear size (Szentagothai, *et al.*, 1965; Khesin, 1967). Serial frontal cerebral sections were used to study the supraoptic and paraventricular nuclei, median eminence, as well as the posterior lobe of the pituitary.

The study of intact rats showed that the activity of the neurosecretory process in these hypothalamic nuclei declines with age.

In 10-month rats, cells in the supraoptic nucleus are found in all phases of the neurosecretory mitotic cycle (Fig. 31-1a). The paraventricular nucleus is less active than the supraoptic. Tissues of the hypothalamo-hypophyseal tract contain small amounts of densely packed neurosecretory granules.

In 24-month-old rats, most of the cells both in the supraoptic and paraventricular nuclei are filled with neurosecretory granules which often merge together into coarse, dense lumps which occupy the peripheral zone of the perikaryon and cellular processes. Thus the latter are distinctly outlined within the nucleus and in the periphery along the hypothalamo-hypophyseal tract. As a result of decelerated secretion there are seen many dark cells with a large quantity of Gomori-positive substance in all the elements of the neurosecretory system (Fig. 31-1b). There are often found degenerated neurons of an elongated angular shape with a poorly outlined pyknotic nucleus. Such cells are located along the periphery of the supraoptic and paraventricular nuclei. In old rats the size of the nuclei of secre-

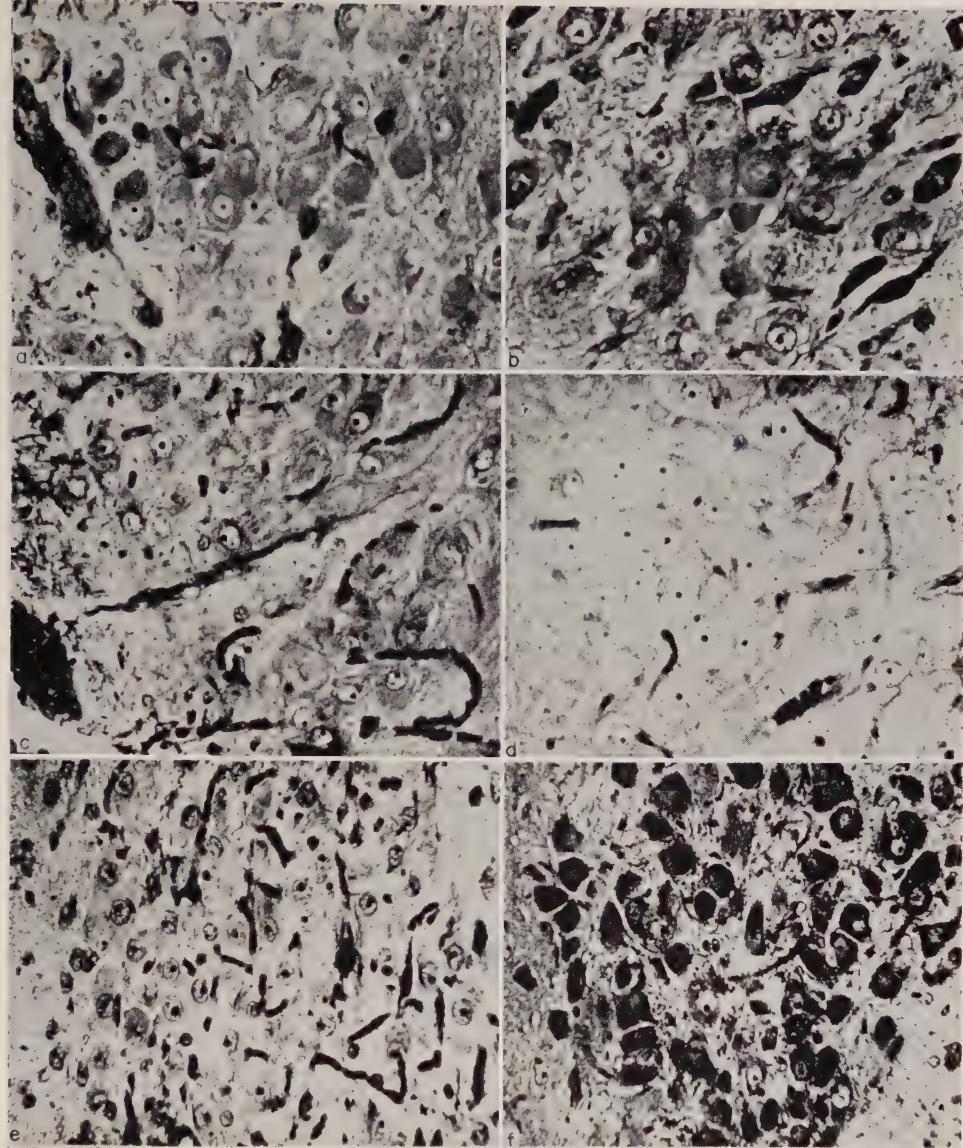


Figure 31-1. Age differences in neurosecretory activity of anterior hypothalamic cells and their reactions to various stimulations (staining with paraldehyde-fuchsin). (a) nucleus supraopticus of the young adult intact rat (10-month male). Large pale neurons prevail. All stages of the neurosecretory cycle can be seen. Magnification 10×40 . (b) nucleus supraopticus of the old intact rat (24-month male). The number of active neurons is significantly decreased. Small- and medium-size cells with dark-coloured cytoplasm prevail. Magnification 10×40 . (c) reaction of nucleus supraopticus of the adult rat on intraperitoneal injection of adrenaline. Magnification 10×40 . (d) nucleus supraopticus neurons of the old rat. Strong activation of synthesis and withdrawal of neurosecretory substance after adrenaline administration. Large, pale neurons with enlarged nuclei prevail. Hyperemia. Magnification 10×40 . (e) reaction of nucleus paraventricularis of the adult rat on electric stimulation of nucleus amygdalaris. Marked hyperemia, neuron bodies being free from neurosecretory substance. Magnification 10×20 . (f) reaction of nucleus paraventricularis of the old rat on electric stimulation of nucleus amygdalaris. Slight hyperemia, neuron bodies being filled with neurosecretory substance. Magnification 10×20 (Reproduced from Frolkis, *et al.*, 1972, with permission of Pergamon Press Ltd.).

tory neurons, and of their nucleoli, is perceptibly reduced (average volume of nucleus is $494 \mu^3$ and of nucleolus $7.6 \mu^3$). As is seen in comparing Figure 31-1a and 31-1b light cells with a large nucleus and two nucleoli, found in a more active phase of neural secretion, occur in old rats much less frequently than in adults.

In old rats the accumulation of neurosecretory substance prevails in all the elements of the neurosecretory system. It may be assumed that the accumulation of neurosecretion is due not to its increased production but to retarded secretion. Thus the fibers of the hypothalamo-hypophyseal tract and of the median eminence in old rats contain large quantities of neurosecretory substance.

In old animals much more neurosecretion has been found in the posterior pituitary than in 10-month rats; pituicytes and endothelial cells of the capillary walls are concealed by great quantities of neurosecretion.

These changes in secretory neurons which we observed in aging are considered by some workers as a sign of reduction in functional activity (Szentagothai, *et al.*, 1965; Polenov, 1968).

Stimulation Effect

In accordance with the literature, the hypothalamic neurosecretory mechanism is of vital importance in coordinating endocrine glands to elicit organism reactions under the action of stress stimuli.

Our tests have shown that a qualitative reorganization of the ability of cells to react to various influences (reduced reaction to reflex stimulations, increased changes due to adrenaline administration) develops against the background of reduced neurosecretory function.

Painful skin stimulation (100 V, 20 pulses per 30 sec, pulse duration 0.25 sec) in adult animals clearly activates the synthesis and discharge of neurosecretory material (Fig. 31-2a).

Similar age differences persist during electric stimulation of afferent fibers of n. tibialis ant.

In old rats painful stimulation does not cause a considerable activation of neurosecretory discharge and this is especially well seen on the preparations of the posterior pituitary (Fig. 31-2b). It appears then that reflex pain stimulation induces a less intensive neurosecretory reaction in old animals.

Similar results have been obtained with afferent stimulation of the hypothalamus. With electric stimulation of the anterocorticomedial group of amygdaloid nuclei, the activation of neurosecretory elements of the anterior hypothalamus was more marked in adult than in old animals (Fig. 31-1e, 31-1f).

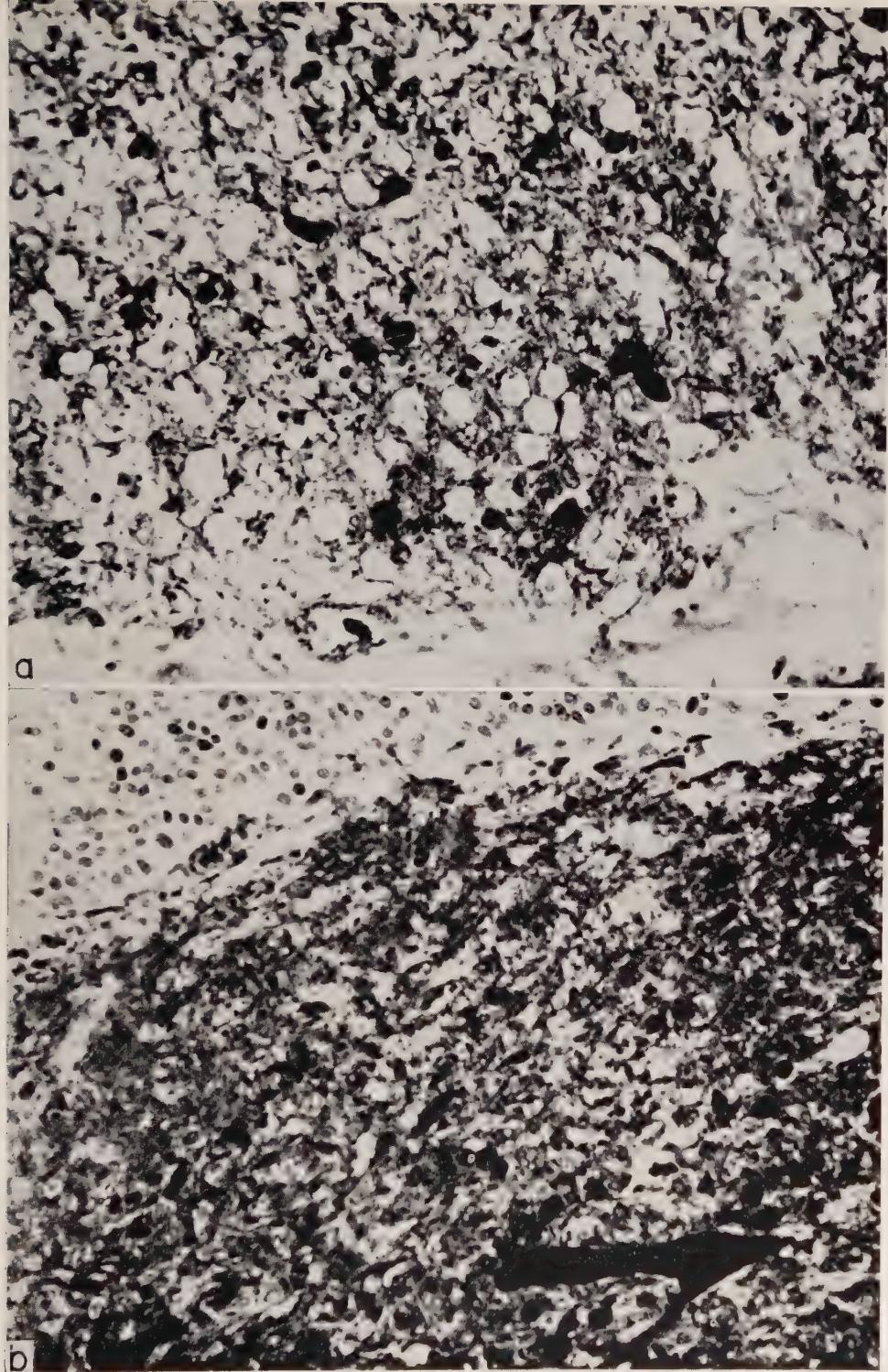


Figure 31-2. Effect of painful skin stimulation on neurosecretion in adult (a) and old (b) rats. a: reaction of posterior pituitary cells of the adult rat (11-month male.) Magnification 10×10 . b: reaction of posterior pituitary cells of the old rat (26-month male.) Magnification 10×10 (Reproduced from Frolkis, *et al.*, 1972, with permission of Pergamon Press Ltd.).

Thus both with reflex stimulation of the hypothalamus and with direct stimulation of the central interneuronal connections in adult animals, the activation of the neurosecretory process was more marked than in old rats.

At the same time the administration of adrenaline causes a greater stimulation of the process of neurosecretion in old rats (Fig. 31-1c, 31-1d).

These data give evidence of qualitative and quantitative changes in the neurosecretory activity of the hypothalamus observed in aging.

The above changes in the neurosecretory process can explain important age features of stress response to various stimulants.

As is seen in Figure 31-3 with reflex stimulants (painful skin stimulation

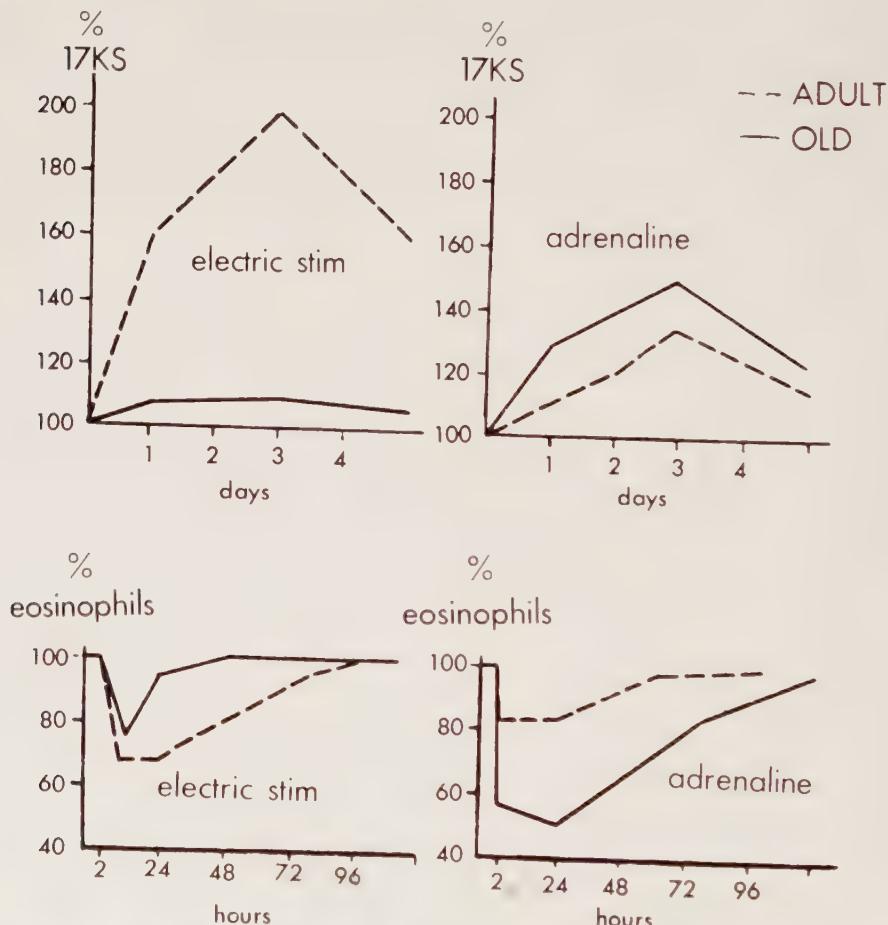


Figure 31-3. Age peculiarities in the percentage change in eosinophil count and 17-ketosteroid excretion in response to the stress of electric stimulation and adrenaline administration ($33 \mu\text{g}/100 \text{ g}$) in adult and old rats (Reproduced from Frolkis, *et al.*, 1972, with permission of Pergamon Press Ltd.).

with electric current), stress symptoms are more openly expressed in adult animals and with adrenaline stimulation in old animals.

Moreover, in old animals, disruption and exhaustion of the adaptive mechanisms due to prolonged stress stimulation occur much quicker than in adults (Frolkis, 1970).

On the basis of these data it seems likely that a leading role in the development of age specific characteristics in the course of the general adaptation syndrome may be ascribed to the hypothalamus, in general, and to specific features of its neurosecretory function, in particular.

ELECTRIC ACTIVITY

Bioelectric activity of various sections of the hypothalamic region in animals has been described by many authors (Grinker and Serota, 1938; Porter, 1952; Green and Morin, 1953; Gromova, *et al.*, 1965; Baklavadzhan, 1967), but age differences are again restricted to the early stages of ontogenesis.

In our experiments with young adult (8-12 months) and old (42-54 months) rabbits implanted monopolar silver or steel electrodes were introduced stereotactically into the anterior, lateral and posterior hypothalamic regions (a steel screw or a silver ball fixed over frontal sinus of the animal was used as the indifferent electrode).

In the acute experiments on 15 adult and 25 old rabbits, the background bioelectric activity of various hypothalamic regions was recorded several hours later, after the narcotization of the animals with urethane (intraperitoneal injection of 0.8-1.2 g/kg). The anatomical control of electrode insertion sites was performed after each experiment. The records obtained from correctly situated electrodes were analyzed. The data received on animals without pathological changes in brain and internal organs were taken into account.

In this case, in adult rabbits, rhythms of 3.5 to 5 Hz were recorded in the anterior and lateral regions of the hypothalamus—supraoptic nucleus, anterior hypothalamic area, medial and lateral preoptic areas, lateral hypothalamic area (amplitude up to 100 μ V) and in the posterior hypothalamus—posterior hypothalamic nucleus, nuclei of mammillary bodies (amplitude up to 200-250 μ V). The frequency of overlapping oscillations was 15 to 25 Hz (amplitude 15-30 μ V).

In old animals, slower rhythms of 3 to 4 Hz (amplitude up to 150 μ V in anterior and lateral and 200-300 μ V in posterior regions) were recorded both in the anterior and lateral and in the posterior regions of the hypothalamus. In the anterior and lateral sections of the hypothalamus the frequency of overlapping oscillations was 10 to 20 Hz.

In the chronic experiments bioelectric activity patterns in old animals stabilized much later (4th-8th day after operation) than in the adults (3rd-5th day).

As compared with the acute experiments, biopotential rhythms were more monotonous, but the main age difference in the rhythm frequency remains: in old animals the frequency of oscillations in various areas of the hypothalamus was lower (3-5 Hz) than in the young adults (4-6 Hz).

There was no distinct differences in wave amplitude.

As in the acute experiments, the electrographic curve was more uniform and monotonous in old animals, and short-term outbreaks of more frequent oscillations occurred considerably more rarely than in the young adult animals. These features of bioelectric traces in various areas of the hypothalamus can be interpreted as a reduction of the background bioelectric activity

ELECTRIC EXCITABILITY AND SENSITIVITY TO SOME HUMORAL FACTORS

The function of a system is assessed by studying the effects of direct stimulation. Electric stimulation was used throughout.

Taking into account the polymorphism of inter- and intrastructural hypothalamic connections, we judged the threshold of excitability of a point in the hypothalamus by the electrographic reaction of a symmetric point or of the neighbouring areas of the hypothalamus, neocortex, amygdala, hippocampus, reticular formations of the mid-brain and medulla oblongata. Stimulation was performed with a current of 100 Hz, pulse duration 1 msec and the whole stimulation period extended over 15 to 20 sec.

It has been proven that the electric excitabilities of various areas of the hypothalamus change differently with age (Frolkis, *et al.*, 1972). The excitability of the posterior hypothalamus in old animals is substantially higher, that of the anterior slightly higher, and of the lateral substantially lower than in the adult animals. Electrographic reaction was recorded after stimulation of the anterior hypothalamus in adult animals with $40 \pm 9 \mu\text{A}$ and in the old, with $23 \pm 4 \mu\text{A}$ ($p > 0.1$), of the lateral hypothalamus with 36 ± 7 and $90 \pm 14 \mu\text{A}$ respectively ($p < 0.01$), of the posterior hypothalamus with 70 ± 13 and $30 \pm 4 \mu\text{A}$ ($p < 0.02$).

To summarize, the obtained data indicate that electroexcitabilities of various sections of the hypothalamus change with age differentially and in many directions.

Adrenaline, noradrenaline, acetylcholine, insulin and some other substances possessing hormonal and mediatory action play an important part in intracentral connections, in the maintenance of a certain functional

TABLE 31-I

THRESHOLD DOSES OF ADRENALINE, NORADRENALINE AND ACETYLCHOLINE
($\mu\text{g}/\text{kg}$ BODY WEIGHT) PRODUCING EEG CHANGES IN ADULT AND OLD
RABBITS AFTER INTRAVENTRICULAR INJECTIONS

Age of Animals	Adrenaline	Threshold Doses (Mean \pm S.E.)	
		Noradrenaline	Acetylcholine
10-12 months (adult)	1.70 \pm 0.61	2.36 \pm 0.88	0.34 \pm 0.05
42-54 months (old)	0.22 \pm 0.03	0.22 \pm 0.03	0.03 \pm 0.02
P Adult-old	< 0.05	< 0.05	< 0.001

state of the central nervous system, and in the transmission of central influences to the periphery.

Smaller doses of adrenaline, noradrenaline and acetylcholine injected intraventricularly produce typical electrographic changes in the hypothalamus of old animals if compared with the adult ones (Table 31-I).

To achieve a more localized action on individual structures of the hypothalamus in our experiments, minimum quantities of acetylcholine and adrenaline were administered with a microinjector into the nuclei of the anterior, lateral and posterior areas of the hypothalamus. Though equal amounts of adrenaline and acetylcholine were injected into the hypothalamus of adult and old animals, the microinjections caused the corresponding changes more often in the old animals and the changes were more marked in them than in the adults.

Thus with 1 μg of adrenaline administered into the posterior hypothalamus, the electrographic reaction occurred in old animals in 8 cases out of 10, and only in 4 cases out of 9 in the adult ones; with lateral hypothalamus injections, in 5 cases out of 5 in old animals and in 1 out of 4 in adults; with anterior hypothalamus injections the respective figures are 7 out of 7 and 2 out of 7.

Thus with age the sensitivity of the hypothalamus to a number of humoral factors increases, the degree of change in sensitivity being largely dependent on the neuropharmacologic and physiologic characteristics of the respective area.

HYPOTHALAMIC CONTROL OF CARDIOVASCULAR SYSTEM

More than 10 years ago, Frolkis (1959) postulated the existence of a hemodynamic center as a complex integration of neurons at various levels of the central nervous system providing for the regulation of hemodynamics, in adapting to the needs of the organism.

With this in mind we deemed it necessary to record in the experiments with hypothalamic stimulations not only arterial pressure and cardiac rhythm, but also the cardiac output and peripheral resistance.

Thresholds of pressor reactions to the stimulation of anterior and posterior hypothalamus of old animals are lower than in the adults. At the same time the thresholds of pressor reactions from the lateral hypothalamus increase with age. Thresholds of depressor reactions do not change significantly with age (Fig. 31-4). Irregular changes in pressor and depressor reactions from the hypothalamus may contribute to the development of arterial hypertension in old age.

With increased stimulating current, old animals exhibited disorders of the ECG such as auricular and ventricular extrasystoles somewhat more often than adults. The recovery period of circulatory and respiratory changes was more delayed and persistent in old animals. However, the hypothalamic control of autonomic balance is characterized not only by qualitative changes.

In the next series of experiments we obtained data demonstrating different hemodynamic contribution to similar arterial pressure reactions in animals of various ages. The results of one such experiment are illustrated in Figure 31-5. Cardiac output was measured in accordance with the ther-

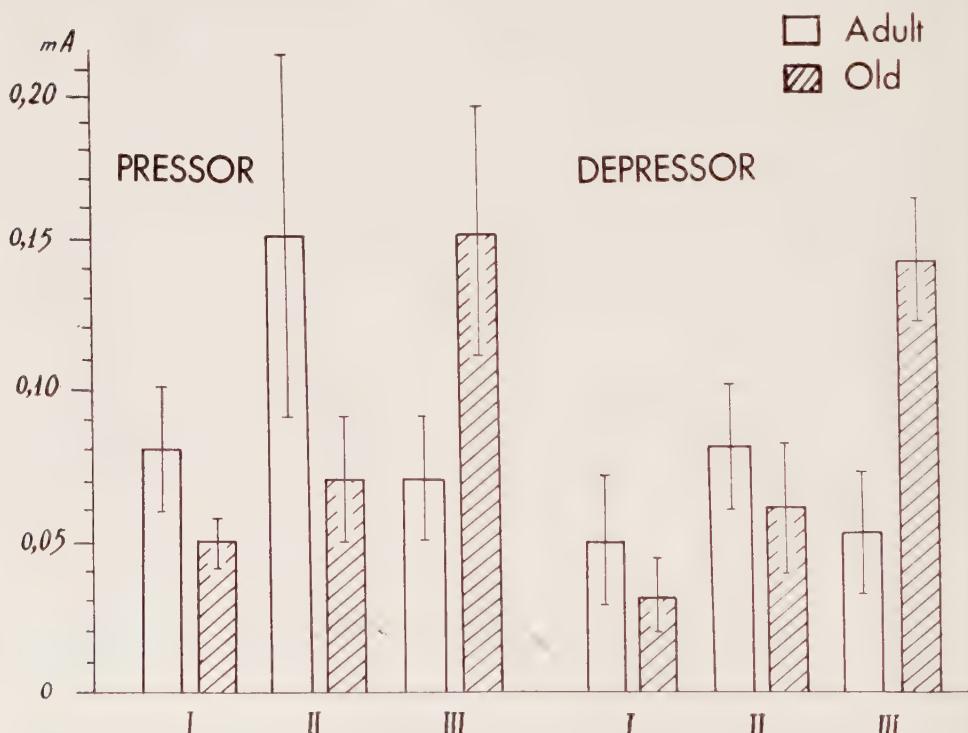


Figure 31-4. Changes in the threshold of pressor and depressor reactions due to electric stimulation of anterior (I), posterior (II) and lateral (III) parts of the hypothalamus of adult (white columns) and old (dashed columns) rabbits.

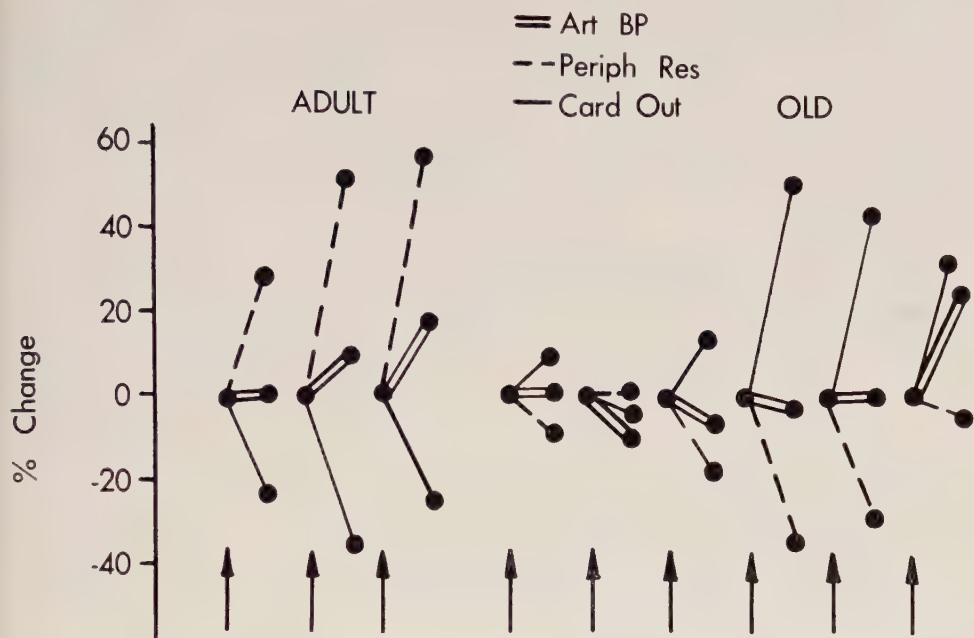


Figure 31-5. Hemodynamic changes (arterial blood pressure, peripheral resistance, cardiac output) in 18-month (ADULT) and 53-month (OLD) rabbits on electric stimulation of nucleus supramammillaris hypothalamus. Arrows show the stimulation with gradually increasing voltage (Reproduced from Frolkis, *et al.*, 1972, with permission of Pergamon Press Ltd.).

modilution method by Fegler (1954) as modified by Gurevich, *et al.* (1967).

On stimulation of the hypothalamus, the increase in cardiac output occurs more often in old animals than in adults.

It should be emphasized that the increased sensitivity of hypothalamic structures to catecholamines and acetylcholine observed in recording the EEG was also noted in recording arterial pressure changes.

Hence, with increasing age the incoming influences from different sections of the hypothalamus change unequally, leading to both qualitative and quantitative changes in the regulatory influences of the hypothalamic area.

DISCUSSION

So, direct experimental data confirm that the hypothalamus undergoes histochemical and functional changes in aging. This is indicated by the demonstrated changes in neurosecretory activity of the hypothalamus, shifts in its bioelectric activity, electrical excitability, sensitivity of the hypothalamus to humoral factors, peculiarities of the hypothalamic control of the cardiovascular system and of the course of stress reactions.

The hypothalamus is the central element in the cerebral integration of autonomic processes, the regulator of organism adaptive processes, and the most important mechanism for homeostatic regulation of its inner medium. The changes found in the function of the hypothalamus are so essential and diverse that in many respects they may well determine the changes of metabolism and functions, and of adaptive capabilities of the organism with age.

Molecular and genetic changes and shifts in protein biosynthesis are quite reasonably considered at the present time as being of major significance in the mechanism of aging. Still, it is usually forgotten that frequently the intracellular changes in protein biosynthesis can be only one of the links in a complex chain of reactions connected with the most important mechanisms of neurohumoral regulation. Hence, shifts in protein biosynthesis during the course of such organism reactions as muscle activity, nutrition and various stress situations would depend both on the condition of intracellular mechanisms of regulation of genetic apparatus and on extracellular, neurohumoral mechanisms. The most important task of gerontology is to find the link which limits to a great extent the range of protein biosynthesis activation.

Our experimental data testifies to the fact that age-related shifts in the function of the hypothalamus cause a number of changes in the reaction of genetic apparatus with aging. In a series of life situations the shifts in the hypothalamus limit the activation range of protein biosynthesis.

So, we have studied in adult and old rats the changes of adaptive synthesis of tyrosine aminotransferase (EC 2.6.1.5), tryptophane pyrrolase (EC 1.11.1.4), glucose-6-phosphatase (EC 3.1.3.9), and fructose-1,6-diphosphatase (EC 3.1.3.11). Changes in content and turnover of RNA in the liver under stress were studied as well. In one series of cases, stress had been reproduced by a humoral factor (adrenaline 30 µg/100 g, intraperitoneally), in the other, by painful stimulation. It appeared that with "reflex" stress the protein biosynthesis activation in liver cells was more pronounced in adult rats than in old ones. With the administration of adrenaline the interrelation became inverse—shifts in protein biosynthesis were more obviously expressed in old animals as compared with the adult ones.

It may be admitted that these differences are connected with age-induced changes in the hypothalamic function. The experiments with the stimulation of the hypothalamus provide direct evidence of age-related shifts in the hypothalamic regulation of protein biosynthesis with aging. As is seen in Figure 31-6 the stimulation of the ventromedial nucleus of the hypothalamus induces in the liver of old animals less pronounced inductive synthesis of tyrosine aminotransferase. Principally similar results have been obtained during the study of age-related peculiarities of hypothala-

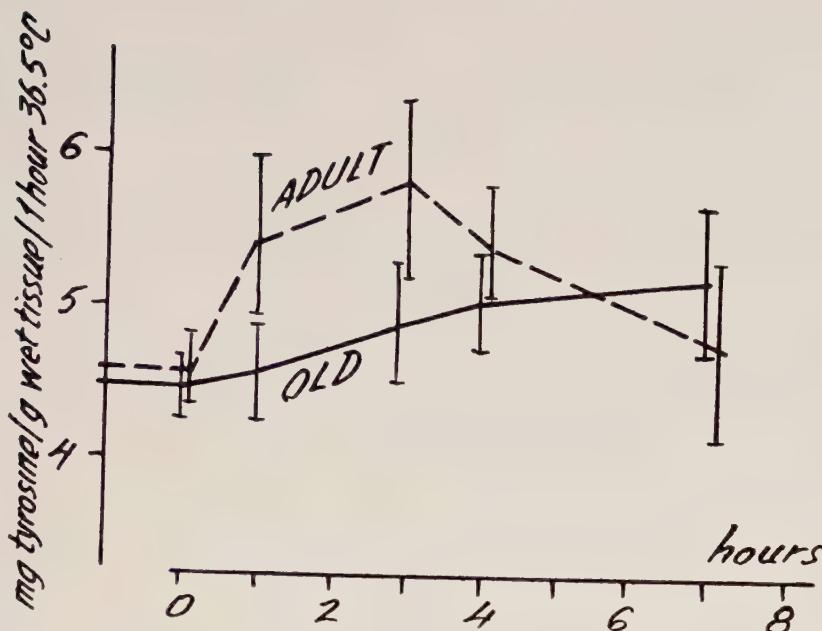


Figure 31-6. Changes in the activity of tyrosine aminotransferase of liver after electric stimulation of ventro-medial nucleus of the hypothalamus of young adult and old rats.

mic regulation of induction of tryptophane pyrrolase, glucose-6-phosphatase in liver (Frolkis, *et al.*, 1974).

The influence of the hypothalamus on protein biosynthesis in liver is mediated through a number of hormonal links. After hypophysectomy and adrenalectomy, the stimulation of the hypothalamus induces no activation of protein biosynthesis. To elucidate which of the links of hypothalamo-hypophyseal-adrenal system limits the protein biosynthesis activation in old age, we have compared the peculiarities of inductions of a number of enzymes in liver after the administration of hydrocortisone and adrenocorticotropic hormone, and by stimulation of the hypothalamus (Frolkis, 1970).

In such a sequence of experiments we managed to compare the age-related changes of genetic induction after switching-on different links of the regulatory system. It was found that with the administration of small doses of hydrocortisone (1 mg/100 g body weight) and ACTH (1 U/100 g body weight), the induction of enzymes in the liver of old rats was more clearly expressed than in adult ones. With large doses of the hormone the interrelation was inverse—the possible range of protein biosynthesis activation in adult animals was wider than in old ones.

The results of experiments indicate that under these conditions (genetic induction of enzymes in liver) protein biosynthesis is limited by the

changes which occur, first of all in the hypothalamus, but not in liver cells, adrenal cortex or hypophysis.

Thus, changes in the hypothalamus can determine essential shifts in the activation of the genetic apparatus and in protein biosynthesis stimulation in old age.

Obviously, the aging of a number of cells can be the alloy of both their own age-related changes as well as the neurohumoral influences (and hypothalamic, in particular) on the processes of metabolism and protein biosynthesis.

The mechanism and functions of the aging organism change heterochronically. This heterochronia is expressed by different degrees, different directions, different time of development of the changes of various systems and of various metabolic cycles of the organism.

In this respect attention should be drawn to the fact that age changes in the hypothalamus also develop heterochronically, irregularly and multidirectionally. Indeed, if, for example, the electric excitability of the anterior and posterior areas of the hypothalamus increases, the electric excitability of the lateral area decreases. Along with this, the sensitivity of all these structures to catecholamines and acetylcholine increases with age.

It may be assumed that these irregular changes in various structures of the hypothalamus may be important in the formation of heterochronic age changes in the systems of the organism.

The irregular changes of the background activity of various hypothalamic structures are combined with the decreased effort required to take them out of this state. Apparently this mechanism makes for a more easy disturbance of different systems, and (in the case of stronger stimulation) of a disruption of regulating mechanisms of homeostasis in old age. It is "a hypothalamic mechanism of reduced reliability of homeostasis in old age." This peculiar instability of the hypothalamic mechanism is a possible cause of the development of hypertensive crises, of essential and sometimes long-term changes of the autonomic functions in people during old age, changes well known to clinicians.

Contemporary physiology has accumulated extensive evidence of a primary relation of one or other of the structures of the hypothalamus to the regulation of emotional (Hess, 1949 and 1956; Sager, 1962), sexual (Hess, 1949 and 1956; Sager, 1962), and nutritional behaviour (Anand and Brobeck, 1951; Sawyer and Robinson, 1956; Nakao, 1958; Wasman and Flynn, 1962; Romaniuk, 1965; Abzianidze, 1969; Brown, Hunsperger and Rosvold, 1969), endocrine (Harris, 1937; Sawyer and Robinson, 1956; Szentagothai, *et al.*, 1965; Eskin, *et al.*, 1966; Polenov, 1968) and autonomic reactions of the organism (Kabat, *et al.*, 1935; Hess, 1949 and 1956; Valdman and Kozlovskaya, 1969).

An analysis of changes in the state of individual hypothalamic structures may draw us nearer to the understanding of specific mechanisms of a number of most important shifts appearing with age.

Thus it is already known that the "hunger center" is located in the lateral hypothalamus, the "satiety center," in the ventromedial hypothalamus (Anand, 1961; Abzianidze, 1969). A reduced excitability of the lateral hypothalamus may be a sign of its reduced functional activity. This should lessen the appetite and reduce the food intake. It should also be taken into consideration that nutrition behaviour is determined by the general state of metabolism of the organism's tissues.

In accordance with our findings, complex changes arise in the neurosecretory activity of the anterior hypothalamic nuclei which trigger the most intricate neurohumoral mechanisms of adaptive reactions of the organism.

It appears that with increasing age, reflex stimuli cause changes of the neurosecretory process which are most patently expressed in adults and the administration of adrenaline produces changes which are greater in old animals. In the formation of stress reactions and of the general adaptation syndrome, many processes depend on the hypothalamo-hypophyseal system. It may be presumed that the above mentioned changes in the neurosecretory activity of the hypothalamic nuclei, due to the action of various stimuli, determine to a large measure age differences in the development of stress reactions.

Thus age changes in this process are characterized not by a simple extinction of the function of the neurosecretory activity of hypothalamic nuclei, but by a complex change of their reactions on the background of the growing degradation.

Hypothalamic structures play an important part in the control of the cardiovascular and respiratory systems. Changes in arterial pressure and respiration result from the stimulation of the most diverse sections of the hypothalamus. Along with this the structures of the postero-lateral and antero-medial regions of the hypothalamus occupy an important position in the control of the cardiovascular system. The results of these experiments bear witness to the fact that in old animals electric excitability of the structures of the anterior and posterior areas of the hypothalamus increases, electric excitability of the lateral area decreases, but sensitivity to catecholamines and acetylcholine of all three, the anterior, posterior and lateral hypothalamus, increases. The cited shifts of excitability and sensitivity of these hypothalamic structures are, in our opinion, the most important mechanism which determines the appearance of stagnant, lengthy, slowly restoring changes of arterial pressure and cardiovascular reactions.

It should be stressed that humoral factors are particularly important

for the hypothalamus. The works of Weil-Malherbe, *et al.* (1959), Weil-Malherbe (1960), Axelrod (1963), Kassil (1963), Samorajski, *et al.* (1964), Maiselis (1965) and others, have shown that precisely in the area of hypothalamus the blood-brain barrier is increasingly permeable to a number of physiologically active agents. The role of cholinergic, adrenergic and serotonergic structures in the regulation of hypothalamic functions is well known. It may be presumed that the increased sensitivity of the hypothalamus to humoral factors with age may help in the appearance here of stable changes of functional activity accompanied by corresponding changes in the organism.

Researchers overlook the fact that with increasing age the quality of hypothalamic control of metabolism and functions may be changed. This qualitative change of hypothalamic regulation can determine the peculiar character of the dynamics of a number of metabolic and functional changes appearing with age.

So, shifts in the hypothalamus appearing with age can be characterized as "disregulation" and "hypothalamic misinformation of the organism." "Disregulation" is expressed by diverse changes of the functions of separate structures of the hypothalamus. "Disregulation" leads to the disturbance of optimal correlations between metabolism and function, to the decrease of adaptive capacities of an aging organism, and creates the prerequisites for disruption of regulatory mechanisms, for the development of pathology in old age and, principally, for the development of arterial hypertension, atherosclerosis, coronary insufficiency, diabetes and pathological climacteric.

"Hypothalamic misinformation of the organism" is the result of shifts in the hypothalamic sensitivity to hormones and mediators against the background of age-related changes in activity of a number of endocrine glands. This hampers the mobilization of a number of most important mechanisms of hormonal regulation in old age.

CONCLUSION

The functions of the hypothalamus are extremely complex and diverse. Individual structures of the hypothalamus are concerned with the regulation of various systems of the organism, various metabolic cycles and behavioral acts. They were formed at different stages of onto- and phylogenesis in relation with different neural centers and peripheral formations. It is high time to pass over from general statements about the character of changes of the "entire hypothalamus" to the analysis of the age dynamics of its individual structures. We consider our work as a step in this direction.

(1) Changes in the hypothalamus determine the important mechanisms

of aging, the shifts in metabolism and functions of cells, the decrease in adaptive capacities of the organism as a whole.

(2) It was noted that there was a certain reduction of the activity of the neurosecretory process in the hypothalamo-hypophyseal system of old animals. However, the changes undergone by the neurosecretory process in adult and old animals differ under the influence of reflex and humoral stimulants.

(3) With increasing age the electro-excitability of some structures of the hypothalamus (anterior and posterior parts) rises, while in others (lateral part) it is diminished.

(4) At the same time, the sensitivity of the hypothalamic structures to the direct effect of adrenaline and acetylcholine increases.

(5) With age, changes are found in the hypothalamic regulation of blood circulation and conditions are created for hypertensive reactions and more frequent changes in cardiac output.

(6) With age, the regulatory hypothalamic influence on the inductive synthesis of a number of enzymes is limited.

(7) The aging process of a cell can be viewed as having two levels. The first is an aging of a cell per se. The second is caused by neurohumoral influences (e.g. hypothalamic) on cellular metabolism and protein biosynthesis.

(8) Functional "disregulation" of the hypothalamus and "hypothalamic misinformation of the organism" create conditions for the development of pathology and, primarily, of arterial hypertension, atherosclerosis, myocardial infarction, diabetes and pathological climacteric.

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CHAPTER 32

THE HYPOTHALAMIC CONTROL OF AGING AND AGE-ASSOCIATED PATHOLOGY. THE ELEVATION MECHANISM OF AGING

VLADIMIR M. DILMAN

SUMMARY

THIS CHAPTER DEALS with data showing that age-associated disturbances of the internal environment of the organism naturally arise as a result of the intrinsic age-related elevation of the hypothalamic threshold to feedback suppression in the three main homeostatic systems of higher organisms: energy, reproduction and adaptation systems. As a result, the compensatory response in the functioning of relevant peripheral endocrine glands arises, thus maintaining the feedback mechanism under the conditions of enhanced hypothalamic activity. This phenomenon is responsible for the stability of the internal environment in the course of the organism's development. In the energy system, compensation is attained by elevation of the blood-insulin level; in the reproduction system, by excessive secretion of nonclassical phenolsteroids; in the adaptation system, by a comparative increase in the cortisol level. However, with time, this compensatory process gradually leads to imbalance of the internal environment and invariably causes homeostatic failure and specific age-related pathology, i.e. diseases of compensation. Stress and some analogous stimuli trigger off processes which are typical of normal aging, thereby achieving the integration of internal and external environmental factors. Therefore, elevation of hypothalamic threshold (or activity) provides a common mechanism for realizing the developmental stage of the neuroendocrine program of ontogenesis as well as the development of age-specific pathology; also it renders these processes dependent on the action of numerous external factors.

INTRODUCTION

Normal aging is accompanied by the gradually developing imbalance of the internal environment of the body, e.g. increased body weight and serum-cholesterol level, decreased glucose tolerance, climacteric, etc. These changes characterize aging as a derangement of homeostasis.

One of the major functions of the endocrine system is to maintain the constancy of the *milieu interieur*. We now know that homeostatic stability

is attained by rhythmic activity of the hypothalamus which controls and integrates the major functions of the body. This integration is effected through the mechanism of negative feedback control.

Theoretically, it is possible to distinguish three main types of disturbances that may lead to hormonal homeostatic failure: Type A—elevation of hypothalamic threshold to homeostatic suppression, i.e. the central type of homeostatic failure. Such disorders are observed in Cushing's disease where suppression by glucocorticoids is lacking; Type B—decreased hormonal action at the hypothalamic level, i.e. the peripheral type of homeostatic failure. Disturbances of this kind are observed, for example after subtotal castration; Type C—impaired hormonal secretion or qualitative shifts in the spectrum of secreted hormones, i.e. the dysfunctional type of homeostatic failure. Such disturbances may occur in congenital defects of certain enzymatic systems, e.g. defective hydroxylation of corticosteroids in patients with congenital adrenal hyperplasia (Wilkins, *et al.*, 1955).

Each organism, and especially a higher organism, exercises a great many functions and thus possesses many homeostatic systems. But in the final analysis the main parameters of organismal function are determined by the three basic homeostatic systems: energetic, reproductive and adaptational. The greatest changes in the course of development and aging of the organism occur in these three systems. A large body of evidence suggests that it is due to changes in the main homeostatic systems that the neuroendocrine program of organism development is realized, leading inevitably to age-specific pathology. Hence, the importance of the elucidation of the mechanism by which disturbances arise in the course of development and aging, especially in the main homeostatic systems, becomes clear. The following data show that although all types of homeostatic failure invariably develop in the course of normal aging, the age-associated elevation of hypothalamic threshold to homeostatic suppression, i.e. the central type of homeostatic failure, is the key factor causing imbalance of the internal environment inherent to normal aging.

THE HYPOTHALAMIC FEEDBACK SYSTEM IN THE COURSE OF NORMAL AGING

Age-Associated Changes in Energy Homeostasis

It is recognized that one of the main processes in energy homeostasis is the inhibition of GH secretion after glucose intake which acts on the hypothalamic receptors. On the other hand, during starvation GH secretion is increased, and this provides energy sources required by the organism by mobilizing free fatty acids (FFA) (Glick, *et al.*, 1965).

Functioning of this extremely important homeostatic system (in which glucose acts as the signal of the negative feedback mechanism) has been

TABLE 32-I
CHANGES IN GH AND FFA BLOOD LEVELS AFTER GLUCOSE LOADING IN YOUNG AND MIDDLE-AGED SUBJECTS

Group	Number of Cases	Average Age in Years	Serum GH Level ^a (ng/ml)		Plasma FFA Level ^b $\mu\text{Eq/L}$	
			Before Treatment	After Treatment	Before Treatment	After Treatment
1. Younger	15	26.6	14.4 ± 2.4 ^c	9.1 ± 1.6	-37	356.0 ± 19.0
2. Young	16	34.8	19.9 ± 2.6	11.8 ± 1.5	-41	—
3. Middle aged	17	52.7	29.8 ± 5.0	44.6 ± 9.1	+50	460.0 ± 17.2
						427.0 ± 18.0
						-7

^a GH was determined radioimmuno logically with the use of amino cellulose as immunosorbent (Bobrov and Patokin, 1971). The absolute value of GH thus obtained was much higher than that with ordinary radioimmuno logical assay. However, in young persons, the physiologic decrease of GH was found at 1 hour after glucose load. This effect was not observed in the middle-aged group.

^b Plasma FFA was titrated by Dole's method (1956). After intravenous glucose load (40 mg of glucose per kg body weight).

^c Standard error.

The ambulatory subjects of Group 1 were examined at 9-10 a.m. before breakfast, after 1 hour's rest in bed. Blood samples were taken from hospital patients of Groups 2 and 3 in bed before breakfast.

found to be disturbed in middle-aged people. As can be seen from Table 32-I, a standard glucose load fails to reduce the level of GH and FFA. This is supported by further GH data given in Table 32-X in the appendix.

On the strength of these data the conclusion may be drawn that in middle-aged people the hypothalamic threshold to homeostatic suppression is elevated.

This homeostatic defect alone suffices to set off a series of metabolic disturbances, such as age-associated increase of the body fat stores, relative refractoriness to insulin, lower carbohydrate tolerance, higher FFA and cholesterol levels in blood, i.e. to cause a number of alterations inherent to both normal aging and age-specific pathology (Dilman, 1968, 1971 and 1972).

A possible sequence of the metabolic disturbances which may be caused by uncontrolled secretion of GH will be considered briefly, below.

Decrease in Tolerance to Carbohydrates

A raised blood-GH level (which manifests itself in the inadequate suppression by glucose) lowers glucose utilization in muscle tissue, mainly by means of the direct contrainsulin effect of GH. In addition there is inhibition of glucose utilization by an excess of fatty acids, i.e. the so-called Randle effect (1965). In other words, carbohydrates do not burn in the flame of fats. These processes result in an inevitable age-connected reduction of tolerance to carbohydrates.

Age Increase of Body-Weight

Insufficient utilization of glucose in muscle tissue leads to compensatory hyperinsulinemia, as shown by relevant data on the age-connected rise in the blood-insulin level (Chlouverakis, *et al.*, 1967; Welborn, *et al.*, 1969). As a result, excessive glucose is mainly utilized in adipose tissue, where glucose metabolizes to fat. This is how fat depots grow with age, the process being a physiological law (Young, *et al.*, 1963). This mechanism ensures constant replenishing of fat stores, despite an intense lipolysis stimulated by excessive GH.

Age-Related Hypercholesterolemia

Reduced utilization of glucose in muscle tissue in turn serves as a secondary cause of the increased requirement of fatty acid mobilization. Increased uptake of fatty acids leads to the accumulation of acetyl-coenzyme A which, due to glucose utilization disturbances, remetabolizes to cholesterol rather than to fatty acids; the synthesis of the latter requires comparatively smaller amounts of nicotinamide-dinucleotide-diphosphate

TABLE 32-II
DIFFERENCES IN AGE-ASSOCIATED METABOLIC PARAMETERS
IN YOUNG AND MIDDLE-AGED SUBJECTS

<i>Parameters</i>	<i>Young</i>	<i>Middle Aged</i>
Average age	34.8	52.7
GH level		
basal (ng/ml)	19.9 ± 2.6	29.8 ± 5.0
1 hour after glucose load	11.8 ± 1.5	44.6 ± 9.1
Insulin-glucose test (blood sugar level, mg/100 ml)		
1 hour after glucose load	112.6 ± 3.1	143.7 ± 3.5*
2 hours	89.3 ± 2.7	114.5 ± 3.1*
Body weight % deviation from ideal weight	+4.7	+18.4
FFA level (μ Eq/l)	393.0 ± 16.0	483.0 ± 16.1*
Blood sugar level		
1 hour after glucose load	114.9 ± 2.7	150.7 ± 2.5*
2 hours	93.6 ± 6.0	131.0 ± 2.6*
After prednisolone glucose test		
1 hour	168.9 ± 4.6	215.3 ± 4.6*
2 hours	113.1 ± 4.4	158.3 ± 5.3*
Serum cholesterol (mg/100 ml)	181.8 ± 6.5	243.9 ± 5.0*
No of observations	35	115

* P < 0.002.

(N.A.D.P. 2H) than the synthesis of cholesterol. This is the way a third metabolic characteristic of aging, i.e. age-associated hypercholesterolemia, develops. It is essential to note that the synthesis of triglycerides in the liver also depends directly on compensatory hyperinsulinemia (Bierman, 1972).

Table 32-II illustrates some differences in metabolic pattern between young and middle-aged people, which supports the above theoretical considerations concerning the sequence of disturbances in energy homeostasis, caused by primary disturbances, i.e. elevation of hypothalamic threshold to feedback suppression.

Therefore, in normal aging, homeostatic failure in the energy system results in metabolic derangement inherent to age-specific pathology, i.e. obesity, prediabetes, adult onset diabetes and atherosclerosis (see below).

Age-Associated Changes in Regulation of Appetite

The activity of the hypothalamic appetite center is known to be inhibited as the blood-glucose level rises. Considering that blood-glucose concentration increases with age, the conclusion that advancing age is associated with a raised threshold of the hypothalamic appetite center to the inhibitory effect of glucose, seems justified (Dilman, 1958 and 1968). Clinical observations also indicate that appetite grows in many persons with age.

Pathophysiological findings of Everitt show that heightened appetite can be explained by increased hypothalamic activity (Everitt, 1970 and 1972).

In this context, increased appetite often observed in aging persons during emotional stress is, in my opinion, a paradoxical response of the hyperstimulated hypothalamus, similar to the "paradoxical" increase in GH secretion after a glucose load. This factor, coupled with the above age related disorders in the regulation of energy homeostasis, predisposes to the development of more profound metabolic disturbances.

Age-Associated Changes in Reproductive Homeostasis

Energy homeostasis is an open system; and it could be claimed that the above regulatory imbalances are caused by external environmental factors. Reproductive homeostasis, however, functions as a closed-loop system; moreover, as it will be shown below, similar regulatory changes occur in this system with advancing age, leading eventually to menopause, the age-specific cessation of the reproductive cycle. As Figure 32-1 shows, the excretion of total gonadotropins and FSH is increased with advancing age (Dilman and Pavlova, 1963; Dilman, 1968).

These alterations are already occurring in an absolutely normal ovarian cycle. Such age dynamics may be connected either with the decrease in ovarian hormone level, which acts as a physiological inhibitor of gonadotropin release (peripheral type of homeostatic failure), or with the elevated resistance of the hypothalamic "sex center" to feedback suppression (central type of homeostatic failure). Since changes in gonadotropin excretion are concomitant with the elevated excretion of ovarian hormones (total phenolsteroids), it may be inferred that the cause of increased excretion of gonadotropins may be attributed to the intensification of hypothalamo-pituitary activity, with a subsequent rise in the activity of the peripheral endocrine gland. Lack of hypothalamic suppression under these conditions is an indication of an elevated hypothalamic threshold to the suppressive effect of phenolsteroids, i.e. the central type of the homeostatic failure.

Over a period of years, compensatory intensification of ovarian activity serves to promote the feedback mechanism under the conditions of age-associated rise in hypothalamic threshold. However, elevation of hypothalamic threshold (which is manifested in the rising excretion of FSH) with age results ultimately in the failure of the feedback mechanism of reproductive homeostasis. In this connection, it may be stressed that the ovulatory surge of LH secretion depends on the rapid rise of estrogen secretion during the late follicular phase of the ovulatory cycle. The increased estrogen level produces a decline in FSH concentration, which may usually be observed 12 to 24 hours before ovulation (Taymor, *et al.*, 1968), but at

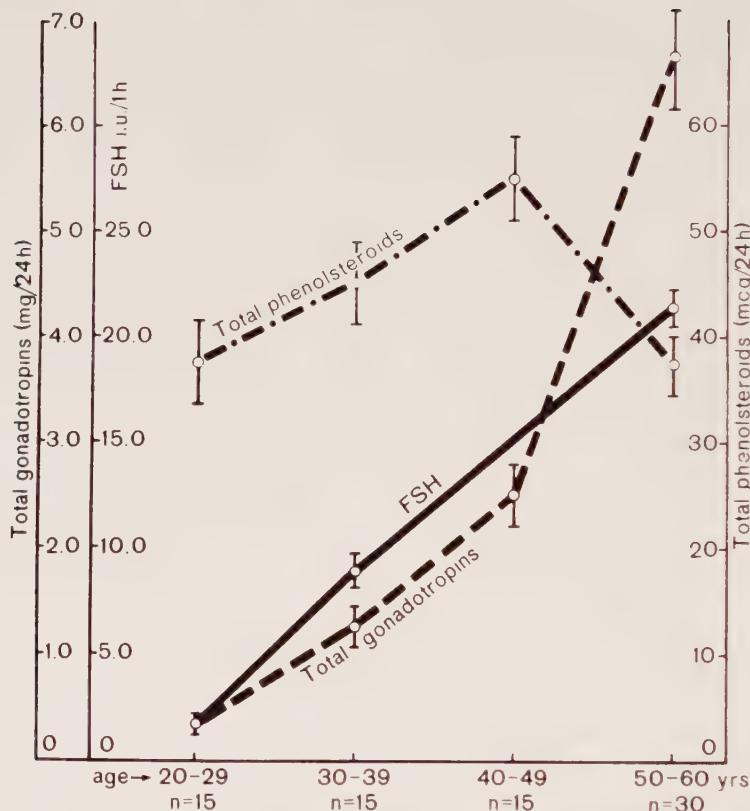


Figure 32-1. Age-associated elevation of excretion of total gonadotropins, follicle-stimulating hormone (FSH) and total phenolsteroids (Reproduced from Dilman (1971), with permission of the *Lancet*). Hormone determinations were made on the 6 to 8th day of menstrual flow. Total gonadotropins were tested by the modified method of Albert (Dilman and Iskakowa, 1965) and expressed in mg of 2nd I.R.P.-HMG Standard. FSH was determined by the method of Steelman and Pohley (1953) and values were calculated per 1 hour—equivalent of 24-hour urine output. Total phenolsteroids, which include both classical and nonclassical estrogens, were determined by method of Dikun and Pavlova (See Dilman, *et al.*, 1968).

the same time it augments the release of LH, thereby inducing ovulation. It may be supposed that these two effects (suppression of FSH and release of LH) are interconnected. Therefore, if the hypothalamic threshold to the inhibitory effect of estrogens is extremely elevated, hypothalamic feedback is disturbed and menopause develops. (See appendix.)

The concept of the relation of age-associated switching-off of the reproductive cycle to elevated hypothalamic threshold to suppression is in good agreement with experimental data showing that this phenomenon is also responsible for the switching-on of the reproductive cycle (Donovan and

Van der Werff Ten Bosch, 1959). However, our findings have shown that the estrogen dose required for the inhibition of gonadotrophic function continues to increase, following the switching-on of reproductive function in rats within the first 3 to 18 months, too. For instance, the inhibitory dose for one month old rats is 0.5 µg of stilbestrol, 2 µg for rats aged 3 months, 8 µg at 12 months and 12 µg at 18 months, respectively (Dilman, *et al.*, 1973). Accordingly, data are required on women of different ages who have normal menstrual cycles, because the menopause may affect the hypothalamic sensitivity to estrogens.

Thus, both age-associated switching-off, and switching-on, of the reproductive system seem to be triggered off by one and the same mechanism. The central type of mechanism of age-connected disturbances of the estrous cycle was demonstrated by convincing experimental data (Kushima, *et al.*, 1961; Aschheim, 1964/65). On the basis of such data, Klotz (1966) advanced the very interesting idea of the potential "immortality" of sex glands, thus emphasizing the key importance of regulatory disturbances in the mechanism of aging.

Compensatory ovarian hyperactivity occurring in the process of aging is manifested in a rather strange manner: under the conditions of gonadotrophic overstimulation, the ovaries secrete the so-called nonclassical phenolsteroids rather than classical estrogens, viz, estrone and estradiol. This conclusion is evident from the widening difference in the rate of excretion of total phenolsteroids and classical estrogens in the course of aging (Fig. 32-2). In the late menopause, the ovaries secrete almost exclusively nonclassical phenolsteroids, leading to the false impression that hormonal activity

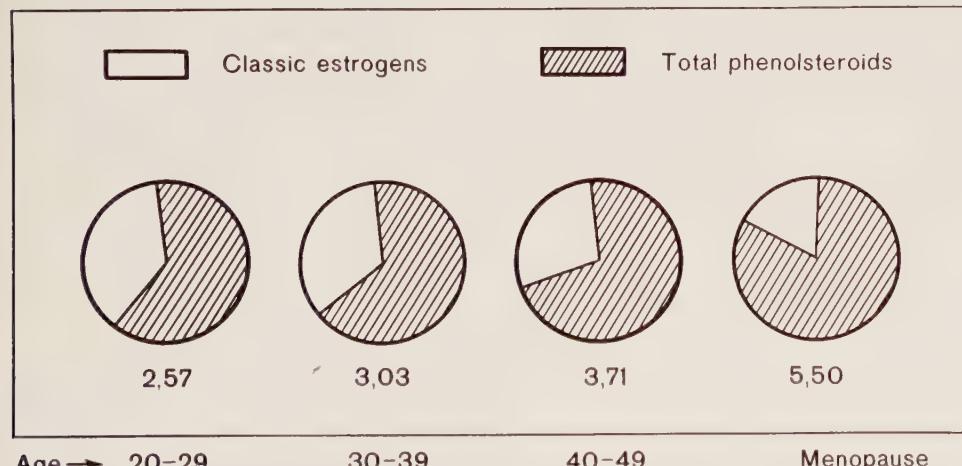


Figure 32-2. Ratio of total phenolsteroids to classical estrogens in different age groups of women with normal ovarian cycle and in menopause.

of the estrogenic type completely ceases in postmenopausal ovaries (MacBride, 1957). In this respect, it is interesting that ovariectomy in senile rats leads to an increased FSH level in the pituitary (Aschheim, 1970), just as ovariectomy in postmenopausal patients with breast cancer results in increased excretion of total gonadotropins (Dilman, 1968). (Nonclassical phenolsteroids are also produced in the adrenals, and, as the result of classical estrogen metabolism, in the liver.) There is evidence that the shift in ovarian production from classical to nonclassical phenolsteroids is due partly to the damage of follicular tissue, and partly to hyperplasia of theca-tissue, which results from ovarian overstimulation by gonadotropins (Dilman, 1968). This assumption may be best inferred from the data on radiation castration, in which both theca-tissue hyperplasia and follicular dam-

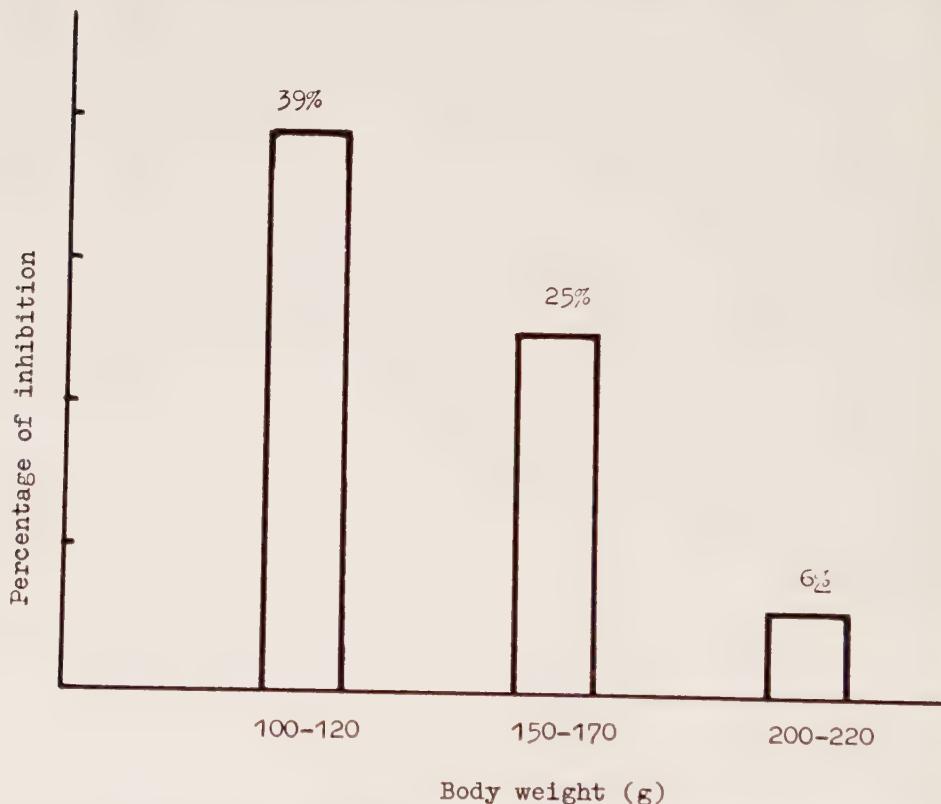


Figure 32-3. Age-associated elevation of hypothalamic threshold to prednisolone inhibition in rats (Ostroumova and Dilman, 1972). Prednisolone (dose of 0.05 mg per 100 g of body weight) was administered at 10 A.M.; blood sample was taken at 12 noon. The tests used female rats in which the body weight of 100 to 120 g corresponds to the age of 3 months, 150 to 170 g to 4 to 5 months, and 200 to 220 g to 6 to 12 months respectively.

age exist, in conjunction with elevated excretion of total phenolsteroids, despite a low level of classical estrogens (Dilman and Pavlova, 1963).

Undoubtedly, nonclassical phenolsteroids are of importance in certain pathophysiological states, being the chief contributors to the activity of sex hormones in the postmenopausal era (see below).

To summarize, the reproductive homeostatic system develops all three types of homeostatic failure with aging: elevated hypothalamic threshold manifested by increased gonadotropic secretion (central type A); insufficient production of classical estrogens (peripheral type B); and qualitative shifts in the spectrum of secreted hormones characterized by the increased share of nonclassical phenolsteroids (dysfunctional type C). Such a combination of disturbances results in the development of persistent changes in the internal hormonal environment.

Age-Associated Changes in the Adaptation System

Clinical experience shows that middle-aged and elderly persons often reveal Cushingoid features. Such analogy between Cushing's disease and aging is due to the resistance of the hypothalamus to feedback suppression in the former, and it is quite probable that the same regulatory changes occur in the latter. Indeed, Figure 32-3 shows that in rats the sensitivity to prednisolone inhibitory action declines with aging (Ostroumova and Dilman, 1972). This is strong evidence that age-associated elevation of hypothalamic threshold to homeostatic suppression does take place, because glucocorticoids exert the central feedback effect only at the hypothalamic level.

Therefore, with aging, the effect of glucocorticoid hormones on the organism increases because homeostatic regulatory disturbances may lead to imbalances in the diurnal rhythm of glucocorticoid secretion. This is corroborated by data given in Table 32-III, which concern middle-aged patients with ischemic heart disease.

Another important consequence of elevation of hypothalamic threshold to suppression in the adaptation system is, presumably, the damaging ef-

TABLE 32-III
LACK OF DIURNAL CORTISOL RHYTHM IN CORONARY
HEART DISEASE

	Number of Subjects	9 a.m.	4 p.m.	Change (%)
Serum Cortisol ($\mu\text{g}/\text{kg}$)	11	16.5 ± 2.2	14.6 ± 1.7	-11.5

Mean age of subjects 50.5 years.

fect of nonclassical phenolsteroid excess, produced by the adrenals in the same cases, owing to hypothalamic homeostatic failure (see below). Both these shifts are likely to suppress cellular immunity.

Age-Associated Changes in Thyrotropin Secretion

Functional activity of the thyroid gland is known to decline with advancing age. The cause of this physiological process has not yet been elucidated. It may be supposed that, in the course of aging, the sensitivity to inhibition in the hypothalamo-thyroid system is raised. In this context it becomes possible to explain why, despite the general 30 to 40 percent decrease in T_3 level in old age, an adequate increase in thyrotropin level does not occur, the higher values being recorded in the earlier and later decades of man's life (Mayberry, *et al.*, 1971). This conclusion is corroborated by findings from our laboratory showing that inhibition of the compensatory hypertrophy of the thyroid (after unilateral thyroidectomy) in young rats is achieved by a greater dose of T_4 than in older animals.

Age-Associated Changes in Secretion of Prolactin and Melanotropic Hormone

It is known that prolactin and melanotropin (MSH) secretion is regulated chiefly by inhibitory hypothalamic hormones, accordingly referred to as PIF and MIF. Therefore, it is reasonable to suppose that age-associated changes in the secretion of prolactin and melanotropin may differ from the age dynamics of secretion of pituitary hormones controlled by hypothalamic releasing hormones. Indeed, if aging is associated with general elevation of hypothalamic activity, it should lead accordingly to enhanced secretion of both inhibitory and stimulatory hypothalamic hormones. While the latter are called upon to raise the output of appropriate pituitary hormones, as is the case with gonadotropins, the increased production of inhibitory hypothalamic hormones may lead to the age-connected decrease in prolactin and melanotropin secretion. Indeed, under certain conditions, the hypothalamus is capable of acting in precisely this manner. For instance, continuous lighting results in a rise in gonadotropins and a fall in MSH secretion. However, direct measurements made recently in rats have shown that prolactin secretion increases with age (Meites, 1972). This is probably due to the fact that TSH-releasing hormones, as shown recently by some investigators, also stimulate prolactin secretion (Turkington, 1972).

To evaluate precisely the nature of hypothalamic changes in prolactin and MSH regulatory systems, it is necessary to use the inhibitory test with levodopa and the stimulating test with chlorpromazine (Kleinberg, *et al.*, 1971), because the basal level of hormone secretion in some cases does not

reflect the state of the regulatory system. It may be true, especially with regard to prolactin regulation, which is carried out not only at the hypothalamic level but also independently, at the pituitary level. Concerning age dynamics of melanotropin secretion, data is lacking.

It is noteworthy that an evaluation of age-linked changes in enzymatic activity has already led to the conclusion that aging is controlled on the supracellular level (Finch, 1972a and b), and that alterations in noradrenalin and dopamine activity (Stoll, 1972) in brain tissue are extremely important. In this connection, of great interest is the concept of Laborit (1969) on metabolic peculiarities in different systems of the brain.

Thus, analogous changes take place in the three basic homeostatic systems of the organism, and they are connected with age-associated elevation of hypothalamic threshold to homeostatic inhibition. Rhythmic activity of the homeostatic system under these conditions can be preserved only by a compensatory increase in the functioning of the corresponding peripheral endocrine glands. This makes it possible for gradual age-associated disturbances of the body's internal environment to arise. Therefore, we shall consider below, some endocrine factors which counteract the intrinsic process of imbalance of the internal environment in the course of development of the organism.

Endocrine Mechanism of Stabilization in the Developmental Program of the Organism

Although the elevation of hypothalamic threshold occurs both in reproductive and energy homeostases, integration between these two main systems of the organism creates a stable condition in which a series of metabolic indices are relatively constant. Some data support the conclusion that this stabilization phase is effected by the inhibitory action of estrogens on some mechanisms of energy homeostasis. In this respect, it may be emphasized that, apart from their properties with regard to reproduction, estrogens can affect a number of important metabolic parameters; thus, obesity and atherosclerosis often arise after ovariectomy whilst, on the contrary, estrogen treatment inhibits the lipolytic action of HGH (Kovaleva, *et al.*, 1965), decreases age related hypercholesterolemia (Barr, 1953) and increases glucose tolerance. Figure 32-4 illustrates the stabilization effect of estrogens in the course of normal development. As can be seen, in childhood when reproductive homeostasis is not yet switched on and in middle age, when it is switched off, the blood level of insulin (Welborn, *et al.*, 1969), cholesterol (Adlersberg, *et al.*, 1956; Hollingsworth, *et al.*, 1965), FFA (Pickens, *et al.*, 1967) and sugar two hours after glucose load (Dąnowski, 1957; Streeten, *et al.*, 1965), are much higher than in the reproductive period. The metabolic disturbances in middle age resemble the so-

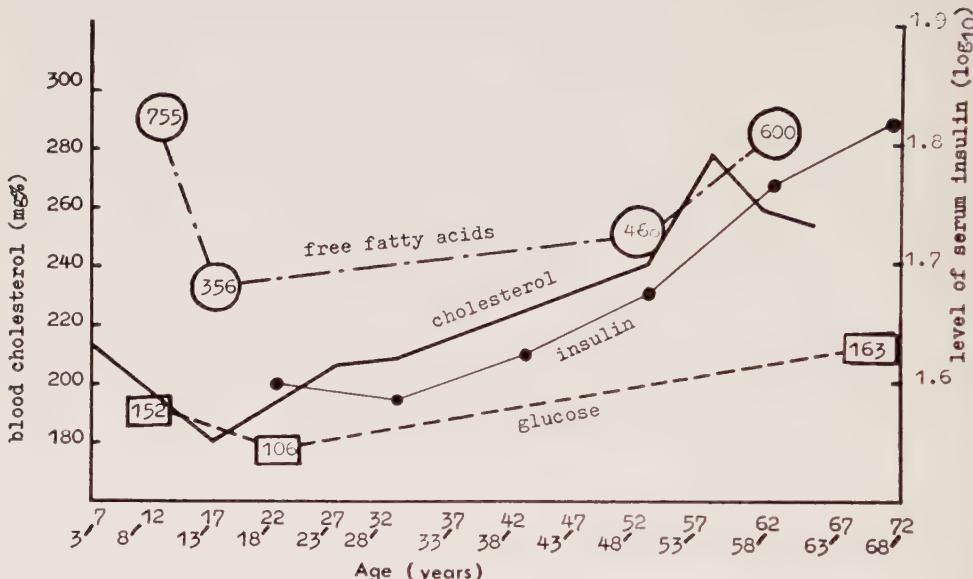


Figure 32-4. Age dynamics of blood-free fatty acids (Pickens, *et al.*, 1967) insulin (Welborn, *et al.*, 1969), cholesterol (Adlersberg, *et al.*, 1956) and sugar level 2 hours after glucose load (Danowski, 1957; Streeten, *et al.*, 1965 and our data) in women. These age changes are characteristic for three different periods of ontogenesis (prediabetes, stabilization and prediabetes).

called "prediabetes"^{*} and, therefore, the similar metabolic pattern observed before puberty may be defined as "pre-prediabetes" (Dilman, 1971). It is in between these two stages that the stabilization period occurs, when physiological parameters are relatively constant.

It is very important to note that energy homeostasis seems to back up reproductive homeostasis and that the reproductive cycle is switched on in young girls when the body weight has reached a certain critical value (Frisch and Revelle, 1971).†

Thus, the interaction between reproductive and energy homeostases fa-

* I regard prediabetes as a normal age-associated physiological process manifested by a delayed and exaggerated insulin-secretory response, raised blood-level of N.E.F.A., age-related gain of body weight, and so on. Disturbances of this kind produce specific age-associated pathology, although hyperglycemia is lacking in the prediabetic states. The progression of prediabetes into subclinical, latent, or adult-onset diabetes is not normally an age-linked process, but depends on the degree of insulin insufficiency.

† The dependence of the immune system activity on energy homeostasis has been also found to exist (Edwards, *et al.*, 1973); we observed that the treatment with phenformin (which is known to improve carbohydrate utilization) resulted in a diminished excretion of total gonadotropins. Consequently, the three basic homeostatic systems interact with one another.

cilitates the distinction of three different periods of ontogenesis: growth and maturation (pre-prediabetes), stabilization required for reproduction, and a period of imbalance of the internal environment (prediabetes) which gradually leads to the development of age-specific pathology.[‡]

Peculiarities of the Male Developmental Program

Some degenerative diseases, and primarily atherosclerosis, develop in the male organism much earlier than in the female, and death from atherosclerosis conforms to the same sex pattern. The average life-span of males is shorter in animals too, particularly in rats. Proceeding from the concept under discussion, this peculiarity may be explained by the different roles of male and female sex hormones in the organism. The ability of estrogens to slow down the rate of aging during the stabilization phase may be connected with the fact that estrogens contribute to the maintenance of the rhythmic activity of reproductive homeostasis, and thus inhibit certain functions of the hypothalamus and secondary metabolic processes. In the male, however, reproductive homeostasis is characterized by a continuous and nonrhythmic pattern which serves to overcome biological limitations imposed on reproduction by the cyclic functioning of the female sexual system. Therefore, testosterone does not exert a stabilizing effect on the energy system. On the contrary, its administration to female animals shortly after birth produces an androgenization of the hypothalamus, i.e. its functioning in the reproductive system acquires the male pattern (Barraclough, 1970). Therefore, the stabilization stage is not inherent in the male organism, and accordingly, castration in the early premature period serves to extend life-span, apparently due to prevention of the damaging effect of testosterone (Hamilton and Mestler, 1969).

The absence of the stabilization phase in the male organism is most evident from the data of Welborn and co-workers (1969), who show a progressive increment in the insulin level in males, whereas in the female or-

[‡] However, some symptoms of elevated hypothalamic activity may diminish with senescence. For example, in the 60- to 65-year age group, some decrease of blood cholesterol has been noted, which could be due not only to natural selection by death, but possibly also to a secondary decrease in hypothalamic activity. This may be due to the accumulation of age-associated defects in the hypothalamic tissue proper. Therefore, probably a fourth period, a period of involution, should be distinguished. We should note that studies of age-linked changes, for instance, of blood cholesterol level involving the comparison of data for young and very old persons sometimes fails to detect any differences, since the latter category of patients pass through the stage of involution, while it is essentially in the prediabetes period when major metabolic alterations occur and diseases of compensation develop. It is quite possible that therapeutic measures taken in the period of involution should be different from those resorted to in the course of prediabetes when the central type of homeostatic failure predominates.

TABLE 32-IV
INCREMENT IN BLOOD-INSULIN AND BLOOD-GLUCOSE LEVELS WITH AGING

Age Group	Increment of Serum-Insulin ^a		Increment of Serum-Glucose ^a	
	Men	Women	Men	Women
21-29	0.0 (110) ^b	0.0 (143)	0.0	0.0
30-39	+0.028 (142)	-0.012 (185)	+ 6	+ 4
40-49	+0.067 (194)	+0.021 (199)	+11	+ 7
50-59	+0.112 (165)	+0.075 (177)	+21	+11
60-69	+0.223 (138)	+0.168 (138)	+28	+26
70+	+0.244 (82)	+0.213 (98)	+45	+43

^a Serum-insulin was tested radioimmunologically 1 hour after glucose load (50.0 g); blood-sugar before glucose intake. Levels of insulin and glucose in age group 21-29 are taken as datum. Increments for insulin are expressed at the Log₁₀ of serum concentration and serum-glucose (in mg%).

^b Figures in parentheses show number of observations.

Reproduced with permission of the authors and Springer Verlag, Berlin. Modified from Welborn, et al., 1969.

ganism, there is a temporary decline at 30 to 39 years of age, i.e. in the stabilization phase (Table 32-IV).

Compensatory hyperinsulinemia is a key-factor in age-associated pathology (see below), and the absence of the stabilization phase in men, is likely to be largely responsible for a more intensified advance of degenerative disease, and a shorter life-span among men than women.

Normal Limits of Physiological Characteristics and Age-Associated Pathology

During normal aging, the rhythmic activities of the three main homeostatic systems become disturbed, which finally leads to imbalance of the internal environment of the organism. It may be pointed out that any deviation from the individual's norm is a step in the direction of age-associated pathology. Therefore, normal parameters of physiological characteristics must be strictly limited within the range of values attained in the phase of stabilization, for women, and the period of cessation of development for men. Thus, the normal level should be considered a narrow-limited constant pattern, and norm limits should be the same for all age-groups. This conclusion is of great importance from the clinical point of view.

The similarity of the mechanisms of age-related homeostatic failure makes it possible to distinguish four stages in the development of homeostatic insufficiency and age-associated pathology (Table 32-V).

Such classification of age-connected changes into 4 stages provides a means for detection of imbalances (homeostatic failure) two stages earlier than when it is done on the basis of the so-called "risk factors."

TABLE 32-V

SEQUENCE OF HOMEOSTATIC FAILURE SYMPTOMS CAUSED BY ELEVATION OF HYPOTHALAMIC THRESHOLD TO FEEDBACK SUPPRESSION

<i>Stage</i>	<i>State of Homeostatic System</i>	<i>Denomination of Stage of Homeostatic Failure</i>
Stage 1	Lack of diurnal rhythmic activity	Dysrhythmic stage
Stage 2	Lack of inhibition or paradoxical response to suppression test	Arrhythmic stage
Stage 3	Presence of metabolic disturbances	Dysmetabolic stage ("factors of risk")
Stage 4	Clinical symptoms of disease	Clinical stage

Table 32-VI summarizes the major tests which may be used for assessment of the main homeostatic systems.

Of considerable interest is a battery of characteristics suggested by Comfort (1969) for the evaluation of homeostatic failure.

It should be mentioned that elevation of the hypothalamic threshold results in resistance to inhibition and, at the same time, under certain conditions, raises sensitivity to the action of stimulatory factors. For instance, a glucose load results in a paradoxical increment of blood growth hormone in endometrial cancer patients. At the same time insulin induced hypoglycemia causes much higher blood HGH levels to occur than in normal persons (Benjamin, 1974). Therefore, both inhibitory and stimulatory tests should be employed for functional evaluation of the homeostatic system. Norm indices may vary between individuals. Consequently, the individual norm should be found for each person at the age of 20 to 25. Repeated

TABLE 32-VI

SUGGESTED TESTS FOR THE ASSESSMENT OF HYPOTHALAMIC FAILURE

<i>Homeostasis</i>	<i>Primary Tests</i>	<i>Secondary Tests</i>
Energy	GH level after glucose load and insulin-induced hypoglycemia	Blood sugar and insulin 2 hours after glucose load; blood cholesterol; weight gain
Reproduction	Suppression of serum FSH and LH by estrogens or hypothalamic-pituitary inhibitors*	Level of total gonadotropin and phenolsteroid excretion
Adaptation	Diurnal rhythm of cortisol. Dexamethasone suppression test and insulin-induced hypoglycemia	Ratio of hydroxysteroid excretion to 17-ketosteroids
Prolactin-MSH system	Levodopa suppression test and chlorpromazine stimulation test	Prolactin and MSH blood level

* Estrogen administration during the first phase of the menstrual cycle may cause an ovulatory release of gonadotropins, even if hormones are administered on days 3 to 5 of the cycle (Yen, S. S. and Tsai, C. C.: *J Clin Endocrinol*, 34:298, 1972). This interferes with the evaluation of the inhibitory effect of estrogens.

analyses should be carried out at certain intervals in order to detect as early as possible, deviations from the constancy of the organism's internal environment.

Remarks on the Nature of Age-Associated Elevation of Hypothalamic Threshold

The age dynamics of the hypothalamic threshold may be defined as the property providing self-development of the basic homeostatic systems. The specific dynamics are connected, in the author's opinion, with the principle by which the stability of internal environment is maintained in higher organisms. The internal environment will remain constant for maximal periods of time if the mechanisms maintaining stability during development, themselves develop with time. The key-role in these homeostatic changes lies in the age-elevation of the hypothalamic threshold of sensitivity to feedback suppression. This property, therefore, must be genetically programmed.

Indeed, in a newly-born organism, the hypothalamic system is at the initial stage of its self-development, and is found to be maximally sensitive to homeostatic suppression (Hohlweg und Dohrn, 1932; Ostroumova and Dilman, 1972). Gradual elevation of the threshold to suppression results in a decreased influence of inhibiting factors on the hypothalamus, and the main self-developing systems, viz. reproduction, energy and adaptation homeostases, undergo an intensifying stimulation by the hypothalamo-pituitary complex. These changes promote the growth and development of the organism. But with time, this hypothalamic process begins to disturb homeostatic regulation when development and growth of the organism have ceased.

It may be assumed that the regulatory mechanism of development and aging is a consequence of evolution of the unicellular organism to a multicellular one, with a specialized homeostatic system intended for the maintenance of a stable internal environment.

At present, there is no evidence that might elucidate the mechanism of the process underlying elevation of hypothalamic threshold to suppression. However, certain observations are of interest in this respect, because they show elevation of hypothalamic threshold to be dependent on functional alterations, rather than on irreversible damage of the hypothalamic system.

Testosterone treatment during the first 5 days of an animal's life causes the so-called "androgenization" of the hypothalamus, an apparent increase in hypothalamic threshold to suppression in reproductive homeostasis (Barraclough, 1970). This effect may be prevented by means of a number

TABLE 32-VII

THE EFFECT OF PINEAL EXTRACT ON HYPOTHALAMIC SENSITIVITY
TO PREDNISOLONE-INDUCED SUPPRESSION IN RATS

<i>Treatment</i>	<i>Blood Corticosterone Level ($\mu\text{g}/\text{kg}$)</i>	
	<i>Before Treatment</i>	<i>After Treatment*</i>
Control	22.8 \pm 4.0 (15) [†]	22.2 \pm 2.8 (15)
Pineal extract	27.0 \pm 3.8 (15)	9.0 \pm 1.0 [‡] (15)

* Prednisolone, intraperitoneally in single doses, 0.05 mg/100 g body weight.

† Number of rats.

‡ $p < 0.05$.

of neurotropic drugs (Kikuyama, 1961). Elevation of hypothalamic threshold to feedback suppression by glucose is found in patients with severe malnutrition, regardless of age and type of disease (Alvarez, *et al.*, 1972). This observation, as well as the "paradoxical" rise in GH level following glucose load (Dilman, 1971), is consistent with the functional nature of hypothalamic changes. It should be pointed out that administration of pineal extract (in an acute experiment) restores hypothalamic sensitivity to prednisolone suppression in old rats (Table 32-VII, Ostroumova and Dilman, 1972). Pineal extract as well as L-dopa and dilantin were found to restore the age-linked decrease in hypothalamic sensitivity to suppression in the reproductive system (Dilman, *et al.*, 1973).

Diseases of Compensation as Age-Specific Pathology

Data presented previously in this chapter show that elevation of hypothalamic threshold to feedback suppression leads to alteration of the rhythmic functioning of the main homeostatic systems, which is retained only by means of compensatory over-stimulation of the appropriate endocrine gland. However, these compensatory efforts, required for the maintenance of homeostasis in the conditions of elevating hypothalamic threshold, also disturb the stability of the chemical environment of the body.

Long-term and persistent imbalance in the internal environment constitute a pathologic state and, therefore, in this sense aging is a disease, or a conglomerate of diseases, which inevitably develops due to the age-associated central type of homeostatic failure. Diseases connected with this mechanism were defined as "diseases of compensation" (Dilman 1968 and 1971). The metabolic disturbances inherent in compensation diseases are present in the main pathologic processes of aging, such as age-related obesity, prediabetes and early stages of adult-onset diabetes, atherosclerosis, climacteric, lowered resistance to infection, hypertension, and many types of cancer (Dilman, 1968, 1971, and 1972). Therefore, it is reason-

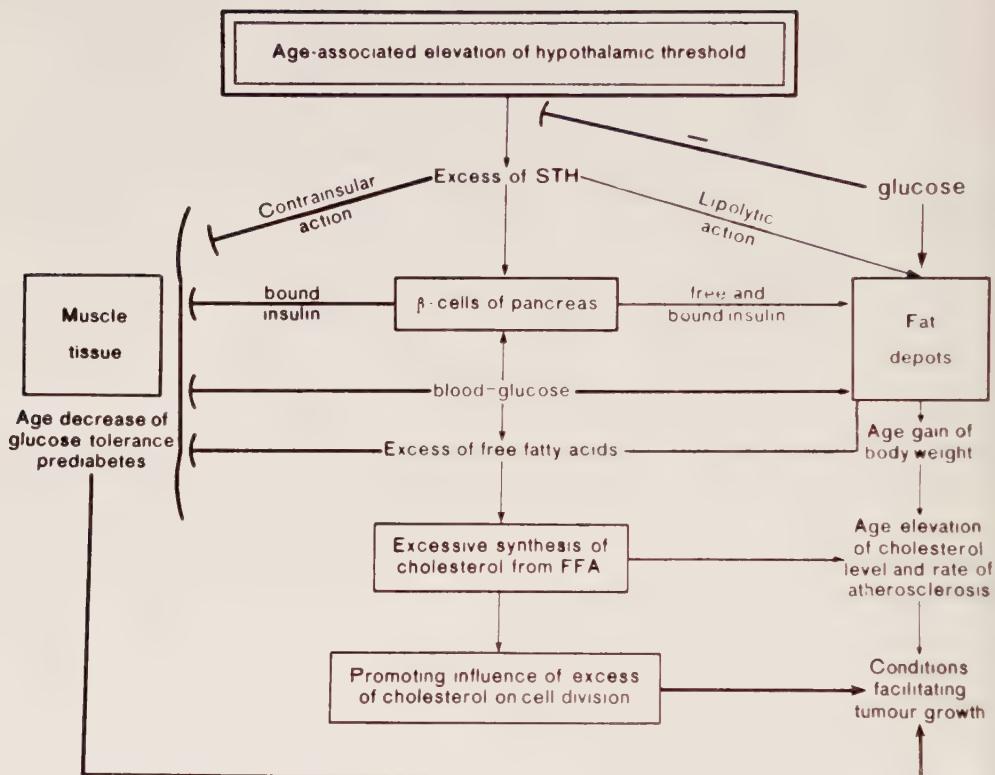


Figure 32-5. Schematic diagram showing the relationship of age-associated hormonal and metabolic disturbances pertinent to the development of prediabetes, obesity and atherosclerosis (From Dilman, 1971 with permission of the *Lancet*).

able to discuss briefly the data available on the role of age-related imbalances of the internal environment in the formation of age-specific pathology in man.

Obesity, Prediabetes, Adult-Onset Diabetes and Atherosclerosis

As mentioned above, age-connected elevation of hypothalamic threshold eventually leads to the development of obesity and prediabetes, and to metabolic disorders inherent to atherosclerosis. A schematic pattern of their relationship is shown in Figure 32-5. Compensatory hyperinsulinemia is a key factor in the development of all of these disturbances.[§] A de-

[§] It is probable that in addition to growth hormone, some other diabetogenic factors (see, for instance, Lawrence, *et al.*, 1971) contribute to the age-associated decrease in the tissue sensitivity to insulin action, thus tripping off a series of compensatory changes, such as hyperinsulinemia, etc. This does not alter, however, our concept of the sequence of metabolic disturbances inherent to compensation diseases, because it is the reactive hyperinsulinemia that is the key factor of the development of these disturbances.

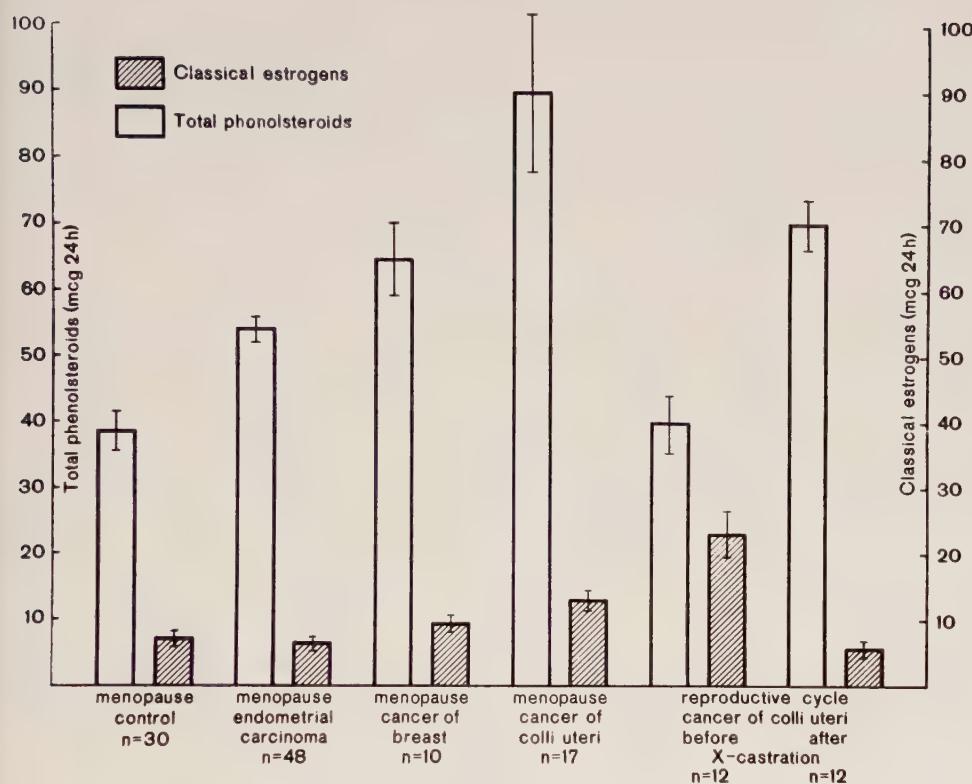


Figure 32-6. Excretion of total phenolsteroids and classical estrogens in healthy menopausal women and in patients with cancer of the breast and corpus and cervix uteri. Classical estrogens were assayed by the method of Brown (1955); total phenolsteroids by the method of Dikun and Pavlova (Dilman, *et al.*, 1968, reproduced from Dilman (1971), with permission of the *Lancet*).

creased level of classical estrogens and a relative excess of cortisol (see above) make important pathogenic contributions to the development of these diseases. There exist, however, many other causes which may lead to atherosclerosis, e.g. hypothyroidism. As far as their mechanism is concerned, such types of atherosclerosis should be referred to as symptomatic, in contradistinction to atherosclerosis regarded in this context as a compensation disease.

Climacteric and Pathology of the Climacteric Period

Compensatory enhancement of secretion of nonclassical phenolsteroids is more pronounced in women suffering from cancer of the reproductive system (Fig. 32-6). These hormones induce endometrial hyperplasia in patients with endometrial cancer in the postmenopausal period (Berstein,

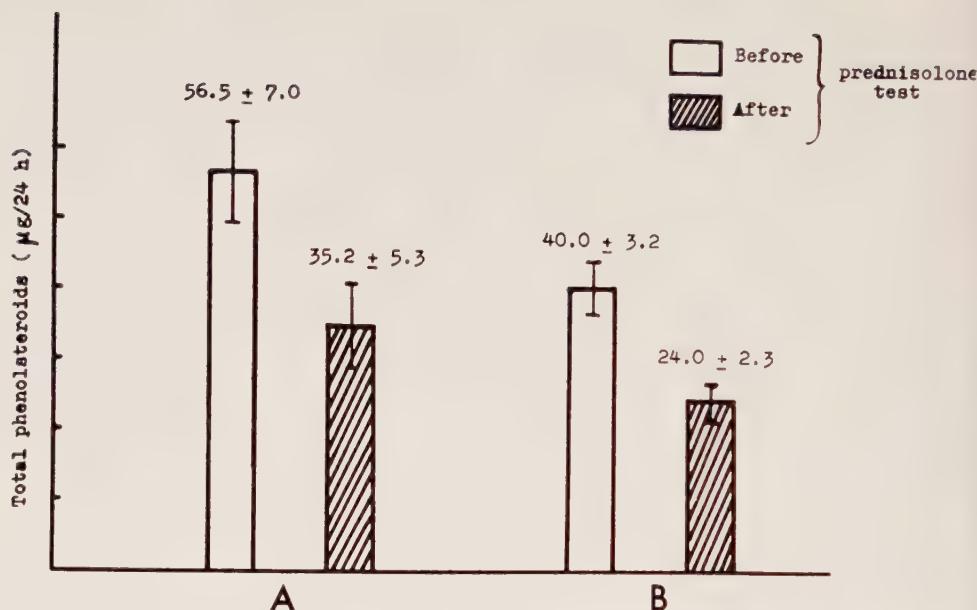


Figure 32-7. Correlation of survival time of breast cancer patients with the level of total phenolsteroid excretion. A—survival time less than 3 years (age range 33-54 years; number of cases 15). B—survival time more than 3 years (age range 29-50 years; number of cases—26).

TABLE 32-VIII
DISTURBANCES OF ENERGY HOMEOSTASIS IN PATIENTS WITH
BREAST AND ENDOMETRIAL CARCINOMAS

Tests	Control Group	Breast Carcinoma	Endometrial Carcinoma
GH basal level	19.9 ± 2.6 (n = 16)	22.6 ± 2.9 ^a (2.1 ± 0.23) ^b	20.4 ± 6.4 ^a (1.4 ± 0.46) ^b
GH level 1 hr after glucose load	11.8 ± 1.5 (n = 16)	27.4 ± 3.2 ^a (2.4 ± 0.26) ^b	22.0 ± 6.8 ^a (1.4 ± 0.49) ^b
Body-weight (% deviation from ideal weight)	+4.7 (n = 35)	+30.2 (n = 68)	+37.0 (n = 168)
Insulin-basal level (μU/ml)	8.5 ± 3.7 (n = 8)	36.9 ± 11.6 (n = 19)	19.8 ± 2.6 (n = 12)
Insulin level 1 hr after glucose load	38.0 ± 3.3 (n = 8)	99.3 ± 16.4 (n = 19)	38.0 ± 5.3 (n = 12)
Incidence of subclinical, latent and overt diabetes (%)	20 (n = 36)	36 (n = 68)	63 (n = 168)
FFA-level (μEq/L)	408 ± 16 (n = 36)	604 ± 75 (n = 27)	915 ± 91 (n = 53)
Cholesterol level (mg%)	203 ± 8.2 (n = 15)	270 ± 19.5 (n = 31)	279 ± 16.3 (n = 128)

^a GH was determined radioimmunologically with the use of aminocellulose as immunosorbent (Bobrov and Patokin, 1971).

^b GH was tested by classic double-antibody method.

et al., 1969); they also affect the course of breast cancer. With increased excretion of nonclassical phenolsteroids, the survival time of such patients has proved to be shorter (Fig. 32-7).

Hence, it is this compensatory mechanism which maintains a normal reproductive cycle under the conditions of elevated hypothalamic threshold, and paves the way to pathologic development.

Age-Associated Rise in Cancer Incidence

Table 32-VIII shows that many cancer patients exhibit metabolic signs of intensified aging.

Hyperinsulinemia seems to be one of the factors contributing to the age-connected increase in tumor incidence. In this respect, our findings showed (Berstein, 1973; Fig. 32-8) that among patients in whom a tumor was revealed after 50 years of age, there was a large group of women who gave birth to children weighing 4.0 kg or more. It is well known that potentially diabetic mothers frequently give birth to large babies; these data demonstrate that metabolic alterations pertinent to prediabetes appear long before tumor manifestation.

A comparative excess of glucocorticoids observed in the course of aging (see above) may also occur in cancer (Table 32-IX). The excess of glucocorticoids, along with diminished excretion of classical androgens, makes the "discrimination function" of Bulbrook negative, which is well correlated with the development of many types of cancer (Bulbrook, *et al.*,

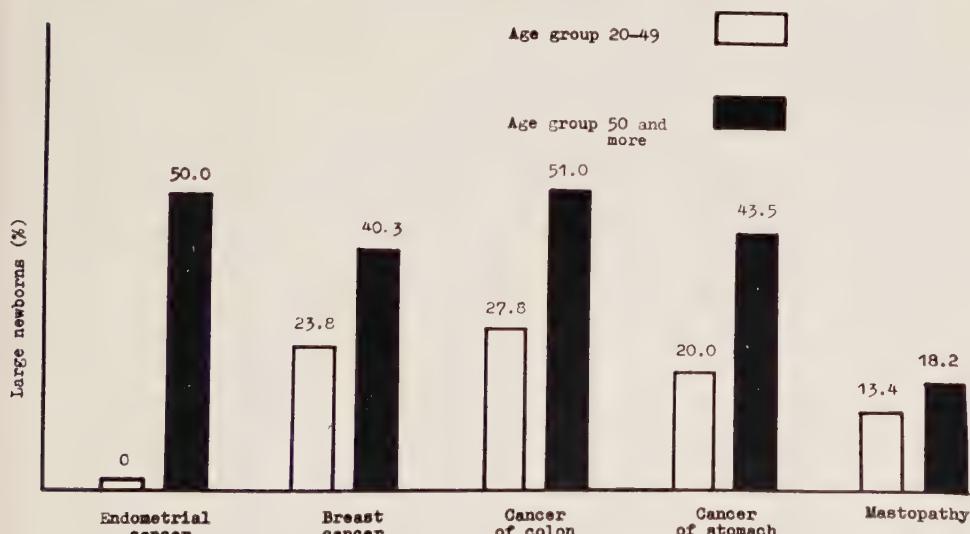


Figure 32-8. Frequency of births of large babies (4.0 kg and more) in two different age groups of cancer patients.

TABLE 32-IX

AGE-ASSOCIATED CHANGE IN THE INHIBITORY EFFECTS OF DEXAMETHASONE IN BREAST CANCER PATIENTS

Group (Mean Age)	No. of Patients	Total Phenolsteroids ^a μg/24 hrs	17-Hydroxy-		17-Keto-	17-OH
			(mg/24 hrs)	(mg/24 hrs)	steroids ^b (mg/24 hrs)	17KS (mg/24 hrs)
Premenopausal (42 ± 1.0)	9	Before	93.0	6.66	7.87	0.85
		After	42.6	3.42	5.05	0.68
		Change (%)	-54.0	-48.7	-36.4	—
Postmenopausal (60 ± 1.12)	7	Before	55.7	5.3	3.33	1.59
		After	80.7	5.5	2.80	1.96
		Change (%)	+45.0	+3.8	-16.0	—

^a Total phenolsteroids were determined in this study without chromatographic purification on Al₂O₃ column; so absolute values are higher than those for standard test methods (see above).

^b The first figure for each test gives a relevant value prior to administration of dexamethasone (0.5 mg/day) for three days and the second—after treatment.

1962 and 1971; De Waard, *et al.*, 1969; Rao, 1970), and a decrease in immunologic reactivity (Mackay, *et al.*, 1971).

Age-related hypercholesterolemia may also be an important factor contributing to tumorigenesis (Dilman, 1967, and 1968). Thus, intense synthesis of cholesterol takes place in organs characterized by a vigorous cell division, e.g. skin, intestines, etc. Many antitumor drugs inhibit the synthesis of cholesterol (Littman, *et al.*, 1966); a cholesterol-deficient diet serves to reduce the frequency of experimentally induced tumors (Szep-senwol, 1966). Moreover, the synthesis of cholesterol has been found to intensify dramatically in malignant cells (Howard and Kritchevsky, 1969; Chevallier and Lutton, 1971).

The current study of the metabolic background of tumorigenesis should receive more attention, because such preparations as phenformin can reduce insulinemia, hypercholesterolemia and body-weight, both in atherosclerotic and cancer patients (Tzagourhis, *et al.*, 1968; Dilman, *et al.*, 1972).

On the other hand, age-linked alterations in the hypothalamic systems regulated by inhibitory factors seem to be capable of suppressing the age-connected dynamics of certain tumors. Thus, if blood MSH level is really relevant in melanoma development, such changes might provide an explanation for the paradoxical decrease in melanoma incidence with advancing age, while other types of tumors become more frequent with aging. Proceeding from this assumption, levodopa should be investigated with a view to melanoma treatment by suppressing MSH secretion (if a reliable drug is available for inhibiting levodopa metabolism at the peripheral tissue level).

Therefore, although carcinogenesis primarily depends on genetic transformation, metabolic disorders arising in the course of aging are likely to substantially affect the clinical manifestation of the tumor process.

In other words, the age-associated elevation of hypothalamic activity leads to the establishment of the pattern of metabolic cancerophilia and to an increased incidence of different kinds of cancer which originate in a hormonal milieu typical of intensified aging (see Dilman, 1973).

Decreased Resistance to Infection

Lowered resistance to infection is frequently observed in adult-onset diabetes mellitus and Cushing's disease, i.e. in metabolic disturbances typical of compensation diseases. Apart from the immunosuppressive action of glucocorticoid excess, age disturbances in the reproductive homeostasis seem to exert a certain effect on the immunological defense mechanism. When the reproductive cycle is switched on, the thymus undergoes involution, and the thymic inhibiting activity of estrogen probably helps to suppress the immunological incompatibility of the mother and fetus. At the same time, the thymolytic action of estrogens inhibits the immunologic response, and this is distinctly observed in pregnancy. A high excretion of estriol-like nonclassical estrogens during pregnancy is very intriguing in this respect, since it is the nonclassical estrogens that are chiefly excreted in the postmenopausal era.

This conclusion seems still more convincing, considering that man is not the only being affected by age-related cortisol overproduction. Death in spawning Pacific Salmon is caused by abnormal metabolic changes resembling those seen in Cushingoid patients (Wexler, 1971; Chap. 17). Experiments in animals suggest that a high population density causes a higher mortality rate, owing to the increased activity of the hypothalamoglucocorticoid complex with subsequent reduction of resistance to infection (Christian, 1968). It is interesting to note that in rats in which increased pituitary activity has been produced by repeated breeding the signs of premature aging develop disguised as arteriosclerosis, hypertension, decreased tolerance to glucose, etc. (Wexler and Kittinger, 1965; Wexler, Chap. 17).

Hypertension

Although this disease should not be classed with compensation diseases directly, the process of aging, by steadily increasing hypothalamic activity, apparently results in a situation where hypertension may be induced, even by rather slight stressor influences. A frequently observed concomitance of hypertension with obesity, hypercholesterolemia, reduced tolerance to carbohydrates (Lang, 1950) and hyperinsulinemia may be attributed to the role of elevated hypothalamic activity in the pathogenesis of this disease.

Relationship between Compensation and Adaptation Diseases

Stressor influences involve disturbances similar to those pertinent to the mechanism of normal aging, but the sequence of changes is different: in aging, the primary elevation of the hypothalamic threshold to feedback suppression leads to the enhancement of hypothalamic activity; while in stressor conditions, elevated hypothalamic activity, which realizes the mechanism of adaptation, secondarily raises hypothalamic resistance to homeostatic suppression. Stress, therefore, may be regarded as a specific case of the intensification of the natural mechanism of aging. Therefore, any factor contributing to elevation of hypothalamic threshold is assumed to promote an earlier and more pronounced development of compensation diseases, and vice versa. Moreover, it should be emphasized that any disease of compensation may develop at any age, if the elevation of hypothalamic threshold is caused by pathologic rather than age-associated processes. However, unlike diseases of adaptation, compensation diseases develop with the natural development of the organism at a varying rate, regardless of the influence of stressors.

Compensation Diseases and Food Intake

In cases of significant nutritional deficiency, one does not observe the pronounced age dynamics of certain secondary metabolic disturbances, e.g. age-related elevation of blood-cholesterol level. Therefore, some researchers attribute age-associated hypercholesterolemia (and atherosclerosis) to the influence of exogenous factors, primarily elevated food intake. However, absence of secondary metabolic disorders is far from being a proof that the functioning of the homeostatic system is not disturbed. Dietary restriction serves to decelerate the realization of internal mechanisms of aging, among other things, owing to diminished hypothalamic activity (Everitt, 1972). However, an assessment of the rhythm of the homeostatic system alone can indicate the extent to which dietary factors can retard the development, not only of secondary metabolic disturbances (like blood cholesterol and insulin), but also of homeostatic deficiency.

Common Nature of Neuro-Endocrine Program of Development, Aging and Age Pathology

One phenomenon alone, i.e. the age-associated elevation of hypothalamic threshold to suppression, provides a common pattern by which basic functions in the homeostatic systems of the growing organism are performed. These include age-connected switching on and off of the reproductive cycle, interaction of energy and reproductive homeostases in the

course of development, regulation of the rate of aging which depends on the pressure of the external environment and the population density, as well as the natural development of those homeostatic disturbances which, under the guise of compensation diseases, lead to age-specific pathology and death from internal causes. It should also be pointed out that age-connected derangement of rhythmic functioning of homeostatic systems inevitably impairs the adaptational potentialities of the organism, since the normal course of adaptative processes requires preservation of the optimal capabilities of homeostatic systems to go through rhythmic cycles. Hence, age-associated elevation of hypothalamic threshold invariably reduces resistance to the damaging effect of external factors. Accelerated development may also be explained in terms of the elevation mechanism of aging. If the expectant mother reveals an age-associated decrease in carbohydrate utilization, this disturbance is likely to result in hyperinsulinemia in the fetus, an increased number of fat cells and finally a fat fetus. This in turn will eventually cause an enhanced fat accumulation, an earlier switching-on of reproductive function and premature development of age-associated pathology. So, it is on these grounds that the author designates it as the elevation mechanism of development, aging and age-associated pathology (Dilman, 1971 and 1972).

So far, it has not been possible to find an alternative specific mechanism to explain all these phenomena, though there may be some other, perhaps phylogenetically more ancient, aging factors operating (at the cellular level for instance) whose action is concomitant with and independent of this mechanism.

HYPOTHALAMIC ELEVATION THEORY OF AGING IN THE LIGHT OF CLASSICAL CRITERIA OF AGING CHANGES

Literature on gerontology contains different criteria for assessing age-connected changes. It is natural that any theory of aging should provide a specific explanation of the development of specific signs (or features) of aging. Therefore, it seems expedient to consider briefly the degree to which certain classical criteria of age-associated changes conform to the elevation mechanism of aging.

Criteria of Strehler (1962)

(1) *Universality*. The change occurs in all older members of the species.

In terms of the elevation theory of aging, this criterion is met by the elevation of hypothalamic threshold to feedback suppression.

(2) *Intrinsicity*. Aging is a built-in process which takes place even when all environmental influences are eliminated.

A phenomenon such as age-connected elevation of gonadotropin secretion undoubtedly satisfies this requirement, since this phenomenon develops in due course, independently of the influence of the external environment.

(3) *Progressiveness*. The onset of the process is gradual and the change is accumulative.

This criterion is met by the phenomenon of switching on and off the reproductive cycle which, according to the elevation concept, is effected by the gradual age-linked elevation of hypothalamic threshold.

(4) *Deleteriousness*. The change must shorten life.

Elevation of hypothalamic threshold in the three main hypothalamic systems leads to compensation diseases which constitute the major cause of death in middle age and senescence.

Criteria of Sobel and Mormorston (1958)

(1) *The process must change at a measurable rate during aging.*

This is satisfied by the age-associated elevation of hypothalamic threshold which finally terminates in homeostatic failure.

(2) *The progression of this change must advance the aging process itself.*

Any acceleration of the elevation of hypothalamic threshold causes a series of metabolic changes which, in turn, speed up the processes of aging and age-associated pathology.

(3) *The progression of this change must also occur in a situation which is known to advance aging.*

Stress, excessive food intake and high population density contribute to the intrinsic elevation of hypothalamic threshold to suppression, and thus advance aging and age-associated pathology.

(4) *The process must be irreversible.*

This parameter of aging is hard to assess in brief. If, for instance, collagen polymerization is considered to be an index of this process, in its advanced stage it is hardly irreversible. However, the rate of collagen polymerization depends, to some degree, on diet and, to be more precise, on hypothalamic pituitary function (Everitt, 1972; Everitt and Delbridge, 1972). In this respect, polymerization of collagen can be regulated, to a certain extent.

A number of factors may influence the rate of hypothalamic threshold elevation or counteract the development of compensation diseases at different stages (Dilman, 1968, 1971 and 1972). Therefore, the degree of reversibility of age-linked changes causing compensation diseases, is subject to further study (Dilman, 1972).

(5) *The event must be representative of the whole organism rather than an individual organ.*

Since a living organism differs from a nonliving object in the three cardinal properties (reproductive ability, metabolism with an adequately regulated stream of energy, and adaptation), age-associated hypothalamic alterations in these three main homeostatic systems are representative of the whole organism.

Criteria of Shock (1960)

In conclusion, it should be noted that some of the major facts which Shock considers indispensable in evolving a theory of aging, can be interpreted in the light of the concept of the hypothalamic elevation mechanism of aging. In particular the following facts, as numbered in Shock's paper, namely the relationship of life-span to diet (4), sex (3), and stress (8), external environmental influences, e.g. population density, have already been discussed in the light of this concept above. A decline in adaptability with aging may also be, to a certain extent, accounted for by the decreased rhythmical activity of homeostatic systems. It should also be taken into account that radiation, in conjunction with other effects, also serves to augment the activity of the hypothalamic-pituitary system (6), i.e. it acts in the same direction as natural aging. A similar effect is exerted by some carcinogens, for instance methylcholanthrene and DMBA, as the data obtained by our staff show.

The relationship of life-span and genetic characteristics (2) is, to a degree, connected with constitutional variations in the rate of age-associated rise of hypothalamic threshold. The regulatory mechanism of age-connected changes also largely accounts both for the greater age changes in total animal performance than in intracellular biochemical processes (9) and the increase of age changes with the complexity of the performance measured (10).

Criteria of Aging Changes (Elevation Mechanism)

However, it should be kept in mind that specific mechanisms of aging in higher animals are conditioned by specific mechanisms of regulation and integration inherent in every species. Therefore, within certain limits, each species grows old and dies in a specific way. Thus, proceeding from the concept of the elevation mechanism of aging, it is possible to outline criteria for evaluation of aging changes in man:

1. characteristics should pertain to intrinsic (endogenous) processes;
2. characteristics should be physiologically substantiated, i.e. they are shown to develop in every person at a rate varying with the individual;

3. characteristics should be closely linked with the mechanism of age-specific pathology;
4. the intensity and rate of change of characteristics should be dependent on the influence of external factors;
5. age-associated changes of characteristics should afford a quantitative assessment;
6. characteristics should reflect, on the basis of one common principle, the condition of the greatest number of organism systems possible (testing the least number of parameters), rather than of a certain isolated system.

In the future, the use of the releasing-hormone assay will make it possible to test the hypothalamic elevation concept of human aging by direct methods.

CONCLUSION

Indirect evidence presented in this chapter suggests that the process of normal aging involves a regular elevation of hypothalamic threshold to suppression in the energy, reproduction and adaptation systems. This phenomenon seems to constitute the neuro-endocrine program of the genetic mechanism of the organism's development which finally terminates in age-specific pathology. Accordingly, prophylactic and therapeutic measures should be largely directed at the restoration of the rhythmic functioning of homeostatic systems, and suppression of their hyperactivity rather than at the stimulation of some endocrine functions as was previously thought. Some therapeutic measures which may be used for this purpose have been considered earlier (Dilman, 1968 and 1972).

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APPENDIX

Serum Growth Hormone and Insulin

It is apparent from Table 32-X that the basal level of growth hormone as measured by the standard double antibody method is decreased with age. There is a statistically significant difference between a young group (4-12 years) with a mean (\pm S.E.) level of 2.6 ± 0.7 ng/ml and a middle-aged group (50-59 years) with a mean of 0.7 ± 0.2 . In the young group, a decrease in HGH level after glucose load was observed in 68.1% of cases, while in the middle-age group only 26.4% ($p < 0.01$).

This decrease may be explained by a rise in blood FFA level due to age-associated overweight. It may be supposed that after the development of obesity, age-associated metabolic disturbances may persist due to a high spontaneous lipolysis which may occur in spite of secondary inhibition of growth hormone secretion. In this respect it is noteworthy that in diabetic patients who reveal shifts in metabolism similar to those inherent in aging, physical strain tends to cause an elevated level of growth hormone (Hansen, 1973) although its basal concentration is low.

The serum insulin level (Table 32-X) rises with age thus confirming the data of Welborn, *et al.*, shown earlier in Table 32-IV.

Reproductive Function

The general concept concerning the age-related mechanism of switching-on and switching-off of the reproductive cycle should be made more precise. It is possible to suggest that the elevation of hypothalamic threshold commences in the tonic center. This process reduces the negative feedback effect of estrogens that leads to the age-connected stimulation of

TABLE 32-X
AGE-RELATED CHANGES IN SERUM GH AND INSULIN
LEVELS AFTER GLUCOSE LOADING

Age (Years)	No. of Observ.	0	GH level (ng/ml) ^a			Insulin level (μ U/ml)		
			60 min	120 min	0	60 min	120 min	—
4-12	19	2.6 ± 0.7	1.1 ± 0.2	—	19.3	37.8	—	—
20-29	14	1.9 ± 0.5	1.4 ± 0.5	1.1 ± 0.2	24.0	58.0	43.0	—
40-49	16	1.19 ± 0.4	0.99 ± 0.3	0.78 ± 0.2	25.0	98.0	118.0	—
50-59	16	0.71 ± 0.2	0.73 ± 0.3	0.95 ± 0.2	37.9	88.5	84.9	—

^a Double antibody method.

TABLE 32-XI

THE INHIBITORY EFFECT OF DEXAMETHASONE^a ON STEROID EXCRETION IN HEALTHY MEN

Age (Years)	No. of Subjects	Percentage Decrease From Basal Level	
		17-Hydroxysteroids	17-Ketosteroids
0-29	16	64 ± 8.5%	58 ± 8%
30-39	12	60 ± 8%	33 ± 8%
40-50	28	42 ± 7.7%	19 ± 6.5%

^a 0.5 mg dexamethasone per day (administered in three separate doses) over a three-day period.

the sex glands. When the estrogen concentration reaches a critical level, estrogen stimulates the cyclic center via the positive feedback mechanism and, in turn, the cyclic center stimulates the tonic center through the catecholamine mechanism. As a result, the ovulatory surge of gonadotropin secretion occurs. However, after the switching-on of the reproductive cycle, elevation of the sensitivity threshold arises in the cyclic center, as well. The age-associated switching-off of reproductive function may be due to this process, because, if the sensitivity of the center becomes low, the estrogen level proves insufficient for the induction of ovulation. Accordingly, L-Dopa treatment of senile rats results in the restoration of the estrus cycle (Quadri, *et al.*, 1973) owing to the tonic center being stimulated without the participation of the cyclic center. Restoration of the estrus cycle may be attained by the administration of a pineal polypeptide extract (Anisimov, *et al.*, 1973), which increases the hypothalamic threshold to estrogen (Dilman, *et al.*, 1973).

The Adaptation System

According to our findings shown in Table 32-XI, the response of healthy men to 0.5 mg dexamethasone per day declines with age, as indicated by the excretion of 17-hydroxysteroids and 17-ketosteroids. These data support the concept of elevation of the hypothalamic threshold in the adaptation system with aging.

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CHAPTER 33

AGE-DEPENDENT HORMONAL REGULATION OF MAMMALIAN GENE EXPRESSION

RICHARD C. ADELMAN

SUMMARY

THE TIME REQUIRED to initiate the inductions of several liver enzymes is lengthened progressively as rats age from 2- to 24-months. This impairment in liver enzyme regulation represents a biochemical parameter of senescence, whose expression may be complicated by a variety of genetic and/or environmental factors.

Essential to the regulation *in vivo* of at least two of these enzymes, glucokinase and tyrosine aminotransferase, is the availability and effectiveness of corticosterone and insulin. The adrenal secretion of corticosterone in response to ACTH, delivery of the glucocorticoid to liver and probably all other tissues, and the hormonal-mediated increase in hepatic tyrosine aminotransferase activity are not impaired during aging. In contrast, the ability to increase corticosterone levels in response to short-term starvation is progressively impaired as rats age, such that at 24-months the response is abolished. However, there is no obvious manner in which the availability to liver or action in liver by this hormone can account for the earlier observation of impaired enzyme regulation.

With regard to insulin, studies are not yet complete. A newly developed bioassay for insulin, based upon the requirement for this hormone in the maintenance of hepatic glucokinase activity, reveals a progressive 70 percent decrease in the circulating level of "functional" insulin as rats age from 2- to 24-months. Current efforts are attempting to distinguish between effects of aging on secretion and turnover of the hormone, its biological activity, and its ability to enhance the rate of synthesis of hepatic glucokinase.

Studies on binding of corticosterone and insulin to their respective receptor systems in liver reveal no significant alterations during aging. However, it is evident that some parameter of hepatic responsiveness to insulin undergoes progressive change as rats age from 2- to 24-months; namely, the

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inability to switch on the mechanism responsible for the inactivation portion of the adaptive increase in tyrosine aminotransferase activity.

INTRODUCTION

The ability to stimulate adaptive increases in the activities of nearly three dozen enzymes is impaired during aging in different tissues of several species in response to a broad spectrum of stimuli (Adelman, *in press*). The beauty of enzyme induction in intact animals as an experimental system in which to study aging is three-fold. 1) Enzyme induction represents a classical biochemical manifestation of adaptive responsiveness, a general feature which probably is impaired universally in aging populations. 2) At least one critical aspect of environmental interaction can be monitored by determining the availability and effectiveness of specific hormones which are essential to the control of certain enzymes. 3) The capability for expression of genetic and metabolic information can be monitored by evaluating the synthesis and post-translational behavior of specific, well characterized proteins. The purpose of the present report is to review briefly current thoughts concerning aging and enzyme adaptation, and to consider the possibility that presumed modifications in target cell gene expression may represent manifestations of alterations in endocrine and/or neural regulatory mechanisms.

AGE-DEPENDENT ENZYME INDUCTION

Effects of aging on enzyme inducibility are divisible into four general categories: inductions which are altered in time course and/or in magnitude of response; and those which apparently are not modified at all (Adelman, *in press*). Emphasis of this author's laboratory has focussed upon several enzyme inductions, in liver of male, Sprague-Dawley rats, whose initiation time is delayed, but whose magnitude of response is not altered during aging (Adelman, 1972). For example, initiation times are delayed during aging for increases in activity of glucokinase (Adelman, 1970), tyrosine aminotransferase (Adelman and Freeman, 1972) and microsomal NADPH:cytochrome c reductase (Adelman, 1971; Adelman, *et al.*, 1972) in response to administration of glucose, ACTH and phenobarbital, respectively. The progressive increase in duration of adaptive lag periods is directly proportional to chronological age from 2- to at least 24-months under experimental conditions employed, and was proposed as a biochemical parameter of senescence (Adelman, 1970; Adelman, 1972). Similar age-dependent lag periods of enzyme inducibility also are documented by several other investigators (Finch, *et al.*, 1969; Rahman and Peraino, 1973; Davis and Pfeifer, 1973; Roth, *et al.*, 1974).

Expression of this phenomenon is extremely complex. For example,

there are apparent sex and strain differences in effects of aging on responsiveness of hepatic glucokinase activity to glucose feeding (Adelman, 1970; Davis and Pfeifer, 1973); genetic and/or environmental factors which influence effects of aging on both basal levels and responsiveness to phenobarbital of microsomal NADPH; cytochrome c reductase (Adelman, 1971; Adelman, *et al.*, manuscript submitted); apparent species differences in initial age of expression of delayed lag period in responsiveness of tyrosine aminotransferase (Finch, *et al.*, 1969; Adelman and Freeman, 1972); etc. It now is evident that expression of at least this aging phenomenon probably can be reproduced exactly only in carefully standardized experimental protocols. This realization prompted the development of a colony of aging male, Sprague-Dawley rats, maintained for this author in the facilities of the Charles River Breeding Laboratories, Wilmington, Mass. These animals are maintained, with continuous independent health evaluation, under as close as possible to constant environmental conditions; even to the extent of providing throughout their lifetime a pasteurized, sterilized diet whose constancy is guaranteed with regard to both percent composition and component source. A detailed description of this rat colony is being prepared for publication.

EXTRA-HEPATIC REGULATORY MECHANISMS

Several experimental observations suggested that the age-dependent delays in liver enzyme induction may be the consequence of alterations in extra-hepatic control mechanisms, and not in the hepatic capacity to synthesize specific enzymes in response to hormonal stimulation. For example, under specific experimental conditions no age-dependence is demonstrable for the increase in glucokinase activity following treatment with insulin (Adelman, 1970; Adelman and Freeman, 1972), or in tyrosine aminotransferase activity following treatment with insulin, glucagon or glucocorticoids (Finch, *et al.*, 1969; Adelman and Freeman, 1972). Subsequent efforts of this laboratory demonstrated the key roles *in vivo* of certain hormones, such as insulin and corticosterone, in the regulation of those enzymes whose inductions are delayed during aging (Adelman and Freeman, 1972). Availability of each of these hormones then was examined during aging.

Corticosterone

The circulating concentration of corticosterone in aorta blood of our rats is approximately 10 µg per 100 ml of serum at 2-, 12- and 24-months of age when exposure to stressful conditions is minimized (Rotenberg, *et al.*, manuscript submitted). The amount of circulating corticosterone bound to serum proteins, as determined by the charcoal binding assay, is nearly

100 percent between 2- and 24-months of age. Of course, this type of measurement does not preclude the possibility that aging may alter quality and/or amount of binding to specific proteins; e.g. transcritin.

The increase in circulating concentration of corticosterone following treatment with ACTH is not impaired during aging (Rotenberg, *et al.*, manuscript submitted). Conflicting earlier claims to the contrary (as reviewed by Rotenberg) failed to evaluate ACTH-stimulated steroidogenesis at appropriate intermediate ages. Thus, we apparently confirm a slight decrease in responsiveness related to growth and/or maturation, between 2- and 12-months of age, but not to senescence.

In contrast to responsiveness to ACTH, the increase in circulating concentration of corticosterone following exposure of rats to short-term starvation is impaired during aging (Rotenberg, *et al.*, manuscript submitted). Corticosterone levels undergo a progressive 6-fold increase during 3 days of fasting in 2-month-old rats. At 12-months of age the corticosterone level rises 2-fold by day 2, but returns to the original fed level by the third day of fasting. There is no detectable effect of a 3-day fast on corticosterone levels in 24-month-old rats. Several possible mechanisms currently are under investigation. 1) Older fasted rats respond to ACTH treatment at least as well as fed rats. Thus, adrenal steroidogenesis probably is not impaired. 2) A neuroregulatory system, at the pituitary level or higher, may undergo a progressive deterioration in its ability to recognize or to respond to the provocation of fasting. 3) Due to their great increase in body weight and fat reserves, our older rats may not consider a 3-day fast particularly stressful. However, recognition of the fast is expressed by decreased blood glucose and insulin concentrations at 2-, 12- and 24-months of age (Freeman, *et al.*, 1973; Freeman, *et al.*, manuscript in preparation). 4) It also is conceivable that the rate of corticosterone turnover in older rats is enhanced when they are fasted. However, the general hepatic enzyme system which catalyzes steroid metabolism is diminished in both rate and responsiveness during aging, as reviewed by Adelman (1971). Furthermore, preliminary data from this laboratory indicate no discernible differences in association of corticosterone with the glucocorticoid binding proteins of rat liver (Britton and Adelman, unpublished observations).

Insulin

The amount of "functional" insulin in our rats decreases progressively, to the approximate extent of 70 percent, as they age from 2- to 24-months (Adelman and Freeman, 1972). This functional assay is based upon the amount of guinea pig anti-insulin serum, administered to rats *in vivo*, required to abolish hepatic glucokinase activity. However, the com-

plex nature of this assay procedure precludes the likelihood of any immediate distinctions between the effects of aging on several parameters which include the following: the steady-state concentration of circulating insulin; the ability of rat pancreas to secrete its insulin store; the quantity of the pancreatic insulin store; the biological activity of circulating insulin; the binding of insulin to its specific receptor on hepatic plasma membrane; and the subsequent message transmission resulting in an enhanced accumulation of active glucokinase molecules. Each of these parameters of insulin function during aging currently is under investigation.

The concentration of immunoreactive insulin in portal vein blood, collected between 8:00 and 10:00 A.M. from *ad lib* fed rats is approximately 250 μ Units per ml of serum at 2- and 12-months of age, and decreases precipitously to 112 μ Units per ml of serum by 24-months (Freeman, *et al.*, 1973). Peripheral levels of circulating immunoreactive insulin exhibit a similar trend, but smaller in magnitude (Freeman, *et al.*, manuscript in preparation). The lower concentration of circulating immunoreactive insulin in 24-month-old rats may not be the consequence of impaired pancreatic secretion, because the increase in immunoreactive insulin levels following administration of glucose is not impaired during aging. The apparent rate of turnover of immunoreactive insulin decreases approximately 50 percent as the rats age from 12- to 24-months. However, the significance of these observations is in doubt until it is ascertained exactly what proportion of the endogenous pool of immunoreactive insulin is biologically active.

HEPATIC CONTROL MECHANISMS

Hormone Receptor Systems

Neither binding capacity nor affinity of cytosol proteins from liver of adrenalectomized rats for cortisol is impaired during aging, according to the *in vitro* studies of Roth (1974). Of possible great significance, at least to examples of age-dependent tissue responsiveness other than hepatic systems, is the apparently reduced binding capacity for glucocorticoids in muscle, brain, epididymal fat pad and prostate in aging rats. However, in agreement with the data of Roth, preliminary *in vitro* studies from our laboratory (Britton and Adelman, unpublished observations) reveal no differences in association of corticosterone to the glucocorticoid binding proteins of rat liver described by Morey and Litwack (1969). In contrast to the studies of Roth and from our laboratory, Singer, *et al.* (1973) observed impaired binding of cortisol *in vitro* to cytosol proteins from autopsy samples of liver from aging humans. In any event, the unimpaired inducibility of hepatic tyrosine aminotransferase by corticosterone in aging rats (described below) confirms at least that any conceivable defect

in this glucocorticoid receptor system does not interfere with the enzyme adaptation during aging.

Binding capacity of purified hepatic plasma membrane for insulin apparently decreases between 2- and 12-months of age in rats, but is not altered appreciably between 12- and 24-months (Freeman, *et al.*, 1973; Freeman, *et al.*, manuscript in preparation). Affinity of the hepatic plasma membrane preparations for insulin is identical as rat donors age from 2- to 24-months. An alteration in responsiveness to insulin during aging, as expressed by the increase in hepatic tyrosine aminotransferase, is discussed below.

HEPATIC CAPACITY FOR ENZYME INDUCIBILITY

The most thoroughly investigated system for assessing hepatic responsiveness to hormonal stimulation during aging is the adaptive increase in tyrosine aminotransferase activity. Based, thus far, on relatively superficial examination, it is generally regarded that responsiveness of this enzyme system to glucocorticoids, insulin and glucagon is not altered during aging (Gregerman, 1959; Finch, *et al.*, 1969; Adelman and Freeman, 1972). However, conflicting studies suggest that under specific experimental conditions differences are demonstrable.

For example, Frolkis (1970) observed age-dependent responsiveness to glucocorticoid treatment that varies with hormone dosage in adrenalectomized rats. However, recent observations in our laboratory (Rotenberg, *et al.*, manuscript submitted) indicate that the apparent dependence on hormone dosage may reflect a greater rate of absorption of intraperitoneally injected corticosterone into portal vein blood of larger, older intact rats. Indeed, when the increase in tyrosine aminotransferase activity is expressed as a function of corticosterone concentration in portal vein blood, it becomes evident that both time course and magnitude of response are identical at 2-, 12- and 24-months of age. Thus, in retrospect, the earlier reports of no age-dependence are the consequence of fortuitously chosen dosages of glucocorticoids; dosages which saturate the adaptive enzyme system, thus making the differential rate of hormone absorption irrelevant. On the other hand, the more thorough study of Frolkis gave no consideration to the possibility of differential absorption. In all fairness, however, any possible contribution by endocrine ablation was not yet considered at all. The tendency to extrapolate from such data the conclusion that the capacity for hepatic protein synthesis may be unaltered during aging also is premature.

A second example is the recent observation that at slightly higher dosages of insulin than previously employed (Adelman and Freeman, 1972), the increase in tyrosine aminotransferase activity occurs to a greater degree

and for a longer duration as rats age from 2- to 24-months (Britton, *et al.*, manuscript in preparation). This phenomenon apparently is related to an age-dependent delay in the inactivation portion of the insulin-stimulated response. Preliminary evidence suggests the progressive occurrence during aging of the inability to generate a specific post-translational modification of tyrosine aminotransferase that probably is essential to enzyme inactivation and degradation. Interestingly, a similarly enhanced response for an extended time by this enzyme also is evoked in stimulated rats whose pituitary glands were surgically removed (Grossman and Mavrides, 1967).

CONCLUSIONS

The progressively impaired ability to regulate adaptive increases in the activities of at least certain hepatic enzymes may be employed as a biochemical parameter of senescence in rigorously standardized animal colonies. Key to the regulation of certain of these enzymes is the availability and effectiveness of corticosterone and insulin. The adrenal secretion of corticosterone in response to ACTH, delivery of the glucocorticoid to liver and probably all other tissues, and at least one recognized manifestation of glucocorticoid action in liver, are not impaired during aging. The circulating concentration of immunoreactive insulin, as well as some aspect of the biological effectiveness of this hormone, diminishes during aging. However, the mechanism is not completely understood.

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CHAPTER 34

CONCLUSION: AGING AND ITS HYPOTHALAMIC-PITUITARY CONTROL

ARTHUR V. EVERITT

SUMMARY

THIS CHAPTER SUMMARIZES the main points developed in the book. Aging is defined as the progressive deterioration of the organism after maturity and involves not only the physiological changes with age, but also the increasing frequency of age-related pathology. The rate of progression of these intrinsic age changes can be increased by a number of hormones.

The hypothalamus and the pituitary-peripheral endocrine system undergo aging changes like other organs in the body. In man the secretion of almost all hormones decreases in old age, except where compensatory changes increase the secretion, as in the case of the pituitary gonadotropins. There is a decline in the daily secretion rate of pituitary growth hormone and the main target gland hormones (thyroxine, cortisol, estradiol and testosterone). However, dynamic tests of hypothalamic-pituitary function have failed to reveal any significant impairment of anterior pituitary function or defects in feedback or neural control of pituitary function. The reduced secretion of hormones in old age may be due to 1) primary aging of the gland, e.g. ovarian and testicular hormones, 2) reduced tissue requirement for hormones, e.g. thyroid and adrenocortical hormones, 3) age changes in the central regulatory mechanism, e.g. estradiol, or 4) reduced blood flow to the gland due to atherosclerosis, e.g. testosterone.

The pituitary gland is essential for a normal life-span. Certain hormones like adrenocortical and posterior pituitary hormones maintain or prolong the life of hypophysectomized or old rats. The same hormone, e.g. cortisol, can have both a life maintaining and an aging effect, presumably due to different modes of action.

Most hormones of the anterior pituitary, thyroid, adrenal cortex and gonads either accelerate physiological aging or increase the incidence of age-related pathology in the kidney and cardiovascular system. Hypophysectomy reduces the secretion of these hormones and so retards aging in the rat and probably other animals. Primary age changes in the hypothalamus modify the secretion of pituitary hormones and thus influence peripheral aging.

Environmental influences such as stress, food supply, temperature and radiation also influence the course of aging and the development of age-related pathology. These factors act on the hypothalamic-pituitary-peripheral endocrine system modifying the secretion of "aging hormones."

INTRODUCTION

This monograph has attempted to define aging, to assess aging in the hypothalamus, the pituitary and its target glands and to determine the role of the endocrine system in aging. The major contribution of this work is to emphasize the importance of the hypothalamus and the pituitary in the regulation of aging processes.

WHAT IS AGING?

Aging may be defined as a progressive deterioration of the organism after maturity, which increases the probability of death. Many physiological functions decline gradually after the cessation of growth (Everitt, Chap. 2) and tissues become more susceptible to disease (Berg, Chap. 3). In the rat, as in man, the major diseases of old age are those affecting the cardiovascular and renal systems, in addition to the neoplasms. In each species, these changes occur usually in an orderly sequence at a set rate, as if programmed.

The life-span is determined principally by the forces that hasten or retard the tissue changes leading to disease (Berg, Chap. 3), and only indirectly by physiological aging. Food restriction delays the onset of pathological lesions and so lengthens the life-span. On the other hand, exposure of rats or mice to ionizing radiation advances the onset of the lethal diseases which shorten their life. These forces act on the immune system.

The causes of aging are only partly known and consequently there are many theories of aging (Everitt, Chap. 2). The primary control of aging appears to reside in the genes, whose expression is programmed as a function of time. The rate of aging and the duration of life appear to be regulated by the interaction between the genetic program in the cell and environmental factors such as food supply, radiation, temperature and stress. The interaction of the environment with the "aging genes" is mediated, at least in part, by the hypothalamic-pituitary endocrine system as presented in simplified scheme in Figure 34-1. Adelman (Chap. 33) has shown that hormones can regulate the expression of genes.

The measurement of biological age (Everitt, Chap. 2) is difficult. There are two ways of assessing the aging process. One is to study mortality in a population and the second is to examine the impairment of particular physiological functions in the individual with advancing age. Life duration is not a measure of physiological aging, but of pathological aging



Figure 34-1. Simplified scheme showing the interaction between the environment and "aging" genes in tissue cells, mediated by the hypothalamic-pituitary-peripheral endocrine system.

since it is determined by the onset of disease. Physiological aging of the whole body can be investigated with a battery of physiological parameters. Pathological age can also be measured with a test battery (Ogura, 1967). Biological age may be estimated at first approximation by summing the physiological and pathological ages (Everitt, Chap. 2).

AGING IN THE HYPOTHALAMIC-PITUITARY-PERIPHERAL ENDOCRINE SYSTEM

Age changes occur in the hypothalamus, the pituitary and its target glands, just as in all other organs. These changes alter the output of "aging hormones" and so influence the course of aging.

"Hypothalamus

Neurons in the hypothalamus are nondividing cells, which accumulate age changes. The individual components of the hypothalamus age in different ways and at different rates. This diversity of age change in the hypothalamus produces a functional disregulation, which may be responsible

for the development of age-related pathology such as arterial hypertension and atherosclerosis (Folkis, Chap. 31).

The threshold of hypothalamic sensitivity to negative feedback of estrogens, corticosteroids and glucose, changes with age (Aschheim, Chap. 19; Riegle, Chap. 28; Dilman, Chap. 32). In man, the data of Dilman (Chap. 32) suggest that the hypothalamic sensitivity to estrogens, corticosteroids and glucose decreases in middle age. However, other workers (Franchimont, 1971; Wise, *et al.*, 1973) report a normal sensitivity to estrogen in most postmenopausal women. In the rat, Aschheim (Chap. 19) finds an increased sensitivity to estrogen and Riegle (Chap. 28) a decreased sensitivity to corticosteroids. Thus there may be differences according to the hormone concerned as well as the species.

Estrogen appears to have an aging action on the hypothalamus of the rat (Aschheim, Chap. 19). Deprivation of estrogen by castration or hypophysectomy preserves the juvenile sensitivity of the hypothalamus to estrogen.

Pituitary

Most studies of age changes in pituitary function and its hypothalamic control have been concerned with changes during early development, growth and puberty (Pecile and Bossa, Chap. 5). However, the recent development of radioimmunoassay techniques permits the measurement in elderly subjects of circulating pituitary hormone levels in response to provocative and inhibitory stimuli (Lazarus and Eastman, Chap. 6). The application of these dynamic tests of pituitary function has not revealed any significant impairment of pituitary hormone reserve in old age. Normal stimulatory and inhibitory mechanisms are retained in the elderly and have not provided any evidence for age-related defects in feedback or neural control of hypothalamic-pituitary function (Lazarus and Eastman, Chap. 6).

Techniques for the measurement of daily secretion rates of pituitary hormones are currently being developed. The secretion rates of all target gland hormones appear to decrease in old age, but the change in pituitary hormone secretion rates are less clear. If primary aging occurs in the target gland, this may lead to a compensatory rise in the secretion, as in the case of FSH and LH, and possibly TSH. However, at present there is no evidence of a rise in ACTH secretion in old age as a result of the decline in cortisol secretion. The probable age changes in secretion rates for both pituitary and target gland hormones in man are shown in Figure 34-2.

Growth hormone production rates in elderly human subjects are reduced, principally due to the diminished secretion of GH during sleep

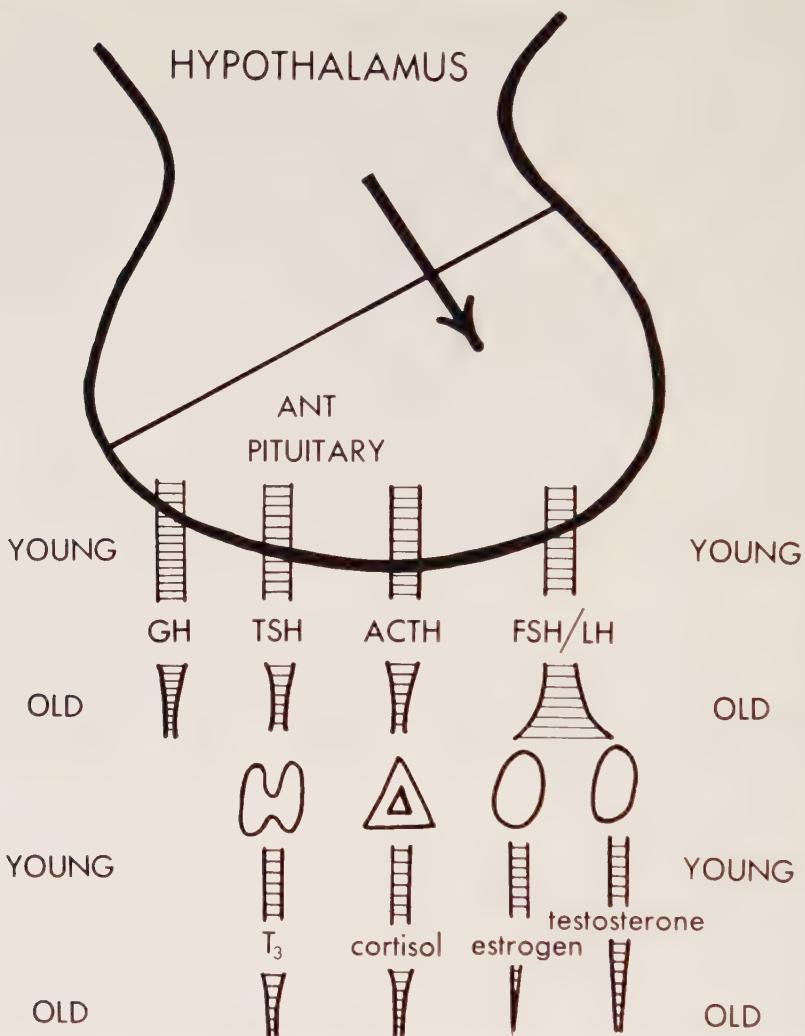


Figure 34-2. The probable effects of age on the secretion of pituitary and target gland hormones in man. The aging pattern is different in the rat (see p. 387). Different pituitary polypeptide species have been detected in senescent mice (M. M. Wilkes, *et al.*, 10th Int Congress Gerontol, Abst Vol II, p. 39, 1975).

(Finkelstein, *et al.*, 1972). The role of the hypothalamus in this age decrement in man is not clear. However in the rat, not only is the GH releasing activity of hypothalamic extracts significantly reduced in old age, but the pituitary gland is less responsive to GRH (Pecile and Bossa, Chap. 5).

Studies of plasma TSH changes with age have yielded conflicting results (Hales, *et al.*, Chap. 27). In the most recent study Blichert-Toft, *et al.* (in press) failed to show any age differences in the fasting morning values of 170 normal subjects aged 18 to 94 years. The stimulation of TSH secretion with thyrotropin releasing hormone is markedly reduced in elderly

males, but not in females (Snyder and Utiger, 1972). In view of the decreased secretion rate of thyroxine in old age (Gregerman, 1967) a decline in TSH secretion is to be expected.

Plasma ACTH levels apparently do not change significantly with age (Jensen and Blichert-Toft, 1971). Because of the difficulty of measuring ACTH in plasma it is common clinical practice to use plasma cortisol levels as an index of ACTH secretion. Such measurements indicate that the secretion of pituitary ACTH in response to hypoglycemia and metyrapone is not reduced in old age (Lazarus and Eastman, Chap. 6; Lockett, Chap. 15). However, the reduced secretion of adrenocortical hormones in old age (Romanoff, *et al.*, 1961) implies that the daily ACTH secretion is also reduced (Blichert-Toft, 1971).

Serum LH and FSH levels increase in postmenopausal women (Lazarus and Eastman, Chap. 6) and in elderly men (Lazarus and Eastman, Chap. 6; Vermeulen, Chap. 22). However, in old female rats, plasma LH levels are diminished, because the mechanism of aging in the hypothalamic-pituitary ovarian axis in rats is different from that in women (Aschheim, Chap. 19).

Basal serum prolactin levels do not change significantly with age in human subjects (Lazarus and Eastman, Chap. 6), but in old female rats plasma prolactin levels are increased (Aschheim, Chap. 19).

Antidiuretic hormone (ADH) secretion in the rat declines in old age. Also the secretion of ADH by the neurohypophysis of the rat in response to dehydration is reduced and delayed in old age (Turkington and Everitt, Chap. 7). However, in man the blood vasopressin level may increase with age (Frolkis, *et al.*, 1973).

The weight of the human male pituitary declines with age after the fourth decade (Rasmussen, 1938). Diseases of the pituitary are not common in elderly human subjects (Duchen and Schurr, Chap. 8), although in rats and mice the incidence of spontaneous pituitary tumors may be very high (Duchen and Schurr, Chap. 8). These tumors usually reduce hormone production by destruction of functioning pituitary tissue. However, in old female rats many tumors secrete prolactin (Aschheim, Chap. 19).

The pharyngeal pituitary consists of adenohypophysial cells in the mucoperiosteum of the roof of the pharynx. After the age of 50 years, especially in women, the pharyngeal pituitary becomes more active (McGrath, 1971). The increased activity may be due to the establishment of direct hypothalamic control in middle age.

Thyroid

In man current measurements of thyroid function indicate a reduction in thyroid hormone secretion in old age (Hales, *et al.*, Chap. 27). There is

a progressive decrease in serum triiodothyronine with human aging (Rubinstein, *et al.*, 1973). However, in the rat a decline in thyroid function with age has not been conclusively established (Everitt, Chap. 26). Both in rat and man the uptake of iodine¹³¹ by the thyroid decreases with age. Hyperthyroidism is a comparatively common disease in elderly human patients (Hales, *et al.*, Chap. 27).

Adrenal Cortex

Plasma corticosteroid levels do not change with age under basal conditions either in man (Grad, *et al.*, 1967; Jensen and Blichert-Toft, 1971) or in the rat (Riegler, Chap. 28). However, following ACTH or stress stimulation there is a decreased reserve adrenocortical secretory capacity in old age (Riegler, Chap. 28). The rate of secretion and metabolism of adrenocortical hormones declines with age probably because of a reduced requirement as the metabolic muscle mass falls (Romanoff, *et al.*, 1961).

Ovary

The life of the ovary is short in women, and is essentially terminated at the time of the menopause at age 50 years, when reproductive function ceases (Everitt, Chap. 20). The secretion of estrogens declines to a very low level at the menopause but the ovary continues to secrete other steroids, such as the nonclassical phenolsteroids (Dilman, Chap. 32). In the rat, the ovary continues to function in old age when the estrous cycle undergoes senile deviations in the form of persistent estrus and repetitive pseudopregnancies (Aschheim, Chap. 19).

Testis

In elderly men the plasma testosterone level decreases rapidly from the 6th decade onwards, and the production rate of testosterone also declines sharply at the same time (Vermeulen, Chap. 22). The response of the Leydig cells to gonadotropin decreases in old age, suggesting primary aging of the testis (Vermeulen, Chap. 22).

Pancreatic Islets

There is a progressive decrease in glucose tolerance with age in man (Burgess, Chap. 25). According to Duddl and Ensinek (1972) insulin secretion declines with age, whereas glucagon secretion appears to remain unchanged. Paradoxically glucose loading produces a greater rise in serum insulin (after 1 hour) in elderly subjects than in young (Dilman, Chap. 32). This hyperinsulinemia may be due to the excessive secretion of biologically inactive proinsulin which is not distinguishable from immunoreactive

insulin (Joffe, *et al.*, 1969). In the rat Adelman (Chap. 33) finds a fall in the blood level of immunoreactive insulin with age.

Conclusion

Although in man there is little evidence of significant impairment of anterior pituitary function in old age, the daily secretion rate of target gland hormones decreases with age. These changes may be due to 1) primary aging of the gland as in the case of the ovary and testis, 2) an adjustment to a decreased demand for hormones by an aged body that has a smaller number of cells requiring hormonal regulation, as proposed for the hormones of the thyroid and adrenal cortex, 3) a reduced blood flow to the gland due to atherosclerosis, as in the case of the testis, or 4) age changes in the central regulatory mechanism, postulated for adrenal and ovarian hormones.

ENDOCRINE EFFECTS ON AGING

Do hormones affect the course of aging? Brown-Séquard in 1889 described a rejuvenating effect of the testicular extract, which he injected into himself, at the age of 72 years. Now, almost a century later, there is a considerable volume of data to show that hormones do in fact affect aging processes. It now appears that most hormones accelerate aging processes in their target organs.

Hormonal stimulation of target organs increases their functional load and in consequence increases the wear and tear or aging of these organs. Therefore one might regard an "aging hormone" as one which either accelerates physiological aging or increases the incidence of age-related pathology. There is little evidence for the existence of a general "aging hormone," but rather there appear to be a number of organ-specific "aging hormones." Thus one may postulate that the thyroid stimulating hormone by stimulating the thyroid gland could be the "thyroid aging hormone." In a similar way thyroxine and the corticosteroids could be termed "collagen aging hormones" because they increase the aging of collagen fibers in rat tail tendon.

However, this concept raises problems. For example, estrogen can hardly be called a "skeletal aging hormone" because even though it accelerates physiological aging of the skeleton (Silberberg, Chap. 12) it may also have a preventive and curative effect on postmenopausal osteoporosis (Everitt, Chap. 20). Furthermore, growth hormone appears to accelerate collagen aging in the skeleton (Silberberg, Chap. 12) and yet retard it in rat tail tendon (Everitt, Chap. 11). In view of these difficulties it is better to refer to the specific aging effects of hormones rather than use the term "aging hormones."

Probably the first association between pituitary function and aging was that reported by Glinski (1913) and Simmonds (1914). In Glinski-Simmonds' disease, the pituitary gland is destroyed and the patient develops physical signs of premature senescence (Herman, Chap. 9). In another clinical condition, Cushing's syndrome, premature aging is associated with an overproduction of adrenocortical hormones (Herman, Chap. 9; Wexler, Chap. 17).

Collagen Aging

Age changes in the physical properties of collagen were first described by Verzár (1955) who used these changes as an index of biological aging. The chemistry of the cross-links, which accumulate during maturation and aging, has been partly elucidated during the last decade (Deyl, *et al.*, Chap. 10). Hormones have been shown to affect not only the metabolism but also the aging of collagen (Deyl, *et al.*, Chap. 10). Recently, it has been demonstrated that hypophysectomy interferes with collagen crosslinkage, probably by inhibiting lysyl oxidase, an enzyme concerned with collagen crosslinkage (Shoshan, *et al.*, 1972). The aging of collagen fibers in rat tail tendon is accelerated by hormones secreted by the pituitary, thyroid, ovary and the adrenal cortex (Everitt, Chap. 11; Árvay, Chap. 18).

Testosterone (Árvay and Takács, 1965) and growth hormone (Everitt, Chap. 11) appear to retard the aging of collagen. This effect is presumably due to the laying down of newly synthesized or "young" collagen under the influence of these hormones.

Skeletal Aging

Hypophysectomy markedly retards the growth and aging of bone (Assling, *et al.*, 1954). In the skeleton the processes of growth and maintenance are inseparably linked to aging (Silberberg, Chap. 12). Thus growth may not be stimulated without acceleration of aging. Growth hormone, thyroid hormone and estrogen accelerate, whilst the glucocorticoids inhibit skeletal aging.

The effects of growth hormone and corticosteroid on the skeleton and on tail tendon collagen are different (see Table 34-1). It is possible that an age-accelerating effect on skeletal collagen is masked by age-retarding effects on other processes involved in skeletal aging, such as calcification or the metabolism of mucopolysaccharides.

Cardiovascular Aging

Hypophysectomy depresses cardiovascular function. There are decreases in cardiac weight, cardiac output, cardiac work, blood volume and blood

TABLE 34-I
THE EFFECT OF HYPOPHYSECTOMY AND HORMONES ON AGING AND AGE-RELATED PATHOLOGY

Treatment	Physiological Aging				Aged-related Pathology				Life Duration
	Tendon	Collagen	Skeleton	Ovary	Kidney	Kidney	Arteries	Tumors	
Hypophysectomy	-	-	-	-	-	-	-	-	-
Posterior pituitary hormones									+
GH	-?	+			+	+	+		
TSH and thyroid hormones . .	+	+			+	-	-		-
ACTH and corticosteroids . .	+	-			+	+			+
Estrogens	+?	+				-	+	+	
Testosterone	-?								-

+ increase. - decrease.

(Reproduced with minor changes from Everitt (1973) with permission of Pergamon Press Ltd.)

pressure (Kovacs and Horvath, Chap. 13). Because of the reduced load on the cardiovascular system in hypopituitarism a slower rate of aging might be expected. A number of studies in the rat seem to indicate that hypophysectomy retards aging in the heart and aorta (Everitt, Chap. 14). The development of atherosclerosis is influenced by hormonal factors regulating lipid metabolism (Katz and Pick, 1967). For example, Wexler (Chap. 17) has described how adrenocorticism associated with reproductive effort is accompanied by hyperlipidemia, hypertension, arteriosclerosis, myocardial infarction and cerebrovascular accidents.

Aging of Lipid Metabolism

The lipid content of many tissues, including the aorta, increases with age, and at the same time the serum cholesterol level rises (Hruza, Chap. 24). In old animals adipose tissue is less responsive to the lipolytic actions of growth hormone and epinephrine. The decreased secretion of pituitary lipolytic hormones, together with the decreased sensitivity of adipose tissue to these hormones may contribute to the development of obesity and atherosclerosis (Hruza, Chap. 24). On the other hand Dilman (Chap. 32) claims that the age-related elevation of the hypothalamic threshold produces a compensatory hyperinsulinemia, which leads to the development of prediabetes and the metabolic disorders inherent in atherosclerosis.

Wexler (Chap. 17) has shown that an excess of adrenocortical hormones, as in Cushing's syndrome, can produce hypercholesterolemia and atherosclerosis. Thyroid hormone may inhibit atherosclerotic changes (Wren, 1968). Estrogens are believed to inhibit and androgens promote atherosclerotic changes.

Hypophysectomy in the rat appears to accelerate the aging process in relation to cholesterol metabolism and atherosclerosis (Hruza, Chap. 24). This effect is in contrast to retarded aging in collagen fibers, the skeleton, the ovary and kidney (Everitt, Chap. 4) and is in conflict with evidence suggesting retarded physiological aging of the aorta (Everitt, Chap. 14).

Renal Aging

There is little information available on the role of hormones in the physiological aging of the kidney (Lockett, Chap. 15). There are large decrements with age in renal function in man. Similar decrements in renal function occur in hypopituitarism and after hypophysectomy in man (Kovacs and Horvath, Chap. 13). It is tempting to postulate that these age decrements are due to a lack of ACTH and growth hormone, the major pituitary hormones concerned with maintaining renal function. However, Lockett (Chap. 15) feels that as there is no evidence of a deficiency of either ACTH or GH in old age, this is not the cause of renal aging.

In the rat, as in man, hypophysectomy produces decrements in renal blood flow and glomerular filtration rate (Kovacs and Horvath, Chap. 13). Because of the reduced work load of the kidney in the hypophysectomized rat it is not surprising to find evidence of diminished renal aging (Everitt, Chap. 4). Hypophysectomy performed early in life prevents the age-related rise in protein excretion in male rats and also significantly retards the thickening of basement membranes in the glomerulus and the proximal tubule.

In small mammals, ACTH and GH produce renal lesions similar to those seen in old age. Prolonged treatment of young mice with ACTH produces glomerular changes resembling the glomerulosclerosis of old age (Christian, Chap. 16), however, these lesions are unlike any common human glomerular lesion or experimental immunologic glomerular lesion in animals. A natural glomerular disease of the woodchuck (resembling the ACTH-produced disease of the mouse) is more prevalent and severe in animals exposed to social strife. Growth hormone has a nephrosclerotic action in the presence of sodium-retaining corticosteroids. In the hypophysectomized rat, long term growth hormone replacement therapy partly restores the age-related excretion of protein (Everitt, Chap. 4). Thyroxine when administered for long periods, elevates age-related protein excretion (Everitt, Chap. 26) and increases the incidence of renal lesions (Berg, Chap. 3).

Ovarian Aging

Jones and Krohn (1961) clearly showed that hypophysectomy in the CBA mouse significantly retards, but does not prevent, the normal progressive loss of oocytes from the ovary (see Aschheim, Chap. 19). Apparently pi-

tuitary gonadotropins accelerate the age-associated loss of oocytes from the ovary. Intrinsic aging of the human ovary may be accelerated by hypothalamic age changes which increase the secretion of pituitary gonadotropins (Everitt, Chap. 20). On the other hand, ovarian aging in the rat appears to be due to hypofunction of the hypothalamic centers leading to a fall in LH secretion (Aschheim, Chap. 19).

Neoplasms

During the course of life the type of cancer and its incidence is clearly related to the endocrine epochs of birth, puberty, maturity and the menopause (Freedman, Chap. 21). Hypophysectomy achieves significant palliation in 40 percent of breast cancer patients, and in a small population of rats hypophysectomy performed early in life reduced tumor incidence to zero in old age (Everitt, Chap. 4). It is quite clear that a number of tumors are dependent on pituitary hormones. Chronic growth hormone injections throughout life have been shown to increase the incidence of neoplasms in many organs of the rat (Moon, *et al.*, 1951). Prolactin and the luteinizing hormone are believed to play a supporting role in certain cancers of the breast and genital tract in women. On the other hand, thyroid hormone may act as an anticarcinogen due to its inhibitory action on the pituitary (Pelner, 1957). Phenolsteroids may be carcinogenic (Dilman, Chap. 32).

Life Duration

Hypophysectomy reduces significantly the life expectancy of the rat and toad, and replacement therapy with certain hormones like ACTH, corticosteroids and growth hormone prolongs their life (Everitt, Chap. 4). The life duration of the hypophysectomized rat is restored almost to normal by cortisone treatment (1 mg per week) (Everitt, 1971). Another adrenocortical steroid, prednisolone, was found by Bellamy (1968) to prolong the life of a short lived strain of mice.* Growth hormone replacement therapy in hypophysectomized rats reduces mortality until middle age, but not after (Everitt and Burgess, Chap. 23). Long term treatment of intact rats with growth hormone did not prolong their life (Everitt and Burgess, Chap. 23). Growth hormone and ACTH increase the survival of hypophysectomized toads (Jørgensen and Larsen, 1963).

Posterior pituitary extract alone (Friedman and Friedman, 1963) or in combination with cortisol prolongs the life of the old rat (see Turkington and Everitt, Chap. 7). There is evidence that oxytocin may be the life-prolonging factor in posterior pituitary extract.

Thyroid hormone was shown to shorten the life of mice (Robertson, 1928) and rats (Everitt, Chap. 26).

* Cortisol increases lifespan of WI-38 cells in culture by 30%. (Cristofalo, V. F. *10th Int Congress Gerontol, Abst Vol I*, p. 94, 1975).

Testosterone is believed to have a life-shortening action in the male. Castration was found to prolong the life of mentally retarded men (Hamilton and Mestler, 1969). In rats Asdell, *et al.* (1967) have shown that androgens shorten whilst estrogens prolong life. Similar effects of gonadectomy and sex hormones on survival have been observed in lampreys by Larsen (1973) and salmon (Wexler, Chap. 17).

In the lamprey, however, hypophysectomy increases the life-span (Larsen, 1973b), and hypopituitary mice are believed to be long-lived (Silberberg, 1972). Thus in some species the pituitary is secreting a life-shortening factor. Life can be prolonged by reduced food intake (rat), gonadectomy (cat, salmon, lamprey), low temperature (annual fishes, lamprey) and hypophysectomy (lamprey). It is tempting to assume that these procedures are all reducing the rate of some process contributing to natural death (Larsen 1973b).

Conclusion

Almost all hormones secreted by the anterior pituitary, the thyroid and the adrenal cortex have been shown to influence aging phenomena in one way or other (see Table 34-I). However, each hormone appears to exhibit specific effects. For example, the adrenocortical hormones inhibit skeletal aging but accelerate the aging of tail tendon collagen, and in large amounts produce cardiovascular disease. Growth hormone appears to inhibit the aging of tail tendon collagen, accelerate skeletal aging, promote cardiovascular and renal disease, and increase the number of neoplasms (in the rat). Thyroid hormone accelerates collagen, skeletal and renal aging, but appears to have inhibitory actions on atherosclerosis and neoplasia. The pituitary secretes both life-shortening and life-maintaining factors, which probably act on the immune system.

THE HYPOTHALAMIC-PITUITARY CONTROL OF AGING

The pituitary gland controls not only the functions of the thyroid, adrenal cortex, ovary and testis, but also regulates the processes of growth, reproduction and metabolism. Evidence presented in this monograph indicates that the pituitary is also concerned in regulating the rate of aging and the onset of age-associated pathology.

The Hypopituitary Hypothesis of Aging

This hypothesis states that the phenomena of aging are due to a lack of pituitary hormones.

Glinski (1913) and Simmonds (1914) observed premature senile changes in patients whose pituitary had been destroyed by disease (see Herman, Chap. 9). Similar observations were made by Pribram (1927) on human patients and by Smith (1930) on hypophysectomized rats. Thus it would

appear that severe hypofunction of the pituitary could cause involutional changes similar to those of senescence. There is no doubt that there is involution of structures (e.g. gonads, accessory reproductive organs, muscles) and depression of functions (e.g. reproductive, metabolic) which are normally maintained or stimulated by pituitary and target gland hormones.

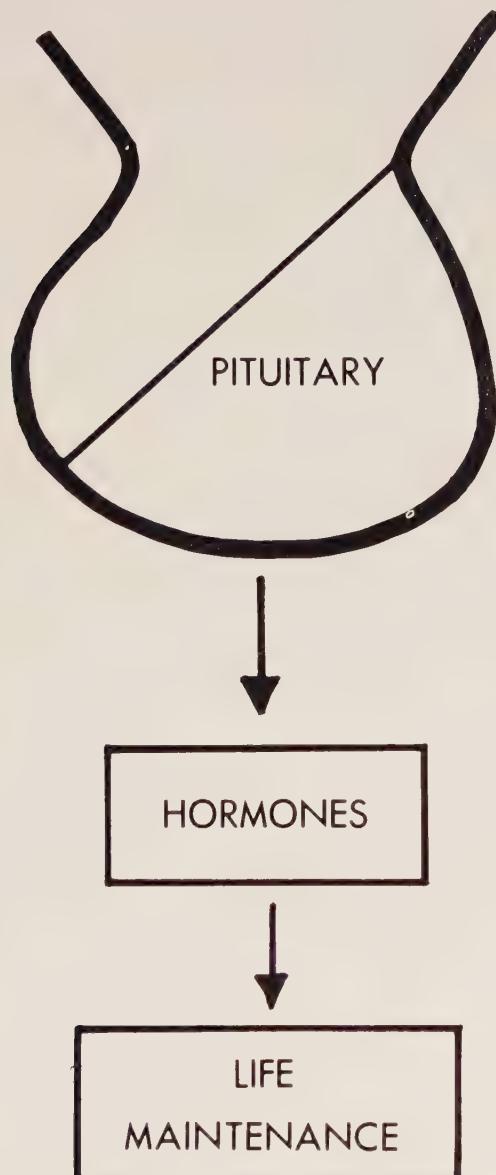


Figure 34-3. The pituitary gland is essential for a normal life-span because it secretes life maintaining hormones in the rat.

Although the involution resembles that occurring in senescence (Herman, Chap. 9), it is not true aging because it can be reversed by appropriate hormone replacement (Taylor, 1956). Aging is an irreversible change according to Strehler (1959). Furthermore, objective criteria of physiological aging in the hypophysectomized rat (Olsen and Everitt, 1965; Verzár and Spichtin, 1966) do not support the subjective observations of premature senility as reported by Smith (1930). There is no direct evidence to support the hypopituitary hypothesis of aging.

Nonetheless, the life duration is significantly reduced both in the patient with Simmonds' disease and in the hypophysectomized rat (Everitt, Chap. 4). Clearly the pituitary secretes a life maintaining factor (Fig. 34-3), which is probably ACTH acting by mediation of the adrenocortical steroids. Small doses of cortisone administered every week were found to restore the life duration of the hypophysectomized rat almost to normal (Everitt, Chap. 4). In the 1930's it was discovered that the adrenocortical steroids are essential for life (Reichstein and Shoppee, 1943). The life shortening caused by hypophysectomy is apparently due to a metabolic disorder, since pathological lesions are very rare. In addition to cortisone, Friedman and Friedman (1963) have shown that posterior pituitary hormones have a life maintaining effect in old rats.

Thus the pituitary gland is essential for a normal life-span in the rat, because it secretes life-maintaining hormones. However, regardless of its life-maintaining action, the pituitary secretes certain hormones which have aging effects (see Table 34-I).

The Pituitary Hypothesis of Aging

This hypothesis states that certain pituitary hormones accelerate the aging process.

Hypophysectomy is known to retard aging processes in collagen, kidney, bone and ovary in the rat. It does this by withdrawing the pituitary hormones that stimulate these aging processes.

Almost all of the anterior pituitary hormones and their target gland hormones have been shown to have an aging effect in one organ or another (Table 34-I).

These hormones accelerate the physiological aging of collagen in rat tail tendon and the skeleton, promote the development of age-related pathology in the kidney and the cardiovascular system, and increase the incidence of tumors (Everitt, 1971). These pathological effects eventually shorten the life-span. Physiological aging probably shortens life indirectly by promoting pathological changes. This model is shown in Figure 34-4.

These aging or pathological effects are probably due to prolonged stim-

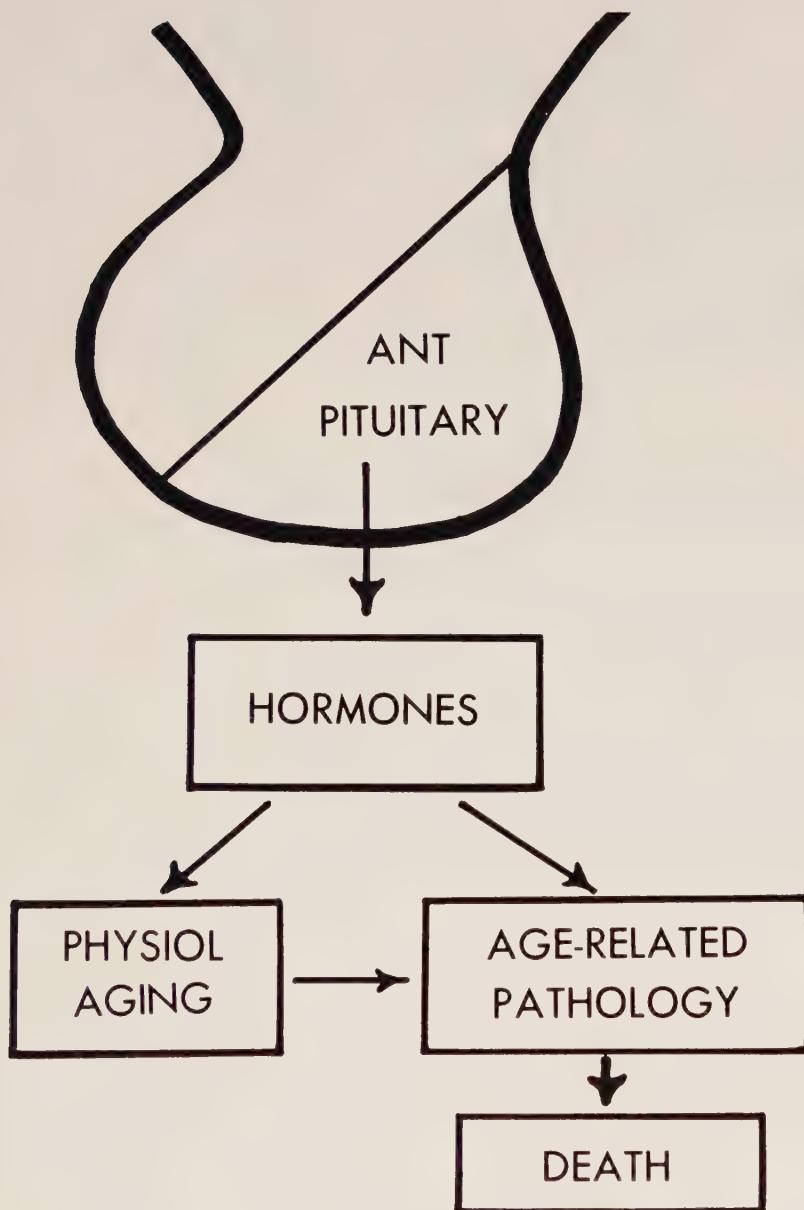


Figure 34-4. The pituitary control of aging. Certain hormones secreted by the anterior pituitary gland either directly or indirectly through the target glands, stimulate physiological aging and promote age-related pathology which ultimately leads to death (Reproduced from Everitt (1973), with permission of Pergamon Press Ltd.).

ulation by hormones and hence overuse of these organs. According to the wear and tear hypothesis of aging, the more an organ is used the faster it wears out. Each organ or function appears to have a limited life and after being used a certain number of times it starts to break down. Selye and Tuchweber (Chap. 29) discuss the wearing out of the adaptive mechanisms as a result of long-continued exposure to stress. Of course, hormonal stimulation may accelerate the aging of organs by mechanisms other than wear and tear. Hypophysectomy, by removing the source of hormonal stimulation, permits body functions to operate at their basal level and thus aging changes proceed at the minimum rate. Aschheim (Chap. 19) regards the aging process in the hypophysectomized animal as "suspended aging." Thus the onset of breakdown phenomena or age-related pathology is delayed.

The Hypothalamic Control of Aging

Primary age changes in the hypothalamus may accelerate the phenomena of aging in the whole body. Like the pituitary, the hypothalamus has many functions, one of which is to control the pituitary gland. The hypothalamus dominates and drives the pituitary.

Endocrine Mechanism

The secretion of the anterior pituitary hormones is controlled by the hypothalamic releasing hormones (see Fig. 34-5). Oversecretion of an anterior pituitary hormone is inhibited by negative feedback of the pituitary hormone, its target gland hormone or a metabolite like glucose, onto the hypothalamus. Dilman (Chap. 32) believes that as a result of intrinsic age changes, the human hypothalamus becomes less sensitive to feedback suppression. The threshold to suppression by negative feedback becomes elevated (Fig. 34-5). This leads to oversecretion of hormones, particularly 1) cortisol due to compensation in the adaptation system, 2) the nonclassical phenolsteroids from compensation in the reproductive system and 3) insulin from loss of rhythmic function in the energy system. Dilman (Chap. 32) claims that in man these three homeostatic systems and the corresponding hormones are the ones which undergo the greatest change with age. This concept of the elevated hypothalamic threshold leading to increased secretion of aging hormones is now incorporated into our model shown in Figure 34-5.

Nonendocrine Mechanisms

The hypothalamus regulates many functions essential for life such as food intake, body temperature, water balance, blood pressure and heart

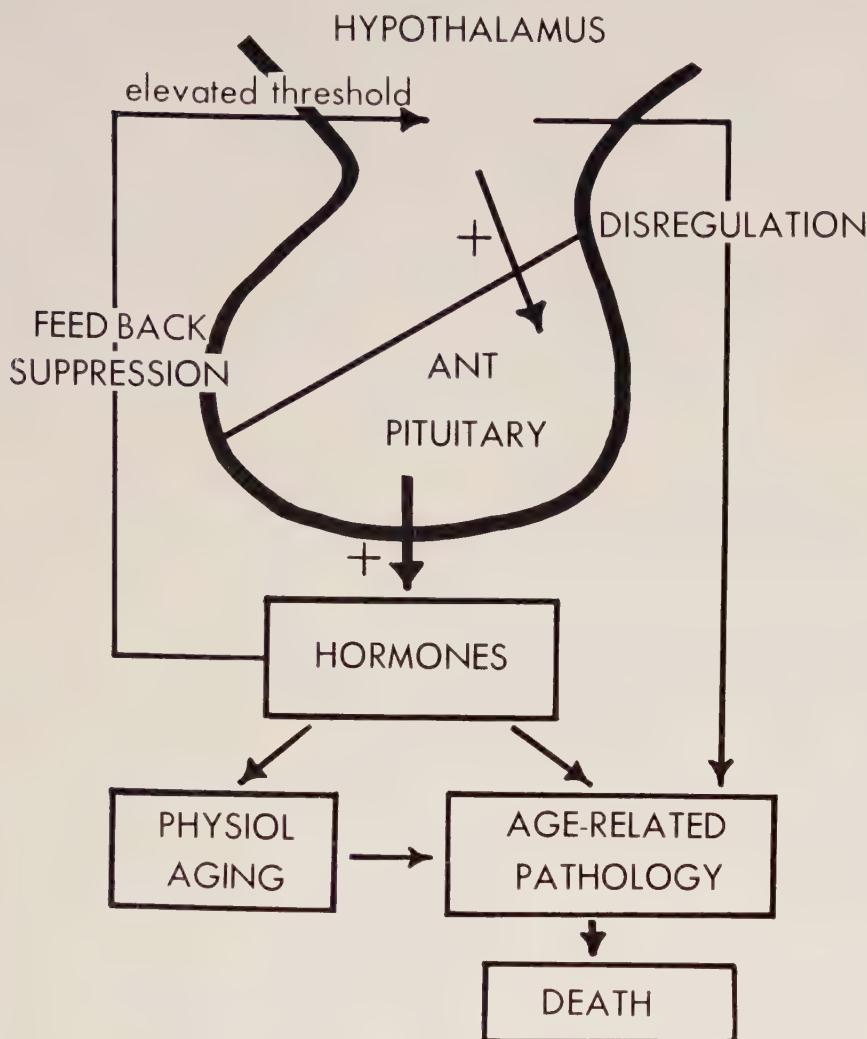


Figure 34-5. The hypothalamic control of aging mediated by anterior pituitary and target gland hormones. Intrinsic aging of the hypothalamus causes elevation of the hypothalamic threshold to feedback suppression, which leads to increased secretion of hormones (Dilman, Chap. 32). Nonuniform aging of the hypothalamus causes disregulation resulting in age-related pathology (Frolkis, Chap. 31) (Reproduced from Everitt (1973), with permission of Pergamon Press Ltd.).

rate. If there is a central regulator of aging then the hypothalamus must be a strong candidate (Everitt, 1972).

Frolkis (Chap. 31) has shown that the individual components of the hypothalamus age in different ways and at different rates. A lack of uniformity in hypothalamic aging would thus create irregularities in hypo-

thalamic function, which could produce the age-related pathology seen in man. This concept is introduced into our model in Figure 34-5 as a disregulation loop from the hypothalamus to age-related pathology. A more general discussion of the effects of primary aging in the brain is made by Still (1969) in the cybernetic theory of aging.

Temperature is a major factor controlling the rate of metabolism and aging (Everitt, Chap. 26). Thus a change in the setting of the body's thermostat, the thermoregulatory centers in the hypothalamus, should have profound effects on both the rate of aging and the life duration. Similarly a change in the setting of the feeding and satiety centers in the hypothalamus would markedly affect life duration (Everitt and Porter, Chap. 30).

THE AGING PROGRAM

Many studies show that aging is an intrinsic process which is genetically determined. There is an orderly sequence of changes which appear to be programmed from the time of fertilization when the genetic code of the individual is laid down. A short list of events in the life program is given in Table 34-II.

Physiological events are precisely timed in the developmental period but not in the involutional period except for the menopause in women (Timiras, 1972). Instead there is a progressive decline in a large number of physiological parameters from age 25 years in man (Everitt, Chap. 2). The type of cancer in man and its incidence changes in a remarkably constant sequence throughout life (Freedman, Chap. 21). The sequence of change and the rate of change in clearly programmed and presumably under genetic control.

A program of aging which is so precisely timed is probably controlled by an "aging clock." If such a clock were located in the hypothalamus (see Fig. 34-6) it could control the aging of a large number of functions.

TABLE 34-II
LIFE PROGRAM

Conception
Embryonic development
Birth
Growth
Puberty
Maturity
Physiological decline
Menopause
Immunological decline
Pathological lesions
Terminal disease
Death

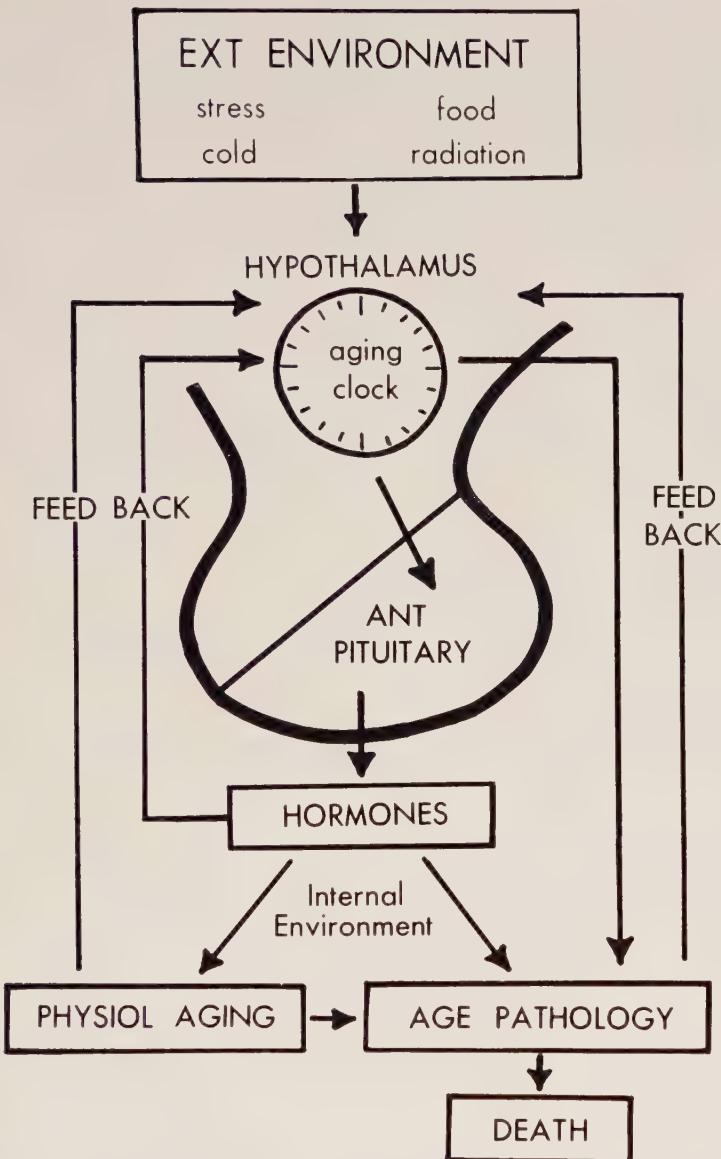


Figure 34-6. The aging effect of environmental factors mediated by the hypothalamus, the anterior pituitary and the peripheral endocrine system. Environmental factors are both internal (e.g. peripheral aging and disease) and external (e.g. food supply, temperature, stress and radiation).

There could be separate clocks* for each function, e.g. one clock controlling the secretion of LRH, another for TRH and so on. Let us consider the possible action in a woman of the gonadotropin "aging clock" controlling the secretion of LRH and hence LH secretion and the ovarian functions

* Pacemakers of aging (Caleb Finch).

of ovulation and hormone secretion. After the gonadotropin "aging clock" has monitored the secretion of (say) 1 g of LRH the onset of puberty occurs. The clock ticks away further quantities of LRH in regulating menstrual cycles until (say) 10 g of LRH, through the mediation of FSH and LH, finally exhausts the ovary resulting in the menopause.

Dilman (Chap. 32) has proposed a different mechanism of programmed aging. He believes that the neuroendocrine program of aging depends on intrinsic age changes in the hypothalamus, which elevate the threshold to feedback suppression. Dilman is convinced that gradual elevation of the threshold to feedback suppression leads to disturbance of the rhythmic activity of some hypothalamo-pituitary systems or to increased stimulation of the pituitary, which promotes growth and development. Continued elevation of the threshold throughout life causes alterations in reproductive, energy and adaptation homeostasis, which is maintained only by compensatory oversecretion of the appropriate endocrine gland. Hormone excess causes the "diseases of compensation" which in man are age-related obesity, prediabetes and adult-onset diabetes mellitus, atherosclerosis, hypertension, climacteric, lowered resistance to infection and some forms of cancer. The terminal diseases are thus caused by oversecretion of aging hormones.

Programmed aging may be modified by environmental factors, both internal (peripheral aging and disease) and external (stress, food supply, temperature, bacteria, viruses, smoke, etc.).

Internal Environmental Factors—Aging, Disease and Pregnancy

Peripheral tissues age and often lose their sensitivity to stimulation by hormones (Gussek, 1972; Hruza, Chap. 24). How does the body adjust to peripheral aging? Adaptation is wholly or partly mediated by the hypothalamic-pituitary-endocrine system, depending on the organ. For example, aging of the ovary and testis reduces the responsiveness to stimulation by gonadotropins. This leads to a compensatory rise in the secretion of gonadotropins in an effort to stimulate the aging gonad. However, adaptation to aging in a nonendocrine organ, like heart or lung, would be only partly mediated by the hypothalamic-pituitary-endocrine system. This concept is represented in Figure 34-6 as a feedback loop from physiological aging to the hypothalamus. Similarly the onset of age-related pathology would stimulate the hypothalamus and pituitary leading to increased secretion of adrenocortical hormones; a greater secretion of hormones would then alter the rate of programmed aging in target organs. Pregnancy increases aging by a similar mechanism (Wexler, Chap. 17; Árvay, Chap. 18).

External Environmental Factors

The rate at which programmed changes develop can be influenced by extrinsic factors such as food supply, environmental temperature, stress and radiation (Fig. 34-6). The hypothalamus and the pituitary play an important role in mediating the effects of extrinsic factors on programmed aging. Endocrine secretion is regulated by the hypothalamus to meet the demands of the environment. Hormones prolong life in a hostile environment but at the same time accelerate aging.

Suprahypothalamic structures are also concerned in mediating the effects of the environment, but their role is omitted due to lack of data on their relation to aging. The strong association between brain weight and longevity in animals (Sacher, 1959) suggests that other areas of the brain may be implicated in the control of aging.

The intake of food has been recognized for a long time as an important factor in aging (Berg, Chap. 3; Everitt, Chap. 30). When there is an abundance of food the intake is usually increased. The greater the quantity of food consumed the faster is the rate of aging, the earlier the onset of age-related pathology and the shorter the life-span. Food restriction has an anti-aging action probably due to the depression of anterior pituitary function (Mulinos and Pomerantz, 1940) and hypothalamic endocrine function (Fig. 34-6). Thus the secretion of many hormones is diminished in the food restricted rat (Everitt and Porter, Chap. 30). Conversely the secretion of a number of hormones is increased in obesity.

Exposure to low temperature for long periods has an aging and life shortening action in the rat (Johnson, *et al.*, 1963; Everitt, Chap. 26). These effects appear to be mediated through the hypothalamic-pituitary-thyroid axis, which increases its output of thyroid hormone. Thyroxine increases heat production and probably acts as a general aging hormone by stimulating metabolic processes in almost every organ of the body. The more work an organ does, the higher its metabolic rate and the shorter its life.

Continuous exposure to stress increases physiological aging, accelerates the onset of age-related pathology and shortens life in animals (Selye and Tuchweber, Chap. 29). The exceptional longevity of inhabitants of the valley of Vilcabamba in Ecuador is attributed in part to the freedom from stress (Davies, 1973). The effects of stress are mediated via the hypothalamic-pituitary-adrenocortical axis. The hormones secreted during stress (ACTH, corticosteroids, growth hormone and catecholamines) produce pathological lesions similar to those seen in old age. Selye (1950) regards the life-span as a protracted general adaptation syndrome that de-

velops in response to the stress and strain of daily life. Adaptation is very low in infancy, rises to a peak in the adult, and is lost in old age. Ionizing radiation, which has a life shortening action (Berg, Chap. 3), acts in part like other stresses through the hypothalamic-pituitary-adrenocortical axis.

Smoke from cigarettes, motor car exhausts and industrial smoke stacks is a major environmental factor which shortens life. It does this by increasing the incidence of lung cancer and cardiovascular disease. It also accelerates the physiological aging of the lung (Dontas, A. S., Webster, I. personal commun.) and probably other organs (Forbes and Gentleman, 1973). The role of the hypothalamus and the endocrines in these changes is unknown.

CONTROL OF THE AGING PROGRAM

Physiological age changes and age-related pathology can be controlled in at least two ways. They can either be prevented or retarded in onset by various treatments described by Dilman (1971) or the aged and defective cells can be removed as they appear by the immune system as described by Burnet (1970). This discussion will be confined to the preventive aspect.

Dilman (1971) has proposed prophylactic and therapeutic measures acting both at the hypothalamic and pituitary levels to slow down the genetic program of aging and age-related pathology. Dilman emphasizes a need for developing drugs that could raise the hypothalamic sensitivity threshold to regulating influences and thus normalize the rhythmical function of the main homeostatic systems, as well as medicaments to reduce the compensatory reactions of the peripheral endocrine glands. Some suggested agents are (a) estrogens or pituitary inhibitors to inhibit the elevated gonadotropin secretion, (b) pineal extract containing the polypeptide fraction or some other drugs to restore the rhythmic activity of the hypothalamic system, (c) phenformin to eliminate compensatory hyperinsulinemia, (d) drugs to inhibit the enhanced secretion of cortisol and to raise the age-linked diminished level of production of androsterone and etiocholanolone, and (e) food restriction which inhibits pituitary hormone secretion.

These measures can only retard the aging program. They cannot prevent the inevitable onslaught of time on the organism. Complete removal of pituitary function in the rat by hypophysectomy only retards aging; it does not abolish aging (Everitt, Chap. 4). It is assumed that this will also apply to the human situation. Only by controlled modification of the genetic code and its expression will it be possible to achieve any major extension of the life-span. Studies on the activation and suppression of normal "aging genes" may therefore lead to significant control over age-related pathology.

and hence to gains in longevity. The pioneering work of Adelman (Chap. 33) may show us how to control the "aging genes" with hormones.

CONCLUSION

The whole life program from conception through growth, development, physiological aging and the onset of diseases leading to death, although primarily under genetic control, appears also to be regulated by the hypothalamic-pituitary-peripheral endocrine system. The very hormones which are responsible for growth and development are those which accelerate the decline phase of life. Hormones by stimulating their target organs, accelerate the life program in those organs and ultimately in the whole body. These effects are probably due to the hormonal regulation of gene expression.

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APPENDIX

PITUITARY-THYROID AXIS AND AGING

W. DONNER DENCKLA

SUMMARY

A NEW FUNCTION of the pituitary has been discovered that apparently decreases the responsiveness of peripheral tissues to thyroid hormones throughout life. The production of a gradually increasing state of hypothyroidism could cause a concomitant decrease in resistance to some diseases.

METABOLISM AND THYROID

Past basal metabolic methods suffer from a lack of precision and endocrine specificity (Denckla, 1973). These liabilities have discredited the BMR as an assay for the thyroid state of the peripheral tissues. However, a new physiological parameter, minimal O₂ consumption (MOC), was discovered that apparently is more specific for changes in thyroid state. Studies with over 70 endocrine and non-endocrine factors indicated that the MOC, unlike the BMR, was altered only when there was unequivocal or strongly suggestive evidence for changes in the thyroid state of peripheral tissues (Denckla, 1970, 1973, 1974; Bilder, 1974; Denckla, 1975a). It is suggested that some of the previous work on thyroid and aging requires re-evaluation when based on BMR or the methods and assumptions underlying BMR-type of measurements.

THYROID AND AGE

Like the BMR, the MOC declines markedly with age (Denckla, 1974). Plasma levels of thyroid hormones do not change with age sufficiently to account for the fourfold greater MOC of young rats. However, it was discovered that there was at least a threefold greater tissue responsiveness to thyroxine (Denckla, 1974) and tri-iodothyronine (unpublished observations) in young rats. This marked decline in tissue responsiveness with age could readily explain the MOC decline with age.

PITUITARY CONTROL OF THYROID

Of all the major endocrinopathies studied only hypophysectomy of young rats arrested the normal age associated decline in tissue responsiveness to thyroxine and only hypophysectomy of adults, if one kept the rats long enough post-operatively, resulted in the full restoration of juvenile re-

sponsiveness to thyroid hormones (Denckla, 1974). A factor has been partially purified from bovine pituitaries which depresses the response to thyroxine of young hypophysectomized rats. Evidence has been given elsewhere that suggests this new function of the pituitary is probably associated with a new hormone (Denckla, 1973, 1974). The substance is tentatively called decreasing O₂ consumption hormone (DECO).

Compared to all other known pituitary hormones, DECO has the longest biological half life of decay in adult rats. Two months after hypophysectomy there is still no return of juvenile responsiveness to thyroxine. It requires either a six months wait for untreated hypophysectomized rats or continuous treatment of such rats with moderately high doses of thyroxine for three months to restore full juvenile responsiveness to thyroxine (Denckla, 1974).

DECO AND DISEASE

A neuroendocrine theory of dying has been presented (Denckla, 1975b). It is suggested that any endocrine system designed to kill mammals has only to cripple the immune and the cardiovascular systems to account for most of the diseases which are the final causes of death as determined at autopsy. The unusually long half life of DECO permitted an attempt to determine if it was responsible for the decline with age of phagocytic and graft rejection rates. After an appropriate post-operative wait, 1.5-year-old hypophysectomized rats had 4.5 and 3.0 fold increases in their rates, respectively, of phagocytosis and graft rejection compared to either hypophysectomized rats kept for only two months post-operatively or intact rats (Bilder, unpublished observations). Studies at various ages indicated that full restoration of function (90-100%) in these two systems had occurred compared to the one-month-old rat, the age at which intact control rats are fastest. Whether the return of juvenile competence in these two parts of the immune system was due to the absence of DECO or the absence of another pituitary hormone remains to be seen. However, it is a rather striking coincidence that in all three cases (return of responsiveness to thyroxine, restoration of competence of two parts of immune system), adult hypophysectomized rats two months after the operation were not different compared to intact rats of the same age, while a longer wait resulted in full recovery of juvenile competence.

CONCLUSION

The above work has two major points: (1) The endocrine system apparently secretes substances which are responsible for the severe age associated decline in three systems studied. (2) The several fold decline in competence in these systems is apparently completely reversible by a specific endocrine ablation.

Acknowledgment: The immune studies are the work of G. E. Bilder.

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