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Cellular Activities During Aging in Mammals

Caleb Ellicott Finch

CELLULAR ACTIVITIES DURING AGEING IN MAMMALS

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Caleb Ellicott Finch, B.S.

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Alfred E. Mirsky
Professor

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Abstract

Although much is known about manifestations of ageing in mammals, the fundamental cause of ageing has remained obscure. Review of a widely scattered literature has demonstrated a histotypic selectivity in the effect of ageing on cell activities. Several recent examples have shown that certain age changes in cell activities can be reversed by transplantation to a young host. Thus, ageing does not appear to diminish the potential for genomic function. The selective and reversible age changes in cell activities are interpreted to be the result of a differential change in gene activity, which appears to be mediated at the supra-cellular level by humoral factors.

Evidence for age changes in regulation at the supra-cellular, physiological level was revealed in an experimental study of the effect of ageing on the response of C57Bl/6J male mice to cold stress. Marked changes in the regulation of body processes were revealed by a study of three parameters. (1) The induction of liver tyrosine aminotransferase (TAT), an enzyme shown to be rapidly induced in a gene-mediated reaction during cold stress in young mice (4-16 months old) was delayed up to 2 hours in senescent mice (26-28 months old); the rate of increase after the delay was similar to that in young mice. Direct challenge of liver cells with injections of insulin and corticosterone, hormones which induce TAT in the perfused, isolated liver, resulted in an identical time course of TAT induction. Hence, the delayed TAT induction during cold stress in senescent mice is probably not the result of a primary age change in the liver; an age change in the regulation of extra-hepatic stimulae which mediate the induction of TAT is implied. (2) In contrast to the regulation of liver cell activities, no age changes were found in the time course of adrenal corticosterone secretion during cold stress. (3) Finally, senescent mice were shown to have a striking inability to maintain body temperature during short exposure to cold. This defect in thermal regulation is the likely cause of the decreased ability of ageing mice to survive cold stress. In conclusion, no evidence for a loss of the potential for cell function was found in senescent mice. However, selective age changes in regulation at a supra-cellular, physiological level were revealed during cold stress.

".....if old age weakens most of our facilities, it is far from paralyzing them all; and rigorous observation shows us that in certain ways the organs of the aged acquit themselves of their tasks with quite as much energy as those of the adult."

Jean M. Charcot, 1881

CONTENTS

I. INTRODUCTION

The cause of old age and the inevitability of death are great enigmas of human life. This essay will present an analysis of the nature of the changes in the cells of man and other mammals during old age, and the relation of these changes, collectively known as senescence, to the increased rate of mortality of old age. It has been widely presumed, with surprisingly little debate, that senescence is in some way an intrinsic property of living protoplasm, and that death is, in consequence, inevitable. In this author's opinion, there is little evidence to support currently given reasons for such a limitation, namely that death of the multicellular organism is due to generalized, irreversible changes in the cell nucleus which progressively limit cell activities, or that the exercise of cell functions results in a general exhaustion of the potential for further cell activity. The cause of senescence, while still obscure, may be derivable from the essential conclusion of this essay: the potential for cell function is not diminished after maturity in the mammal. However, the patterns of cell activities and interactions are altered. Senescence may then result from changes in regulation at a supra-cellular physiological level which are, in principle, reversible.

II. COMPARATIVE BIOLOGY OF SENESCENCE

It is useful to view the senescence and mortality of old age in man and other mammals in the perspective of senescent death as it is distributed in the plant and animal kingdoms. The following evidence will make plausible the notion that senescent death has not been an obligatory feature in the evolution of multicellular organisms.

A. Survey

1). Protista

In order to acquire a consistent perspective on the senescent death of multicellular organisms it is essential to recognize the capacity of some unicellular protista to proliferate indefinitely without the necessity for intervening sexual processes. This proposition has been controversial in the past. The classical studies of Bütschli (1876) and Maupas (1888, 1889) showed that various strains of ciliates undergo a gradual degeneration

after a certain number of divisions which results in a senescent death. However, if conjugation occurs, the daughter products appeared rejuvenated. Thus, a general analogy can be construed to the rhythm of growth and fecundity, senescence and death in the life cycle of higher organisms. (See Wilson, 1924, for a first rate discussion of the history of this notion.) However, the number of cell generations before the clone becomes senescent was observed to vary widely according to the species.

It was subsequently found that under certain culture conditions, some protistan strains conjugate very rarely, if at all. Woodruff's celebrated culture of Paramecium aurelia, descendants of a single cell, proliferated freely from 1907-1943 (21,800 calculated generations) without conjugation apparently having ever occurred (Woodruff, 1943). However, periodic depressions in the growth rate of Woodruff's cultures led to the discovery of a process called endomixus which was thought to involve degeneration of the macronucleus and the formation of a new macronucleus from the division products of the old micronucleus (Woodruff and Erdmann, 1914). Endomixus was thought to be analogous to parthenogenesis. Later studies by Diller (1936) and Sonneborn (1947) cast doubt on the existence of endomixus as described by Woodruff and Erdmann (1914), by the finding that the early micronuclear division products appear always to fuse. This is a process of self-fertilization (a type of sexual process) and was given the name autogamy by Diller (1936). Thus, the long maintained cultures of Woodruff underwent a form of sexual process at regular intervals.

Protistan strains lacking a micronucleus have long been in existence. Such strains can not undergo any sexual process; they are sexually inert. The most familiar are the various amicronucleate Tetrahymena strains which have multiplied vigorously for probably 40 years or more (Ferguson, 1940; Corliss, 1953). The exact length of time that the currently amicronucleate strains of Tetrahymena have existed is difficult to establish because of the apparent ease with which normal Tetrahymena strains can shed their micronuclei. Hence, one can not be sure that a strain was amicronucleate from the time it was established. Amicronucleate protistan strains were also described by Woodruff (1921), although at that time it appeared that such strains had a very limited ability to proliferate.

In sum, the descendants of a complex cell with a nucleus and cytoplasmic organelles can proliferate indefinitely. Evolution of the eukaryotic cell was not achieved at the expense of obligatory cell senescence which requires some form of sexual cycle for periodic rejuvenation.

The existence of varied patterns of senescence in protista (e.g., see the recent review of Ciliate senescence by Siegel (1967) and descriptions of senescence in the Suctorian Tokophora by Rudzinska (1961; a, b; 1962)) hint that senescent processes may be the result of unique selective forces for each species during its evolution.

2) Immortality of plants and animals

Before considering the distribution of senescent death in the Plant and Animal Kingdoms, it is useful to consider the idea of an upper limit to longevity which is characteristic for some species. The concept of an upper limit to the life-span for individual organisms derives simply from tabulations of the longest lived individuals of a species which have been found. For many species, under optimum circumstances, the survival of a population of adult organisms may be nearly 100% up to a certain age after which, as time progresses, there is a steadily increasing rate of death. For many plant and animal species, this increasing death rate is concurrent with the onset of obvious degenerative and morbid changes. In contrast, the rate of death for many other species does not appear to show an obvious increase at any age, but may be more or less constant. It is my opinion that if such species do not also show degenerative changes characteristic for all members of the species after achieving sexual maturity, then one might consider such species to be potentially immortal. However, the onset of senile degeneration and increased mortality may possibly occur at a time in excess of the length of an ordinary human life, and hence may be difficult to establish for some very long lived species.

Unfortunately, there is such a dearth of data on the distribution of senescent death among multicellular organisms that one can feel sure that at the most only a few multicellular species are potentially immortal in nature.

In the Plant Kingdom the best known examples are conifers, e.g., the redwood (Sequoia sempervirans), the bristlecone pine (Pinus aristata), and the giant Mexical cyprus (Taxodium distichum); individuals of these species are thousands of years old and seem to be capable of indefinite growth. Also noteworthy are naturally occurring, asexually reproducing plant clones, e.g., the Buffalo grass (Spartina), clumps of which slowly enlarge by rhizomal extension to form the characteristic "fairy rings" of the great plains in the western United States; grass in the rings of large circumference represents a "clonal" descent of many hundreds and probably thousands of years (Chase, 1934). Other anciently established, asexually reproducing plant clones are cited by Sax (1962).

In the Animal Kingdom one can be sure that certain hydroid species, e.g., Cyania capillata and Clytia johnstoni (Brock and Strehler, 1963) are potentially immortal. An interesting conclusion which can be drawn at present from records of longevity is that among those evolutionarily kindred species with a well established upper limit to longevity, the maximum adult lifespan can vary astoundingly. For instance, Lepidopterans in general have a lifespan of 6-12 weeks, whereas certain Coleopterans observed in captivity have lived up to 10 years (Comfort, 1964). The reason for the attenuated lifespan of the Lepidoptera is that most species of this group of insects, and others as well, suffer reduction of the mouth parts and alimentary canal during metamorphosis. Numerous species of Diptera and Hymenoptera utilize only carbohydrate foodstuffs (Trager, 1953). Longevity is therefore determined by the nutritional reserves established during development when feeding was active. Death is generally concurrent with exhaustion of the nutritional reserves of the fat body. The cause of death of the long lived Coleoptera is not known. It is easy to find other great contrasts in maximum recorded longevity between species which have a fairly close evolutionary relationship (see tables in Comfort, 1964). Unfortunately, only in a few species is the cause of senescent death as clear as in the aphagous insects. The cause of senescent death remains obscure for most multi-cellular organisms.

B. Higher organisms whose lifespan limit can be experimentally manipulated

In certain plant and animal species, it is possible to intervene experimentally and escape the normal limit to lifespan. Such individuals may be infinitely longevous.

1) Plants

Molisch (1928) has noted that the century plant Agave americana under the best of conditions flowers, comes to fruit and then dies at an age of 8-10 years. However, if conditions are not favorable for flowering, vegetative growth may continue indefinitely. Death is also a consequence of flowering in many annual plants: if the new buds are removed as often as they reform, the individual plant may continue to grow for some time (see discussion in Varner, 1965). Senescent death of these plant species can not be due to an intrinsic property of the plant tissues, but is triggered by certain developmental events (see Woolhouse, 1967, for a valuable discussion of this point).

2) Animals (non-mammals)

a) Planaria

The acoelomate flatworm or planarian was an object of intensive study in the early part of this century because of its capacity for regeneration and rejuvenescence. Many species have a well defined senescent involution, during which feeding ceases, reproduction wanes, and death of the individual ensues by disintegration. However, if a pre-senescent planarian is starved, the usual course of the life cycle can be arrested: upon refeeding, the planarian eventually regains its original size and soon exceeds the chronological age at which it would have become senescent. By repeating this process many times, Child obtained individuals of Phagocata velata which were truly ancient, having achieved an age of 2 years, manyfold greater than the usual life limit of 3 weeks when food is continuously available (Child, 1914). The fragmentation products of some turbellarian species, such as Phagocata velata, are capable of encystment and can reorganize to form a tiny worm identical to the one hatched from an egg capsule (Child, 1914). This process can be repeated successfully a large number of times, if not indefinitely. Child indicates that this cycle of senescence and rejuvenescence in Phagocata velata occurs naturally in this species' habitat of temporary pools near Chicago, Illinois (Child, 1913).

b) Insects

The female dog tick, Rhipicephalus sanguineus Latr. usually dies 4-5 days after oviposition. However, Achan (1961) found that injections of sucrose into the body cavity could prolong life up to 3 days more. Not only did the ticks injected with sucrose live longer, but they were also capable of re-infesting their host, the dog. The longevity of the dog tick would seem to be determined by nutritional reserves as in the other species of insects previously described.

Williams has grafted adult decapitated bees to pupae and finds that the tissues of the body survive 10 months or longer, in contrast to the usual adult lifespan of 10 days (Williams, 1958).

c) The European eel

The European eel (Anguilla anguilla) matures in the freshwater streams and lakes of western Europe and then, on dark nights, begins a remarkable journey back to the Sargasso Sea to spawn and start a new cycle of life. No food is eaten during this long journey. The digestive tract atrophies and general demineralization sets in. Upon spawning, eels die (see Bertin's monograph (1956) on eels for an excellent description of the life of the eel).

An impressive number of cases document the ability of female eels to achieve relatively enormous lifespans if they are entrapped and prevented from returning to the sea (Roulé, 1933; Gandolfi-Hornyold, 1935· Lekholm, 1939). One such eel, familiarly known as "Putte", resided for many years at the museum in Helsingborg, Sweden (Lekholm, 1939). "Putte" finally died at the ancient age of 88 years (Vladykov, 1956). In nature, the life-span of the European eel is less than 15 years (Bertin, 1956). Bertin (1956) points out that degenerative changes closely similar to those found in the intestinal epithelia of the pre-spawning silver eel, which is nearing the time of its return to the Sargasso Sea (Berndt, 1938), can also be induced in the immature yellow eel by simple starvation (D'Ancona, 1921). Thus, at least some of the senescent changes of the European eel depend on its nutritional circumstances.

d) The Pacific salmon

Certain other fishes besides the eel, notably the lamprey, the Pacific salmon, the candlefish (or eulachon) and a small group of annual fishes of the killifish family (Myers, 1952), die shortly after spawning (Robertson, 1957). Robertson and his associates have made a remarkably successful investigation into the cause of the death of the Pacific salmon (genus Oncorhynchus). The adrenal cortex was found to become hyperactive during sexual maturation (Hane and Robertson, 1959; Robertson and Wexler, 1960; Robertson et al, 1961). Serum levels of 17-hydroxy-ketosteroids, identified as cortisone and hydrocortisone, may increase up to ninefold during the spawning of king salmon (Oncorhynchus tshawytscha) (Hane and Robertson, 1959). Widespread degenerative changes characteristic of adrenal corticoid toxicosis and similar in most respects to Cushing's syndrome occur at this time (Robertson and Wexler, 1960). Death normally occurs within two weeks of spawning. In migratory rainbow trout (Salmo gairdnerii), which usually survive the first spawning, the levels of 17-hydroxy-ketosteroids are elevated to a much lesser extent and the degree of degenerative change in the tissues is correspondingly slighter (Robertson et al, 1961). Most of the same histological changes which preceed death in spawning Pacific salmon can also be induced in immature rainbow trout by implanting hydrocortisone pellets (Robertson et al, 1963). Thus, the senescent changes of Pacific salmon are the result of a change in physiological state at the time of maturation and spawning.

Robertson (1961) has found that castration of immature kokanee salmon (Oncorhynchus nerka kennerlyi), a landlocked variant of the blueback salmon which normally dies upon spawning at the age of four years, may prevent adrenal hyperplasia. Those castrated fish of either sex in which adrenal hyperplasia does not occur continue to grow and generally retain their juvenile appearance. (Robertson (1957) has also observed that precociously mature male king salmon, bearing all other juvenile characteristics, do not die after spawning. These occasional specimens escape the usual degenerative sequelae to spawning and continue to grow for at least five months). Some castrated kokanees lived for eight years, about twice the normal lifespan. In about one-half of the castrated fish which died after the age of four years, partial regeneration of gonadal tissue and adrenal hyperplasia had occurred; these fish had acquired the usual senile appearance (Robertson, 1961). Histological examination of other senile appearing castrated kokanees,

in which there was no detectable regeneration of the gonads, also revealed a marked adrenal hyperplasia and the usual degenerative changes (Robertson and Wexler, 1962). Although no castrated kokanee salmon has survived to as great a multiple of its normal lifespan as the landlocked European eel, such might be possible if one could prevent adrenal hyperplasia from occurring. In any case, senescent death in intact or castrated Pacific salmon can be ascribed to a non-mysterious and definable change in physiology.

e) Conclusion

Despite the extension of life for various annual plants, planaria, insects, salmon and eels, one must allow the possibility that the "immortal" individuals of these species may eventually die from another type of senescent death. For instance, if it were possible to infuse the aphagous adult Lepidopterans with adequate nutrients, would such individuals eventually succumb to another type of senescent death which might be postulated for kindred but longer lived genera such as the Coleoptera?

These varied examples of species in which it is possible to escape the natural limit to the lifespan clearly show that the limit to their lifespans is not determined by some intrinsic and irreversible feature of their cells. Senescent death for these species would seem to be the result of a developmental process leading to a physiological state (adrenal corticoid toxicosis or inanition) which resulted in the death of the organism. For these species, natural death can not be ascribed to exhaustion of the potential for cell activity or to irreversible damage to the cell nucleus.

In considering next the senescent death in man and other common mammals and the upper limit for longevity characteristic of each mammalian species it will be useful to keep in mind the escape from the longevity limitation which is possible for certain plant and animal species. We can at once discern that senescent death may have many origins and should not be regarded as a uniform phenomenon among multicellular organisms.

3) Mammals

The length of life can be considered in several ways. Most rudimentary is the average age at death or, conversely, the average life expectancy at birth. A more cogent measure, considered by Lankester (1870), is the normal

potential lifespan, defined as the life expectancy of individuals who have survived to some arbitrary age in early maturity. Finally, Lankester defined an absolute potential longevity, an approximate upper limit to the lifespan which is characteristic of the species in many different environments (Lankester, 1870). Although some individuals in various populations seem to approach the same maximum age limit, the average lifespan and per cent distribution of a population in different age groups may vary as can be readily seen by comparing the population structures of the United States and Ceylon: about 20% of the United States population is older than 65, whereas only 2% is older than 65 in Ceylon. Thus, there are some individuals in each nation who attain an age greater than 100 years, although the average age at death is 68 in the U.S. and 57 in Ceylon (Rosset, 1964).

In all mammalian populations, the basis for diminishing membership in the older age groups arises from the increasing mortality rate with age. It was first observed by Gompertz that the logarithm of the rate of mortality is roughly proportional to the age in adult humans (Gompertz, 1825). Analysis of national populations reveals them to be aggregates of subpopulations, each of which may have its own mortality characteristics (e.g., the average longevity of the Negro in the U.S. is 10 years less than the non-Negro (Rosset, 1964). Jones has developed a valuable method of analyzing subpopulation mortality characteristics which is based on the mortality characteristics of groups born at the same time, called cohorts (Jones, 1956). The interesting conclusion which emerges from analysis of mortality rate by cohort in comparatively homogenous countries is that the logarithmic mortality rate, while still proportional to the age, is decreasingly less for cohorts of the same age which were born more recently. This decline in death rate is slight per calendar year, but can be clearly seen in comparisons of death rates from different centuries, data for which only exist in a limited number of countries. Thus the death rate per year per 1000 individuals at the age of 50 in Sweden was 25 for those cohorts born 1816-1840, 9 for those born in 1875, 7 for those born in 1895, and is projected to be 5 for those born in 1905 and 4.0 for those born in 1915. The rate of progress in, or acceleration of, the death rate is apparently unchanged and doubles in approximately 8.5 years for all human populations (Jones, 1956). Thus,

prolongation of adult life has been achieved by postponing the age at which a particular mortality rate is reached, not by altering the rate of acceleration of the death rate. In other words, an individual born more recently in Sweden stays youthful (e.g., as judged by the incidence of cancer at that age in his cohorts) for a longer time than his ancestors born a century before. The absolute potential lifespan of humans, based on the decreasing mortality rate of the last century, is obviously increasing. However, the death rate of all human cohorts studied so far increases logarithmically and does set a statistically definable upper limit to the lifespan of that cohort.

Jones has also analyzed the incidence of disease by cohort. It is clear that cohorts with smaller death rates at a particular age also have a delayed onset of various chronic diseases (Jones, 1956, 1959). The coexistence of cohorts with markedly different disease patterns in the same environment implies that the lifelong pattern of health may be primarily determined by environmental experiences early in life, although later in life environmental experiences (e.g., urban vs. rural environment) may alter the time of onset or condition the expression of the disease. These same phenomena have been observed in various rat populations in the studies of Osborn and Mendel (1910, 1915, 1917), McCay et al (1935, 1943), Ball et al (1947), and Berg and Simms (1960, 1961) which demonstrate that the average longevity of the laboratory rat can be significantly increased and the age of onset of degenerative disease postponed with certain diets. Calculations made on the basis of the published data of Berg and Simms (1961) show that the increase in mortality rate in dietarily restricted, as well as in control groups, is nonetheless logarithmic and proportional to the age. Thus, environmental factors may modify the time of onset of chronic disease, the incidence of which parallels the increased rate of mortality.

An intriguing fact is that puberty occurs progressively earlier in the same human populations in which the onset of disease occurs progressively later. Tanner's demonstration of the increasingly earlier age of the adolescent growth spurt and of puberty was based on records which included a sample from Swedish populations over the last 100 years (Tanner, 1962). Jones' studies showing the progressively later onset of chronic disease were also based on Swedish records which probably sampled from the same

populations as the ones analyzed in Tanner's study during that same historical period. Thus the total span of healthy adult life is increasing for two reasons: the increase is due to both an earlier attainment of maturity and the postponement of the period of chronic disease.

Although the mortality rate increases exponentially with age in all known populations of mammals in the laboratory or in nature, there is no reason to believe that the limit of the span of healthy adult life is being approached. In fact, Tanner (1962) has shown that the earlier age of menarche is a linear function of time between 1800 and the present. Similarly, the chronological age at which the death rate for that age group reaches 1% or 2% per year (an arbitrary definition of the end of youthfulness) may be calculated from the data of Jones (1956) to be a linear function of the time between 1830 and the present, as shown in figure 1. On the basis of these figures, the total period of youthfulness is thus increasing at the rate of 0.5 years/decade of historical time because of the earlier age to puberty and at the rate of 2.5 years/decade because of the later age of senescence.

Tanner suspects that puberty occurs earlier because of decreased exposure to disease in childhood. Jones' analysis has actually shown that the mortality of cohorts as children can be used to predict the mortality of these same cohorts as adults (Jones, 1956). If both conclusions prove true, there may be a common site of action of the environment on the organs or organ systems governing the length of mature life. Needless to say, it would be of the utmost interest to identify the regions of the body concerned. Therein might be found a way of achieving control over the length of life.

III. AGEING CHANGES IN MAMMALS

A. Introduction

The major question before us concerns the origin of the exponentially increasing mortality in ageing mammals. Although the same mathematical laws apply equally well to the mortality of annual plants, flies, rats, and men, the diversity of senescent death (discussed in Section II) provides a powerful argument against a universal cause of ageing and senescent. However, a common course of events during ageing can be

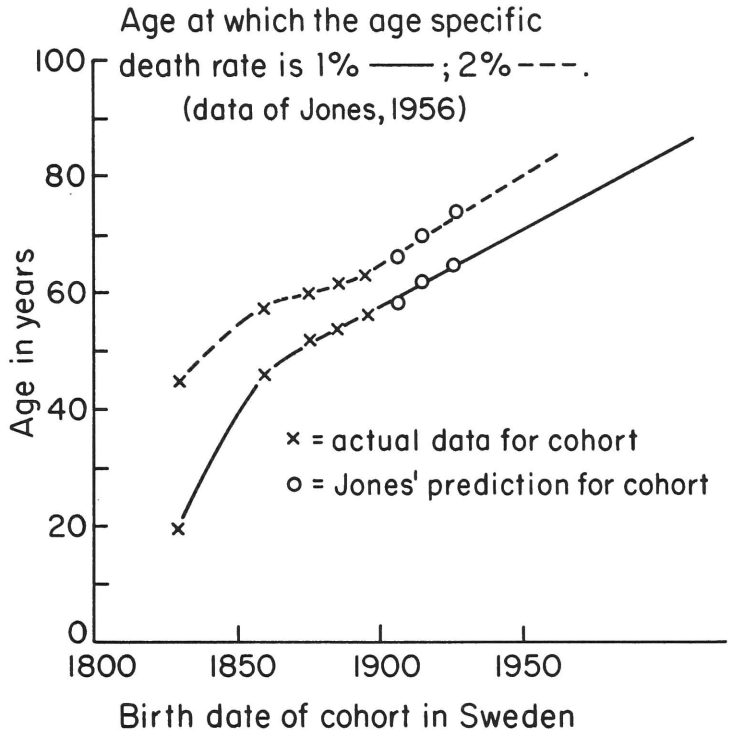


Figure 1: Increase of the healthy lifespan in recent history. See plate for details.

described for man and other homeothermic mammals which are closely similar in anatomy, physiology, and pattern of early development.

Comparison of the course of ageing changes among mammalian species, which differ in large multiples of lifespan, as do mice and men, can not be expressed on a scale of absolute, or sidereal time, but may be expressed as some fraction of the lifespan characteristic of the species. Assignment of an appropriate length of time for the lifespan unit of individual mammalian species is difficult because of the large variations in average lifespan of different populations. Changes of ageing, notably incidence of certain diseases, do not occur at a fixed and immutable time, but appear to vary markedly in time of onset from population to population in humans (Jones, 1956). Moreover, studies of human twins have indicated the importance of hereditary influences on longevity (Jarvik et al, 1960) and disease present at death (Kallman et al, 1956; Jarvik, 1962). Thus, analysis of changes during the lifespan might best be made in terms of successive, lifelong measurements on the individual subject. These measurements might subsequently characterize age changes according to the fraction of the lifespan of the individual in which they occurred.

Analysis of ageing changes by the longitudinal method can yield entirely different conclusions from the usual cross-sectional analysis of a population of different age groups. This is demonstrated in Everett's studies of body weight changes during ageing in rats (Everett and Webb, 1957; Everett, 1957). Weight averages of adult rats at different ages in a population do not significantly differ. However, when body weight is analysed in terms of days of remaining life (obtained from lifelong, consecutive weight measurements), a very clear loss of weight in the 3-4 months before death is revealed.

Another aspect of the difference between longitudinal and cross-sectional studies is given by Storer's observations on the variance in survival time of highly inbred mice exposed to 100r of x-rays per day as a function of age: if the mice are chosen from a single cohort tested at various ages, the age-wise increase in variance is markedly less than if the mice were randomly chosen from a colony of mixed ages (Storer, 1965). From this one might infer that cohorts coexisting in the same population may differ because of genetic fluctuations from generation to generation due to sampling error (a type of founder's principle) or that coexisting

cohorts, if genetically equivalent, may vary from generation to generation because of fluctuations in biological environment (disease, etc.) during the critical early period of life.

B. Phases in the mammalian lifespan

It will become apparent from species to species, and among subspecies and strains as well, that the events of the lifespan, such as puberty or changes in the connective tissue of the bone, may vary in the exact percentage of the lifespan in which they occur. Moreover, the events of the lifespan may also vary from species to species in their relative order of occurrence. However, most of the events and processes characteristic of the life pattern of a particular mammal appear to occur during the lifespan of other mammals as well.

Events during the course of ageing will be located in four phases of the total lifespan, which are marked by the onset of puberty, early maturity, late maturity, and senescence. This convention will be used henceforth. The length of the total unit in physical time will be set arbitrarily according to the normal potential longevity in longer-lived populations of the species. Laboratory or clinical studies, with few exceptions, do not mention the average lifespan in the population from which the experimental subjects were drawn. One can then only guess the physiological ages of the subjects from their chronological ages. Optimally, one would wish to know the age at maturity, the average lifespan, and the longest lifespan in the population from which individuals were drawn for study. For the purposes of this analysis, the normal potential longevity will be arbitrarily taken as 26 months in mice, 32 months in rats, and 70 years in humans.

Puberty, a major developmental landmark, occurs between 10 and 25% of the normal potential lifespan of an extremely wide range of mammalian species (Asdell, 1945). This event marks the end of the first phase of the mammalian lifespan (I). It is very difficult to assign appropriate landmarks for the rest of the lifespan. The following intervals may be tentatively considered: early maturity (II), during which growth continues until maximum skeletal muscle mass is reached (this is the period of the bearing and rearing of the young); late maturity (III), during which

reproductive capacities of the female diminish or are lost; senescence (IV), during which is the onset of chronic disease and death of 25% or more of the members of the original population who survived to reach early maturity.

1) Fertilization to puberty (phase I)

Asdell (1945) has considered the spacing of developmental processes in this phase in a broad comparative perspective. Gestation, for instance, may vary within the wide limits of 0.75% of the lifespan of the Australian opossum, Trichosurus vulpecula (which is born precociously and continues early development in the mother's pouch), to 9.0% of the lifespan of the fisher, Martes pennanti (in which there is delayed implantation of the embryo). Another example of variability among species in the timing of developmental events is given by the degree of development of the nervous system at birth: in guinea pigs, born with nearly full size brains, the amount of post-natal neurogenesis is much less than in mice and rats, which are characterized by their greater relative immaturity (Altman and Das, 1967). In contrast to these examples indicating much interspecific variability in the developmental program, the calculations of Moulton indicate that the time at which limiting adult values of total protein (nitrogen) and ash content are approached (denoted as "chemical maturity") is a relatively invariant percentage of the total lifespan (3.9%-4.6%) (Moulton, 1923).

2) Post-maturational changes (Phase II, III, IV)

Rather than attempting description of each remaining phase of life according to its characteristic changes, which may vary considerably in time of incidence with different populations, strains, and species, certain illustrative examples of ageing changes at the anatomical, physiological and behavioral levels of organization will be described, and their location in the lifespan will be indicated as precisely as available information permits.

a) Anatomical changes

(1) Bone structure

Age changes in bone structure have been described for mice and men. The most persistent feature is the general tendency for bone rarifaction, which affects all individuals of older ages. Such changes occur in tibias of the C57Bl, DBA, and A strains of mice during senescence

(Silberberg and Silberberg, 1962). Garn has studied bone density of the second metacarpal in human populations in Ohio, El Salvador and Guatemala. Despite presumed major differences in the timing of sexual maturation, in the composition of diet, and in the disease profiles of these populations, the time of onset and magnitude of bone loss appear to be very similar (Garn et al, 1967). Significantly, females of both species show more extensive rarefaction of bone and degenerative joint disease than do males.

(2) Connective tissue

Tonna has studied age changes in the connective tissue of mice (Tonna, 1964) and men (Tonna and Hatzel, 1967). The birrefringence of toluidine blue stained sections of the femur diminishes after 60 years of age in man (senescence) and after 1 year in mice (late maturity). These changes in optical properties imply changes in the organization of the collagen - mucopolysaccharide framework of the bone connective tissue. The onset of these changes in different phases of the lifespan of men and mice provides a good example of species differences in the timing of ageing changes.

b) Physiological changes

(1) Reproduction

One of the most characteristic features of ageing in female mammals is the waning and cessation of the ability to bear young. This age change is well known to occur (e.g., in mice (Ingram et al, 1958; Thung et al, 1956), hamsters (Soderwall et al, 1960), and humans) during the period of late maturity.

(2) Post-prandial lipemia

During late maturity in dogs (Sobel et al, 1963; Sobel and Thomas, 1963) and man (Hertzstein et al, 1953; Brown et al, 1961), blood lipid increases which follow ingestion of a fatty meal return to the control levels more slowly.

(3) Response to cold

Senescent mice, rats, and men have a reduced response to cold. The survival of mice exposed to temperatures of 6-11°C declines during late maturity, as do other aspects of the physiological response to cold, such as oxygen consumption, ability to maintain body weight, increase in blood glucose, etc. (Grad and Kral, 1957). Similar observations on rats have

been made by Kibler et al (1962). Although most of these same parameters have not been measured in cold exposed humans, it is clear from the studies of Krag and Kountz (1950) and Hovarth et al (1955) that the response to cold is similarly diminished in late maturity and senescence.

(c) Behavioral changes

(1) Psychomotor reactions to stimulae

The delay in reaction time to auditory stimulae has been observed to increase progressively during late maturity in rats (Birren, 1955) and humans (reviewed by Welford, 1959).

(2) Voluntary physical activity

Measurements on rats indicate progressive decrease in the amount of voluntary activity (measured by exercise wheels) during late maturity and senescence (Slonaker, 1912; Richter, 1922), nest building activity (Richter, 1922) and exploratory behavior (Goodrick, 1967a). The human analogue of such changes is given in the results of a questionnaire-based study of the use of leisure time: throughout life after maturity the preference for pasttimes requiring vigorous activity and rapid adjustment (e.g., driving an automobile, playing tennis, pursuing bandits in a sheriff's posse, or engaging in arguments) progressively declines, whereas the preference for less active pasttimes (e.g., visiting art galleries or museums, or bird watching) gains ascendancy (Strong, 1931).

(3) Sexual behavior of the male

Larsson's study (Larsson, 1958) of the same rats tested in early maturity and late maturity showed a definite decline in the frequency of ejaculation and an increase in the refractory period before ejaculation is possible again. This result closely parallels the progressive decline in frequency of orgasm for men, from early maturity through late maturity (Kinsey et al, 1948).

d) Incidence of disease and ageing

A major event of ageing in mammals is the increased incidence of disease. It was first discovered by Nathan (1924) and later amplified by Simms (1946) that designated causes of human mortality from most specific diseases can be described by the same mathematical relationship as the

age-wise increase in mortality from all causes. Both investigators recognized that certain malignancies (e.g., of the digestive tract or female reproductive organs) decline in frequency as the designated cause of death after certain ages.

Jones implicitly assumes that the incidence of the pathology, designated post mortem as the cause of death, increases according to some function of the mortality rate. Simm's further analysis showed in fact that the logarithmic increase of mortality in ageing rate is preceeded by a closely parallel logarithmic increase in the incidence of a variety of new pathological lesions (Simms and Berg, 1957).

The statistical incidence of particular diseases and the severity of the course of these diseases appears to vary from population to population depending on such factors as historical period, geography, and socio-economic status (Jones, 1956). Moreover, genetic factors within the same species are of great influence for the incidence of pathology as is revealed by comparison of diseases present at death in various mouse strains (Storer, 1966) or by diseases designated as the cause of death of human twins (Kallman et al, 1956; Jarvik, 1962). Finally, diseases of some species, such as arteriosclerosis in humans, occur in few other mammalian species (Leader, 1964).

Although the particular diseases found in different ageing populations may vary greatly and indeed will not affect all individuals, the existence of the phase of increased disease incidence which preceeds the onset of the major increase in mortality in rats (Simms and Berg, 1957) and humans (Jones, 1956) is characteristic of the senescent state. In sum, the most important manifestation of mammalian senescence may be the exponential increase of disease.

e) Ageing and variability

The diversity of diseases in ageing populations of the same or different species is perplexing in view of the approximate uniformity of the events of ageing at the anatomical, physiological and behavioral levels. From a pathologist's point of view, diseases of ageing have a pronounced random character (with statistical bias according to genetic tendencies of individuals for particular diseases): the same pathological events will not occur in all individuals. However, from an anatomist's, a physiologist's,

or a behaviorist's point of view, other changes of age occur with such regularity that they may be considered collectively as a post-maturational developmental pattern.

The variability of non-pathological changes in age is a function of each particular parameter. For instance, the variance of glomerular filtration rate and renal plasma flow is smaller in senescent humans than in young adults (Davies and Shock, 1950). Contrarily, the variances in body weight of mice (Storer, 1965), cardiac age pigment accumulation of humans (Strehler et al, 1959), maximum breathing capacity of humans (Norris et al, 1956), length of the phases of the cell division cycle of the intestinal epithelium of mice (Fry et al, 1966), and hematocrit values of mice (Storer, 1965) show definite increases with age.

The stringent internal boundary conditions for maintaining life in the homeothermic mammal exclude much change in certain parameters, e.g., blood pH and carbon dioxide tension (Shock and Yiengst, 1950). The individual variability of these parameters must fall within highly restricted limits: those individuals whose values departed from a certain range would soon expire. Similarly, Norris and Shock (1966) observe that tolerated limits of variability of other parameters which do change with age (e.g., maximum breathing capacity) appear to exclude values outside a certain range, inasmuch as individuals with such values are not found in older populations.

The origins of variability during ageing derive from individual differences of experience and genetic proclivity. Variability of age changes is obviously at its greatest in the pattern of specific diseases which statistically increase in mammalian populations after a certain age. From one point of view, the limited extent to which individual variability obscures the profile of ageing changes is most remarkable, for it is easy to guess a man's age within a few years. This fact underlines an essential feature of mammalian ageing: the regular and developmental nature of the ageing process.

The pattern of ageing changes is most closely similar in genetically identical individuals. As in the case of other developmental patterns, the patterns of ageing changes are closely congruent if one compares different populations of the same strain or species of the same genus or

family. However, as one proceeds to compare species of different classes, orders, and phylla, the patterns of post-maturational change probably will be found to be as divergent as are the species differences in the patterns of development, the anatomy, and the physiology.

f) Inquiry into currently held assumptions about ageing

The great questions now before us concern the nature of the post-maturational age changes and the relationship of these changes to the increasing incidence of mortality. It may be that many age changes, such as the decreased elasticity of the skin, do not contribute significantly, if at all, to the increased force of mortality.

An aspect of the environmental contribution to ageing can be evaluated from studies of germ free mice. Such mice have the same spectrum of pathologies at death as well-kept conventional mice (renalopathies, neoplasms, etc.) apart from the obvious peculiarities of germ free life (enlarged caecum, etc.) (Gordon et al, 1966; Walburg and Cosgrove, 1967). Germ free mice die at approximately the same maximum potential lifespan as other mice of the same strain (Gordon et al, 1966; Walburg and Cosgrove, 1967). Symbiotic bacteria and other small organisms, exclusive of viruses, can not therefore be the generative cause of the usual course of mammalian ageing.

Disease may be the ultimate cause of non-accidental death in senescent mammals (Aschoff, 1938; Henschen, 1968). The renowned pathologist Ludwig Aschoff has stated (Aschoff, 1938):

> "...we in Freiburg have seen no true death from age alone. It is my conviction that natural death in human beings never occurs, or only in rare instances. Autopsies which have been made on the very old always show a pathological cause. In life the severe disease changes of those advanced in years are usually not felt. When I visited a 97 year old man two days before his death, he showed so little the symptoms of a serious illness that I was convinced on hearing of his demise that at last I had seen a case of natural death. I was very surprised when I found at the autopsy a severe lobar pneumonia of at least four to five days' duration and numerous metastases from a malignant tumor of the thyroid gland."

This claim is also bolstered by the fact that the incidence of chronic diseases parallels in advance the logarithmic increase in mortality in rats (Simms and Berg, 1957). The alternative position, that death is due to some intrinsic cause apart from the debilitating effects of disease must

be considered further.

Current theories of senescence have proposed that cell death is a major cause of the death of the whole organism. Cell death is known to be a normal and programmed event in animal morphogenesis (Glücksmann, 1951; Saunders et al, 1962). Senescent death is commonly considered to be the result of cell death, which might be, in turn, the result of a "suicidal" genetic program in all (or certain) cell types, or which might result from damage to the cell nucleus. This latter possibility is conjectured to result from such causes as accumulation of mutational damage in the genome (Szilard, 1959) or of cross-linking of the chromatin which might restrict genomic expression (von Hahn, 1966). Any of these alternative changes could lead to an upper limit in time for normal cell function. Such a limit has indeed been observed in the limited growth potential of euploid mammalian cells in culture (Hayflick and Moorehead, 1961). If this process of ageing of cultured cells in vitro is a correct analogy of ageing of cells in the entire organism, it may be supposed that the species lifespan is determined during embryological development by variations in the number of stem cells whose clonal descendants, as long as they proliferated, would sustain the vitality of the organism. Another in this family of similar propositions is that cells in non-dividing or slowly proliferating tissues might have a limit to the total amount of activities that can be sustained. The exercise of cell function during the lifespan might then exhaust the potential for further activity and eventually result in death of the cells. If these functionally exhausted cells can not be replaced because they are non-dividing (e.g., nerve and myocardial cells) or because the population of stem cells which normally replace functionally exhausted cells was approaching depletion, then the tissue might cease to function satisfactorily and death of the organism might ensue. This possibility was first stated by Weismann (1891). According to this premise, death could result from a pure, senescent exhaustion of the potential for cell function. Disease leading to death might also strike because of the dwindling potential for cell function. In either case, the limit for longevity would have been predetermined by an ageing process intrinsic to the cell.

There is another common premise about the nature of ageing which should be considered at this time. Age changes in the nature of the extra-

cellular ground substance have long been familiar to histologists. Certain molecules in the ground substance, such as collagen, are ordinarily remote from genomic control because of extremely slow turn-over rates (Neuberger et al, 1951; Thompson and Ballou, 1956) and hence can accrue changes which are not corrected through the normal process of molecular turn-over. The cross-linking of the polypetide chains of collagen has been inferred to increase with age (Bjorksten, 1968; Verzár and Meyer, 1961). Furthermore, it is known that components of pre-existing collagen fibrils can be re-assembled without degradation of these components to amino acids in vivo (Klein and Weiss, 1966). The limitation on mammalian lifespan has been, therefore, inferred to result from changes in the molecules of ground substance which in consequence hinder diffusion of metabolites and hormones essential to cell maintenance and essential for the integration of organ function in the body (Kohn, 1966).

The crux of these preceeding arguments lies in the demonstration of an exhaustion, disintegration, or termination of cell function in the organism during or preceeding senescence. The effect of ageing in the cells of the organism will now be considered in detail. It should become clear that there are no generalized cellular ageing changes throughout the body. Ageing will be shown to have a highly selective effect on cell activities: not all cells appear to be affected and not all activities of affected cells appear to be changed. No evidence will be found for a generalized or regional cell death during the period preceeding the death of the organism. If this conclusion is accepted, then the limitation on lifespan can be inferred to result from the increased proclivity to disease. Senescence might then result from changes in the setting of control mechanisms in some regulatory center, which could affect the function of other cells of the body in such a fashion as to decrease resistance to disease or to decrease resistance to the fuller expression of latent pathology.

C. Age changes of the activities of individual cells

1) Cell composition

a) DNA content of the cell nucleus

The first fact one might wish to know about the effect of ageing on the cell concerns the status of genomic information in the cell nucleus. It is frequently considered possible that ageing is the result of changes in the DNA of the cell nucleus.

The conservation of the genomic information of the fertilized egg throughout early development has been directly demonstrated by the celebrated experiments of Gurdon, in which nuclei were transplanted from the differentiated cells of tadpole intestinal epithelium to enucleated eggs; the transplanted nucleus behaves as a typical synkaryon and can direct the normal development of the egg into a sexually mature frog (Gurdon, 1962; Gurdon and Uehlinger, 1966). If it was possible to perform such an experiment with nuclei from the tissues of senescent mammals, many conjectures about the effect of ageing on the cell nucleus could be set aside. To date, it has not been possible to do nuclear transplant experiments on any mammalian species of any age.

Other lines of evidence have also led towards the conclusion that each differentiated nucleus has the same genomic information. The amount of DNA has long been known to be a constant quantity in the nuclei of differentiated tissues (Mirsky and Ris, 1949; Boivin et al, 1949). Recent studies have shown that the quantitative distribution of single copy and redundant sequences of nucleotides in DNA is the same in various differentiated tissues of several mammals (Britten and Kohne, 1968). These same measurements might be made on tissues of ageing mammals; so far, only information on the quantity of DNA per cell nucleus is available.

Direct microspectrophotometric studies on the DNA per diploid liver cell nucleus of one 90 year old man (Swartz, 1956) and in the liver cells of a larger sample of senescent rats (Enesco, 1967) demonstrate that the quantity of DNA per cell nucleus does not change during ageing. Other measurements of the DNA per liver cell nucleus, calculated from the concentration of nuclei and DNA content in a tissue (Jacob et al, 1954; Barrows et al, 1960) or estimated from bulk DNA content of isolated, purified nuclei (Falzone et al, 1959; Kurnick and Kernan, 1962) have also

also indicated the constancy of DNA of the cell nucleus during ageing. Enesco's figures show in particular that the standard error of measured DNA content per nucleus does not change with age. Thus, the DNA content is not only maintained on the average per liver cell nucleus during ageing, but appears to be maintained uniformly in all nuclei. Multiples of the diploid DNA content (2n, 4n, 8n ploidy classes), characteristic of the adult liver, also persist through senescence (Swartz, 1956; Falzone et al, 1959; Post et al, 1960; Enesco, 1967).

There are few precise measurements of DNA cell nucleus of other cell types during ageing. The recent microspectrophotometric study of Lapham (1968), showing the existence of tetraploid Purkinje cell nuclei in adult humans, indicates no departure from the expected 4n quantity of DNA in 8, 33, or 53 year old subjects. Any quantitative measurements of DNA in other cell types would indeed be welcome.

b) Cellular content of RNA

(1) Neurons and myocardial cells

The quantity of RNA per cell varies from tissue to tissue. In some tissues, the RNA content varies according to the physiological condition of the animal. For instance, liver RNA content and total mass drop precipitously in the first 48 hours of a fast, but the DNA content is unchanged (Davidson, 1947). The example of loss of RNA in the liver of a fasted animal may be a useful model for interpreting changes of cellular RNA content during ageing in the following survey.

There are relatively few direct microscale analyses of the RNA content or other cell constituents of single cells in old animals. Only such an analysis can guarantee that age changes in the chemical composition of a tissue compare changes in the composition of the same cell types at different ages. It is always possible that there has been a shift in the relative proportion of cell types in a tissue with age. This phenomenon has been noted in ageing mammals, e.g., increased connective tissue in liver (Hinton and Williams, 1968) and blood vessels (Movat et al, 1958) or invasion of liver by lymphocytes (probably a pathologic change) (Andrew et al, 1943), but the generality of such changes and their relation to pathological events is unclear. In any case, measurements made on extracts from whole tissues in which a change in the relative proportion

of cell types has occurred might give very misleading results.

Hydén's microspectrophotometric measurements on the RNA content of anterior horn neurons in the human spinal chord revealed a gradual increase from birth through midlife (3-30 years of age), followed by a period of no change during late maturity (40-55 years), and ultimately a progressively decreased RNA content during senescence (after 60 years) (Hydén, 1960). This same general pattern of change was observed in neurons of the rat hippocampus: individually dissected neurones of 2 month old rats (early maturity) have about twice as much RNA as nerve cells of senile rats, 36 months old (Ringborg, 1966). Microspectrophotometric techniques for measuring RNA (based on the loss of stainability of tissue sections for toluidine blue after ribonuclease treatment) indicate a slight loss of RNA during senescence in the cervical spinal cord lower motor neurones, the dorsal root ganglion motor neurons, and the Purkinje cells of the cerebellum of the rat and a major loss of RNA in cardiac ventricular muscle in the rat (Wulff and Freshman, 1961; Wulff et al, 1963). In contrast, the RNA content of neurones in the supraoptic nucleus of the rat hypothalamus does not change with age (Wulff et al, 1963). One can conclude from these data that age changes in RNA of non-dividing cells (neurones and myocardial cells) may vary according to the particular cell type; massive loss of cellular RNA, such as occurs in the hippocampal cells (Ringborg, 1966) is not an obligatory phenomenon in all cells of ageing mammals.

The trend for decreased RNA in neurons of senescent mammals has often been noticed in non-quantitative histological studies. For instance, Andrew's plates of whole brain sections or micrographs of different regions of nervous tissue demonstrate a marked decrease in tinctorial affinity for cresyl violet (an acidic dye which stains for RNA) in senescent mice (Andrew, 1959). Marked decrease in Nissl substance, known to represent rough surfaced endoplasmic reticulum (Palay and Palade, 1955) was noted in old humans (Hodge, 1894), in rats and mice (Andrew, 1936), and in many other creatures (see review of Andrew, 1956).

An interesting feature mentioned by Hodge (1894) and Andrew (1936, 1938) is the paleness of stained nucleoli in some ageing nerve cells which is suggestive of decreased nucleolar activity. The nucleolus is known to be the site of ribosomal RNA synthesis (Brown and

Gurdon, 1964) and is rich in ribosomal RNA (Muramatsu et al, 1966) distinguished by a high G+C/A+U ratio in mammals and other deuterostomes. Although the changes in base composition of ageing hippocampal neurones reported by Ringborg (1966) indicate a shift towards an increasing G+C/A+U ratio (opposite to that expected with less active nucleoli) changes in the genomic activity of the nerve cell nucleus are implicated in either case.

(2) Liver: RNA concentration and weight loss

Many estimations of the RNA in the liver have been made on the basis of bulk chemical analysis. These data can best be expressed as the DNA/RNA ratio. No change in DNA/RNA ratio, total RNA, or total DNA was found in livers of senile Wistar male and female rats, Sprague-Dawley male rats and C57Bl/6J female mice (Beauchene et al, 1967), of liver DNA/RNA of senescent wild rats (Rattus norvegicus) (Barrows et al, 1962a), and in senescent C57Bl/6J male mice (Lang, personal communication). In contrast, Detwiller and Draper have found a major increase in DNA/RNA ratio from 0.25 to 0.33 in livers of senescent rats from their colony; base composition changes of the RNA in various cell fractions were also found (Detwiller and Draper, 1962).

I do not believe that these contradictory results are attributable to faulty measurement. Detwiller's and Draper's observations were made in senile animals which were losing body weight: the livers of these creatures weighed less and contained less protein/cell nucleus than livers of younger rats (Detwiller and Draper, 1962). In certain respects, the condition of these livers appears to be similar to the condition of livers in fasted animals. The wild rats observed by Barrows et al (1962a), although apparently losing body weight, did not have smaller livers. Prof. Lang has also communicated to me that the senescent mice from his colony do not show loss of liver, or body weight, or DNA, RNA and DNA/RNA. It may be that changes in DNA/RNA occur only when the liver is losing weight.

Tauchi's micrometrical studies of senile human liver show that very old humans (80 years or older), whose livers weigh less than half that of a group younger than 50 years, display a marked tendency for an elevated nucleocytoplasmic ratio in both central and peripheral zones of the hepatic lobule (Tauchi and Sato, 1962). The DNA-RNA ratio would probably be increased in this old group.

A brief survey of the occurence of weight changes during senescence reveals that loss of body weight and loss of weight of some tissues, particularly skeletal muscle, may be widespread. However, the time of onset and extent of body weight loss (and presumably weight changes in individual tissues as well) may vary greatly with the conditions of rearing and the diet. This question is discussed at greater length in section III F 2 C 5. Atrophy of organs and tissues during ageing has long been described in terms of weight loss (see Korenchevsky's compendious monograph which contains tabulations of organ weight changes in humans; Korenchevsky, 1961). Skeletal muscle, liver, kidney, and adrenals show the greatest net weight loss in humans. Brain weight declines significantly but less than in these previously mentioned organs. There is no comprehensive tabulation of organ weight changes for mammals other than man, although it is probable that skeletal muscle atrophy is a general feature of mammalian ageing.

It is significant that the weight loss of many organs and tissues begins between the 5th and 7th decade in humans, a period of dramatically increased mortality. Heart weight, on the other hand, does not decline during this period; in fact the ratio of heart to body weight increases. Furthermore, although decrease in weight of bones (e.g., the ribs) generally occurs, some bones (e.g., the sternum) become progressively heavier with ageing in humans (Ingalls, 1931). Atrophy (as weight loss) is not, therefore, a generalized phenomenon throughout the body. Ageing may be said to have selective and specific effects on the different anatomical elements of the body.

(3) Muscle

The biochemistry of the weight loss in skeletal muscle in rats has been investigated by Detwiller and Draper (1962), who have found a net loss of DNA, RNA and nitrogen content. The DNA/RNA ratio increases from .43 to .62 and the G+C/A+U of RNA from the whole tissue changed from 1.46 in young rats to 1.7 in senescent rats (Detwiller and Draper, 1962), implying a greater preponderance of ribosomal RNA. It is difficult to imagine this, however, because about 90% of the RNA of most eukaryotic cells is ribosomal RNA.

(4) Heart

In accordance with the absence of weight loss in the heart, it has been found that the DNA, RNA and protein nitrogen concentration in rat hearts do not change with age (Lang, personal communication).

(5) Fat

The DNA content of adipose tissue in the rat does not change with age (Gellhorn and Benjamin, 1966; Placer and Slabochova, 1961), although the RNA content (Gellhorn and Benjamin, 1966) and the nitrogen content (Placer and Slabochova, 1961) decline.

(6) Kidney

The kidney weights of wild female rats (Rattus norvegicus) (Barrows et al, 1962a) and of McCollum strain laboratory rats (Barrows et al, 1960) do not change in senescence. Correspondingly, no changes in protein nitrogen or DNA content per gram tissue were observed in either strain of rats.

(7) Summary

In general, if there is loss of weight in any tissue, it appears that there are concomitant changes in the nucleo-cytoplasmic ratio, as evidenced by DNA/RNA ratios, DNA/protein N ratios, etc. in the liver. From the fragmentary evidence available, one is given the impression that the loss of cells, measured as decreased total tissue DNA, may be a smaller component in the decline of weight in the whole organ than is the loss of cytoplasm in the remaining cells of the organ. Such weight loss does not appear to occur uniformly throughout the ageing mammal, although a particular anatomical element may indeed show such changes.

2) Cell enzyme profiles

A fact about ageing mammals, which has no less importance than its self evidence, is that differentiated cell characteristics persist in mammals to the end of life. Thus, the stem cells of the bone marrow produce erthyrocytes containing hemoglobin, the liver produces albumin and other serum proteins, the pancreas produces its characteristic enzymes and hormones, etc. Changes in body function definitely do occur, but they are in general quantitative in nature. The obvious and perhaps sole exception to this proposition is the absolute loss of reproductive ability in mammalian

females. Such a change does not occur in male mammals; testicular spermatogenesis proceeds even in the most extremely aged human individuals.

Biochemical changes during ageing are given in tabulations comparing enzyme and hormone contents at different ages in the liver (table I), kidney (table II), heart (table III), brain (table IV), skeletal muscle (table V), and erythrocyte (table VI). Age changes in the enzyme profile of human arterial tissue have also been thoroughly studied (see review by Kirk, 1966). However, a tabulation of these changes will not be given here because of the well known increase in connective tissue in the arterial wall during ageing (Movat et al, 1958). Many of the enzyme changes in arterial tissue may originate from changes in cell population, rather than a shift in the enzyme constitution of a given cell type.

The basic conclusion one can draw from this compilation is that certain enzyme levels may be altered during ageing. The changes may be positive or negative, but tend to be small. For instance, Zorzoli observes that enzyme activity per unit liver protein does not change during ageing for glucose-6-phosphatase (Zorzoli, 1962), is increased by 30% for alkaline phosphatase (Zorzoli, 1955), and is decreased by 20% for acid phosphatase (Zorzoli, 1955) in the C57Bl/6J mouse. Many other examples of the differential effect of age on tissue enzyme profiles can be found in these tables. It is also clear that the same enzyme may be affected oppositely in other tissues. Thus, glucose-6-phosphatase which is increased during senescence by 30% in the liver (Zorzoli, 1962), is decreased by 10-20% in the kidney of the senescent C57Bl/6J mouse (Zorzoli and Li, 1967), and does not appear to change at all in the erythrocyte (Bertolini et al, 1964). Enzyme changes during ageing appear therefore to have <u>histospecific</u> patterns, as do enzyme changes during earlier stages in development.

Conflicting results are also apparent in this tabulation. For instance, liver succinic dehydrogenase levels are not altered with age in the wild rat (Barrows et al, 1962a) and McCollum rat (Barrows et al, 1958, 1960) but show a 10% increase in a Russian strain of white rat (Sazonova, 1960). It may be that the changes are not statistically significant. Alternatively, the chronological age at which biochemical changes became manifest may vary from cohort to cohort as do other features of the ageing process (e.g., the time of incidence of disease, as previously discussed). If this is so,

Table I

LIVER Species	Strain	Sex	Compared in Months	% change	Change given per unit	Reference
acid DNA-se						
mouse	CF-1	M	12 vs 20	0	DNA	Kurnick & Kernan, 1962
acid phosphatase						
mouse	C57Bl/6J	M	12 vs 24	-20	protein	Zorzoli, 1955
"	"	F	"	-20	"	" "
rat	Wistar	F	12 vs 24	0	"	Franklin, 1962
"	"	"	"	0	DNA	Barrows et al, 1962a
alkaline phosphatase						
mouse	C57Bl/6J	M	12 vs 24	+30	protein	Zorzoli, 1955
"	"	F	"	+30	"	" "
rat	wild	F	14 vs 34	0	DNA	Barrows et al, 1962a
alkaline DNA-se						
mouse	CF-1	M	12 vs 20	-15-20	DNA	Kurnick & Kernan, 1962
D-amino acid oxidase						
rat	wild	F	14 vs 34	0	DNA	Barrows et al, 1962a
"	Wistar	M	6 vs 21	+20%	DNA	Ross and Ely, 1954
arginase						
rat	not spec.	F	7 vs 15	0	dry wt	Lightbody, 1938
"	" "	M	"	-15%	" "	" "
cathepsin						
rat	wild	F	14 vs 34	+50	DNA	Barrows et al, 1962a
"	Wistar	F	14 vs 31	+11	"	Beauchene et al, 1967
"	"	M	13 vs 25	+27	"	" "
"	Sp.-Daw.	M	13 vs 27	+15	"	" "
"	Wistar	F	12 vs 24	+40	"	Barrows et al, 1962a
DNA-se I & II						
rat	Holtzmann		10 vs 14	+100	protein	Mukudan et al., 1963
fructose-1, 6-diphosphatase						
rat	Wistar	M	12 vs 15	-20	DNA	Singhal, 1967a
glucose-6-phosphatase						
rat	Wistar	M	12 vs 15	-30	DNA	Singhal, 1967a
"	C57Bl/6J	M	12 vs 24	0	DNA	Zorzoli, 1962
"	"	F	"	0	DNA	" "
glucose-6 phosphate dehydrogenase (relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
histidase						
rat	Wistar	M	6 vs 21	+130%	DNA	Ross and Ely, 1954
lactic dehydrogenase						
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1966
"	"	F	12 vs 24	-10	"	" " "
"	"	M	6 vs 21	+10	DNA	Ross and Ely, 1954
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
malate dehydrogenase						
rat	Wistar	F	12 vs 24	-10	protein	Schmuckler and Barrows, 1966
"	"	M	6 vs 21	+20	DNA	Ross and Ely, 1954
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
RNA-se I & II						
rat	Holtzmann		10 vs 14	+100%	protein	Mukudan et al, 1963
succinic dehydrogenase						
rat	Wistar	F	12 vs 24	-10%	DNA	Barrows et al, 1962a
"	wild	F	14 vs 34	0	"	Barrows et al, 1962a
"	McCollum	M	13 vs 26	0	"	" ", 1960
"	Wistar	M	6 vs 21	0	"	Ross and Ely, 1954
succinic dehydrogenase (in mitochondria)						
rat	McCollum	M&F	13 vs 26	0	protein	Barrows et al, 1960a
tryptophan pyrrolase						
rat	Wistar	F	13 vs 26	0	protein	Gregerman, 1959
tyrosine aminotransferase						
rat	Wistar	F	13 vs 26	0	protein	Gregerman, 1959
mouse	C57Bl/6J		9 vs 26 16 vs 26	-20% 0	DNA "	Finch et al, 1969 "

Table II

KIDNEY			Compared in		Change given	
Species	Strain	Sex	Months	% change	per unit	Reference
acid DNA se						
mouse	BF-1	M	12 vs 20	+50%	DNA	Kurnick and Kernan, 1962
rat	Wistar	F	6 vs 24	-25-30%	protein	Franklin, 1962
acid phosphatase						
rat	Wistar	F	6 vs 24	-11%	protein	Franklin, 1962
"	"	M	6 vs 21	0	DNA	Ross and Ely, 1954
alkaline DNAse						
mouse	BF-1	M	12 vs 20	+50%	DNA	Kurnick and Kernan, 1962
alkaline phosphatase						
rat	Sp.-Daw.	F	12 vs 24	0%	DNA	Barrows and Roeder, 1961
"	Wistar	M	6 vs 21	-30%	"	Ross and Ely, 1954
arylsulphatase B						
rat	Wistar	F	6 vs 24	0	protein	Franklin, 1962
ATPase (Na-K activated)						
rat	outer layer	F	13 vs 20	0	protein	Beauchene et al, 1965
"	Inner layer	F	13 vs 20	-30%	protein	" " "
cathepsin						
rat	Sp.-Daw.	F	12 vs 24	+50%	DNA	Barrows and Roeder, 1961
rat	wild	F	14 vs 34	0	"	Barrows et al, 1962a
fructose 1, 6-di-phosphatase						
mouse	C57Bl/6J	M&F	6 vs 23	0	protein	Zorzoli and Li, 1967
fumarase						
mouse	C57Bl/6J	M&F	6 vs 23	-12%	protein	Zorzoli and Li, 1967
glucose-6-phosphatase						
mouse	C57Bl/6J	M	6 vs 23	-20%	protein	" " "
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
isocitric dehydrogenase (Relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
lactic dehydrogenase						
mouse	C57Bl/6J	M	6 vs 23	-14%	protein	Zorzoli and Li, 1967
"	"	F	"	-15%	"	"" " "
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1967
"	McCollum	F	13 vs 26	10%	"	" " "
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		" " "
lysozyme						
mouse	B10-LP		14 vs 25	+500-1200%	protein	Troup and Walford, 1967
"	F1(B10-LPxC57Bl/10)		9 vs 25	+260%	"	" " , 1967
malate dehydrogenase						
rat	Wistar	F	12 vs 24	-10%	protein	Schmuckler and Barrows, 1966
phosphoenolpyruvate kinase						
mouse	C57Bl/6J	M	6 vs 23	0	protein	Zorzoli and Li, 1967
6 phosphogluconate dehydrogenase (relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1967
pyrophosphatase						
rat	Sp.-Daw.	F	12 vs 24	+20%	DNA	Barrows and Roeder, 1961
succinic dehydrogenase						
rat	Sp.-Daw.	F	12 vs 24	0	DNA	Barrows and Roeder, 1961
"	McCollum	F	13 vs 26	-10%	DNA	Barrows et al, 1960
"	wild	F	14 vs 34	-20%	"	" " "
succinic dehydrogenase in mitochondria						
rat	wild	F	14 vs 34	0	protein	Barrows et al, 1962a
"	McCollum	F	13 vs 26	0	"	" 1960,
triose phosphate isomerase						
mouse		M&F	6 vs 23	-10%	protein	Zorzoli and Li, 1967

Table III

HEART			Compared in		Change per	
Species	Strain	Sex	Months	% change	per unit	Reference
lactic dehydrogenase						
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1966
"	"	F	"	-10%	"	" " 1967
"	not given		7 vs 19	-43%		Kanungo and Singh, 1965
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1967
H_4	Not given		7 vs 19	-12		Kanungo and Singh, 1965
M_4	" "		"	-85		" " 1965
succinic dehydrogenase						
rat	Wistar	F	12 vs 24	-10%	weight	Barrows and Roeder, 1961

Table IV

BRAIN			Compared		Change given	
Species	Strain	Sex	Months	% change	per unit	Reference
lactic dehydrogenase						
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1966
"	"	F	12 vs 24	-10%	"	" " , 1967
"	not given		7 vs 19	-10%	weight	Kanungo and Singh, 1965
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
H_4	not given	F	7 vs 19	+10%		Kanungo and Singh, 1965
M_4	" "	F	"	-50%		" " "

Table V

SKELETAL MUSCLE			Compared in		Change given	
Species	Strain	Sex	Months	% change	per unit	Reference
glucose 6 phosphate dehydrogenase (relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
isocitric dehydrogenase (relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
6 phosphogluconate dehydrogenase (relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0		Schmuckler and Barrows, 1967
lactic dehydrogenase (total)						
rat	Wistar	F	12 vs 24	0	protein	Schmuckler and Barrows, 1966
rat	Wistar	F	12 vs 24	-10%	"	" " , 1967
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0	"	" " , 1967
malate dehydrogenase (total)						
rat	Wistar	F	12 vs 24	-10%	protein	Schmuckler and Barrows, 1966
(relative proportion of isozymes)						
rat	Wistar	F	12 vs 24	0	"	" " , 1967

Table VI

Changes in the Enzyme Activity of Total Erythrocyte Population from Humans of Different Ages

Enzyme	Nature of change	Reference
glucose-6-phosphate dehydrogenase	no change	Bertolini et al., 1964
alkaline phosphatase	no change	" , 1962
catalase	"	" , 1964
acid phosphatase	decrease 30% after 60 years	" , 1962
pyrophosphatase	increase 40% after 60 years	" , 1962
diaphorase I (NAD-dependent)	increase 40% 30 vs. 70 years	" , 1964

see reviews by Bertolini (1964, 1966)

one would need to sample members of a given cohort throughout their lifespan to establish the chronological time at which members of the cohort undergo a particular biochemical change. The mortality characteristics of the population under study which might allow comparison of the program of ageing from population to population are seldom given. Simple interpretation about quantitative ageing changes in cell proteins (enzymes, hormones, etc.) can not be made because many enzymes and some hormones have several biologically active components. It is possible that compensating changes occur in the relative proportions of these components without any net change in enzyme activity having been detected. Alternatively, the quantitative changes in total cellular content of a particular enzyme may result from complex changes in the proportions of multiple components. Such changes in the relative proportion of the iso-enzymes of lactic dehydrogenase have been detected in the heart and brain of the senescent rat (Kanungo and Singh, 1965). However, no changes in the relative proportions of the isozymes of lactic dehydrogenase, malic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and isocitric dehydrogenase have been detected in heart, brain, liver, kidney and skeletal muscle of the senescent rat by Schmuckler and Barrows (1967). Another example of a multi-component enzyme system which does not change is given by the multiple transfer RNA activating enzymes for leucine, which appear to be present in identical amounts in old and young kidney and old and young heart of the rabbit (Strehler, 1967).

The array of ageing changes of enzymes in various tissues does not appear to indicate a recognizable pattern of metabolic change, although the increases of cathespsin in liver and kidney and of lysozyme* in kidney suggest an increase in degradative and catabolic processes (for a discussion of this notion, see Lang, 1967). A relative dominance of catabolism is consistent with the loss of body weight and appetite and the atrophy of various organs which occurs in later mammalian life. It

* Infiltration of a tissue with lymphocytes of macrophages, which contain high concentrations of such enzymes, would also produce this result. Such infiltrations have been observed in liver (Andrew et al, 1943).

is difficult to relate changes in enzymes to the precipitous increase in death rate which occurs at the same time.

3) Ageing pigments

A consistently noted change in cell composition during ageing is the increase of substances known variously as ageing pigment, age pigment, lipofuscin, or Abnutzungspigment (wear and tear pigment). These brown, fluorescent, intracellular pigment particles have long been recognized to be increased in diverse vertebrates during ageing in a great variety of dividing and non-dividing cell types: nerve cells, myocardial cells, striated and smooth muscle cells, spleen cells, liver cells, adrenal cells, etc. (Toth, 1968; Reichel, 1968).

Despite numerous histological descriptions, ageing pigment has been isolated and characterized only from the myocardium (Siebert et al, 1955; Bjorkerud, 1964). It is clear that the ageing pigments have a complex composition: lipoproteins complexed to chromophores are found together with various enzymes. The origin of ageing pigment is obscure at present but is generally assumed to be intracellular: the mitochondrion, the Golgi apparatus, and the lysozome have all been considered (see references given in Toth, 1968). Despite the general supposition that ageing pigments represent the accumulation of cellular waste products (Strehler, 1960) there is little or no information on the turnover of any components of ageing pigments which would indicate their metabolic inertness. The fact that active enzymes are found associated with pigment particles implies some metabolic activity. Ageing pigments have been considered a possible cause of senile changes in the function of non-dividing cell types such as myocardial cells and neurons. If ageing pigment accumulation beyond a certain point were toxic, it might impair the normal function of these cells or cause their death. This phenomenon could be of lesser importance to organs containing cell types which were capable of dividing, such as the liver, as these cells might be replaced. The evidence against a generalized impairment of function in non-dividing cell types during ageing is impressive: in particular, human, rat and mouse myocardia show undiminished ability to hypertrophy throughout adult life (Hugin and Verzár, 1956; Swigart, 1969), despite the occupancy of 2-3% of the volume of its cells by pigments in senescence (Strehler et al, 1959; Munnell and Getty, 1968). Strehler's interesting analysis shows no correlation between heart disease, heart

failure, and the amount of ageing pigment (Strehler et al, 1959). The association of nerve cell degeneration with pigment accumulations so extreme as to displace the cell nucleus has been observed (e.g., Morrison et al, 1959; Few and Getty, 1967). One cannot be certain, however, that the cell was degenerating because of excessive pigment accumulation. It is possible that the cell was degenerating from other causes and the process resulted in the pigment mass.

It was thought at one time that the decrease in nerve cell RNA during ageing might result in the increase in ageing pigment (Hydén and Lindström, 1950). However, Hydén himself later showed that there was no inverse relationship between the two: the pigment and the RNA per cell increase in a parallel fashion into the 5th decade of human life. The RNA content eventually decreases, but the pigment shows no further increase (Hydén, 1960).

An intriguing fact about ageing pigments in the nervous system is their regional pattern of accumulation (Vogt and Vogt, 1946). Survey of a number of different mammals shows that in all species, little or no ageing pigment can be found in some regions of the nervous system of the oldest individuals. For instance, Wilcox (1959) could not detect ageing pigment in the cochlea, the dorsal motor nucleus of the vagus, or the Edinger-Westphal nucleus, in contrast to many other regions of the senile guinea pig nervous system. Similarly, little or no pigment was detected in the dorsal motor nucleus of the dog or hog (Whiteford and Getty, 1966), the supraoptic and paraventricular nuclei of the human hypothalamus (von Buttler-Brentano, 1954), or the human cerebellum (Andrew, 1956).

The rate of ageing pigment accumulation in other parts of the nervous system has repeatedly been found to vary from region to region with respect to the percent of pigmented cells as well as the amount of pigment in any cell (White, 1889; Höpker, 1951; Andrew, 1956; Wahren, 1957; Wilcox, 1959; Whiteford and Getty, 1966; Few and Getty, 1967; Reichel et al, 1968). This phenomenon is clearly demonstrated in the figures of Few and Getty (1967), which are presented in table VII. Linear increase of pigment throughout life has, however, been demonstrated in human myocardium (Strehler et al, 1959), canine myocardium (Munnel and Getty, 1968), and for dorsal ganglia and cerebellar Purkinje cells in the mouse (Samorajski et al, 1968).

Table VII

Differential Accumulation of Ageing Pigment in the Neurones of the Dog

Age (months)	Per Cent Pigmented Neurones: Autonomic cells (A)	Spinal cord ventral horn (B)	Ratio A/B
2	0	0	0
8	1	15	.067
12	1	55	.018
35	40	65	.615
105	75	85	.88
157	85	95	.895

(data of Few and Getty, 1967)

Wilcox (1959) has suggested that the activity of the nerve cells might be related to the amount of pigment they accumulate. The most active cells are supposed to be less prone to accumulate pigment than those which are relatively inactive for long periods of time. Wilcox evolved this theory from his observations of the guinea pig cochlea which appears to remain unpigmented throughout life. This notion does not seem plausible for many reasons, foremost among which is the fact that the nerve cells in the cochlea of aged dogs are found to become substantially pigmented (Whiteford and Getty, 1966). It seems highly unlikely that the cochlea of the dog is less active than that of the guinea pig. Other interspecific differences such as the amount of pigment accumulated in cerebellar Purkinje cells of aged mice vs rats (Reichel et al, 1968) or in the cerebral cortex of rats vs humans (Kuhlenbeck, 1954), make it impossible to generalize about the meaning of ageing pigment distribution at this time.

The deposition of ageing pigments in other organs of the body also appears to be localized in particular cell types. Thus, the zona reticularis has 5 times more pigment than any other zone in the adrenal gland of the senile rat (Reichel, 1968); in the ovary of the senile rat, the pigment is found exclusively in the peri-follicular macrophages and not in the ovarian follicles themselves (Reichel, 1968). Another example is the preferential increase of pigment in the muscles concerned with locomotion as opposed to those concerned with the maintenance of posture (Kny, 1937). Given

the fact of regional differences in the rate of accumulation and the fact that some tissues do not accumulate pigment at all, it would seem best not to assume, as is commonly done, that ageing pigments are a homogenous group of substances whose accumulation in different cells of the body results from the same process.

Although pigment accumulations are a progressive change of ageing, it has been found possible to induce substances in the nervous system which are superficially identical to ageing pigments through the imposition of various stresses on young animals. Once induced by such stressors as acetanilid, hypoxia, vitamin E deficiency, unilateral nephrectomy, or ACTH administration, the pigment apparently remains in those nerve cells throughout the lifespan (Sulkin and Srivanij, 1960). The effect of precocious pigment accumulation on the subsequent life pattern or longevity of the young stressed animal was not stated by the authors. Other situations resulting in the production of ageing like pigments are given in the review of Toth (1968). The entirely regular course of pigment accumulation in laboratory animal populations (Munnel and Getty, 1968; Samorajska et al, 1968) and in human populations (Strehler et al, 1959) implies that accumulated traumata from random stresses of life are not a likely cause of pigment accumulation during ageing. Occasional traumata may, however, be the basis for individual differences in pigmentation of some organs; e.g., the aged human pituitary is not always pigmented (Parsons, 1936), although the possibility of genetic differences in pigment accumulation between individuals of any species remains unexplored.

In conclusion, the accumulation of pigment during ageing shows the same type of specificity as do the ageing changes of RNA and enzyme concentrations: not all cell types are affected and those which are show specificity in the time of onset and final extent of change.

4) Activities of metabolic pathways: respiration

a) Respiration of the whole organism

The pace of body metabolism measured as total oxygen consumption of the whole body has often been shown to decline during ageing in rats (Kibler et al, 1962) and humans (Shock et al, 1963). If the basal respiration is expressed in terms of some function of the metabolically active body mass, then the basal oxygen uptake per unit of functioning

cellular mass does not appear to decline significantly during ageing in humans (Shock et al, 1963), and may even be increased in the rat (Ring et al, 1964). Shock and co-workers (1963) point out that the parameters, in which basal metabolism is commonly expressed, such as body mass or surface area, may give rise to spurious values, because these parameters are measures of the sum of all body components regardless of the content of living cells. Intracellular water (Shock et al, 1963) or total body potassium (Ring et al, 1964), 90% of which is intracellular, give better estimates of the active cellular mass in theory.

b) Respiration of isolated body components

(1) Intact tissue

Measurements of oxygen consumption of tissue slices from individual tissues with glucose added as a substrate indicate a varied pattern of change (table VIII). At least one tissue, the ear skin of the mouse, does not appear to decline in respiratory rate after maturity. In the brain of the senescent dog, regional differences in the extent of metabolic decline are evident. In the liver, conflicting results are apparent. In general, there is a trend towards decreased oxygen consumption when the respiring tissue is measured in terms of its mass or its number of cells.

(2) Homogenates

Cell free homogenates of liver (glucose added as a substrate) show a similar trend towards declining oxygen consumption with age (see table IX). One study (Barrows et al, 1960) shows that such changes may not necessarily occur simultaneously in all organs (e.g., liver vs kidney).

(3) Mitochondria

In cell free homogenates, the mitochondria are the dominant oxygen consumers. Explanation of the decline in respiration, therefore, might be sought in terms of decreased number of mitochondria per cell, or alternatively, as a decreased metabolic activity per mitochondrion.

(a) Oxidative phosphorylation in isolated mitochondria

The P:O ratio of whole homogenates was not found to change during senescence in rat liver and kidney (Barrows et al, 1960) with glucose as a substrate. This is an indication that mitochondrial respiration is not impaired during ageing. However, mitochondrial P:O ratios cannot be

Table VIII

Age Change in Oxygen Consumption of Tissue Slices with Glucose as Substrate (except as indicated)

	% change	Change given per unit	Ages compared	Reference
Liver				
1. rat	-35%	tissue mass	7 vs 55 wks	Pearce, 1936
2. rat	-40%	DNA	"adult" vs 24 mos.	Jacob et al, 1954
3. rat (no substrate added)	-20%	tissue mass	5 vs 21 mos	Ross & Ely, 1954
4. rat (+ succinate)	+10%	tissue mass	5 vs 21 mos	Ross & Ely, 1954
5. rat	0	DNA	13 vs 26 mos	Barrows et al, 1960
Myocardium				
1. rat	-30%	tissue mass	7 vs 55 wks	Pearce, 1936
2. guinea pig	+5%	tissue mass	2½ vs 5 yrs	Wollenberger and Jehl, 1952
Brain (dog)				
caudate nucleus	-15%	tissue mass	"adult" vs 10 yr	Himwich and Himwich, 1959
cerebral cortex	-50%			
medulla oblongata	-85%			
thalamus	-15%			
Kidney				
1. rat	-10%	tissue mass	7 vs 55 wks	Pearce, 1936
2. rat F	-10%	tissue mass	13 vs 26 mos	Barrows et al, 1960
rat M	- 5%	tissue mass	"	"
Ear skin				
mice	0		5 vs 26 mos	Mundy & Krohn, 1965
Articular cartilage				
cattle (n.b., no exogenous substrate added; glucose found to inhibit)	-55%	DNA	3½ vs 9½ yrs	Rosenthal et al, 1941

Table IX

Age Change in Oxygen Consumption of Cell Free Homogenates with Glucose as Substrate

	% change	Change given per unit	Ages compared	Reference
Liver				
1. guinea pig	-25%	tissue mass	12 vs 23 mos	Rafsky et al, 1952
2. rat	-20%	tissue mass	15 vs 24 mos	Reiner, 1947
3. rat	0	tissue mass	13 vs 26 mos	Barrows et al, 1960
Kidney				
1. guinea pig	-35%	tissue mass	12 vs 23 mos	Rafsky et al, 1952
2. rat	-35%	tissue mass	13 vs 26 mos	Barrows et al, 1960
Brain				
1. rat	-35%	tissue mass	15 vs 24 mos	Reiner, 1947

satisfactorily measured in whole homogenates because of microsomal hexokinases which tend to reduce the value of the total phosphate esterified (Umbreit et al, 1964).

The careful and detailed studies of Weinbach and Garbus amply demonstrate that the metabolic activities of isolated mitochondria persist throughout life. Measurements of P:O ratio in mitochondria isolated from rat liver with succinate, α-ketoglutarate, glutamate, or malate as substrates have not revealed any differences between young or senescent rats (Weinbach and Garbus, 1959a). With the exception of β-hydroxybutyrate, which is oxidized at a decreased rate, all other substrates were oxidized at identical rates. No age changes in P:O ratio were found with any substrate.

The decreased oxidation of β-hydroxybutyrate appears to represent a unique and selective age change of the liver mitochondrion. Further investigation revealed that senescent kidney mitochondria also have a decreased ability to oxidize β-hydroxybutyrate (Weinbach and Garbus, 1959b), although the decrease is greater (30-40%) in the liver than in the kidney (20%). Weinbach and Garbus conjectured that this smaller age decrement

might result from the established ability of the kidney (but not liver) mitochondria to metabolize β-hydroxybutyrate beyond the level of acetoacetate (in the liver, acetoacetate accumulates stoichiometrically during the oxidation of β-hydroxybutyrate). In fact, no age difference in oxidative phosphorylation with acetoacetate as a substrate in kidney mitochondria was found (Weinbach and Garbus, 1959b).

The association of coenzyme A with the coupled oxidative phosphorylation of β-hydroxybutyrate in mitochondrial fragemnts led Weinbach and Garbus (1959b) to investigate coenzyme A levels. A consistently obtained but not statistically significant difference was found between coenzyme A levels of mitochondria from old and young rat kidneys. Weinbach and Garbus concluded that this difference could not by itself cause the decrease in β-hydroxybutyrate metabolism with age.

The decreased capacity for metabolising β-hydroxybutyrate appears therefore to be an unique and selective enzymatic change during senescence in rat kidney and liver mitochondria, but not in rat brain mitochondria. Supplied with other substrates in vitro, brain, kidney and liver mitochondria appear to be completely competent throughout life.

(b) Number of mitochondria per cell

Various studies have indicated a decreased number of mitochondria per cell measured as the decreased amount of cell nitrogen associated with mitochondrial fractions in senescent rat liver (Griswold and Pace, 1957; Detwiller and Draper, 1962), decreased activity of succinic dehydrogenase (a mitochondrial enzyme) per cell in kidney (Barrows et al, 1960) and liver (Barrows et al, 1962a), or from direct microscopic count of mitochondria in periosteal osteoblasts of rats (Tonna and Pillsbury, 1959). In conflict with these data are observations that succinic dehydrogenase activity per cell does not change during ageing in rat liver of other laboratory populations (Barrows et al, 1958, 1960).

The decrease in number of mitochondria per cell during ageing may vary according to the tissue examined, the population from which the animal was drawn, or the strain of animal. A similar situation was enountered previously during considerations of the effect of ageing of RNA per cell and on the activities of various enzymes. Differences in the extent of weight loss during ageing may be the primary cause of this variability.

(4) Conclusion

The decreased oxygen usage of the aged organism cannot be attributed to a failure of respiratory metabolism at the mitochondrial level. In certain instances, cited above, the number of mitochondria per cell may be less in older animals, but those remaining mitochondria in general appear to be fully functional. The causes of the larger decreases in the respiration of intact tissues in vitro during ageing (table VIII) remain unexplained.

c) Physiology of axonal excitation

The effect of age on the electrophysiological properties of excised nerves was the object of an excellent study by Birren and Wall (1956). Action potentials were produced in a 60mm. long excised segment of rat sciatic nerve by a square wave potential. The conduction velocity of the impulse was found to increase to a maximum velocity in male and female rats at about ten months and did not change thereafter up to at least 26 months. The temperature coefficient of the conduction velocity was the same in 8 and 21 month old rats between 17°C and 37°C. The absolute refractory period and the 50% recovery time did not change between the ages of 12 and 22 months. In sum, the ability to support the conduction of nervous impulses and the ability to regenerate axonal excitability after the passage of a nervous impulse is not altered by age. According to the ionic theory of the action potential, these electrophysiological properties indicate that those aspects of intermediary metabolism and the molecular architecture of the nerve fibre which are concerned with selective changes of ionic permeability do not change during the life of the mature rat. Consideration of these facts suggests that the slowing of psychomotor reflexes during ageing does not result from a degeneration of the excitable membrane of nerve trunks. It is possible that the age change in reflex speed results from a change in the integrative properties of the central nervous system or a change in the threshold of the sensory receptors.

d) Active transport

The active transport of 6-deoxy-D-glucose by the small intestine of the mouse in vitro may even be increased in senescent mice (Calingaert and Zorzoli, 1965). In contrast, the total active transport of p-amino hippuric acid or α-amino isobutyric acid was decreased by 40-50% in kidney slices

from senescent vs young rats, although the kinetics of transport were unaltered (Beauchene et al, 1965). The decreased transport capacities of senescent kidney slices probably results from the age related loss of nephrons and tubules (the active cells of transport in the kidney) which will be discussed in section IIIC10a. The stability of the transport system in the remaining tubules persists throughout the lifespan, as is shown by the absence of p-amino hippuric acid leakage during prolonged incubation of kidney slices from rats of all ages (Beauchene et al, 1965).

e) Activities of metabolic pathways generally considered

A summary of the changes in various metabolic pathways is given separately for catabolism (table X) and anabolism (table XI). The previous tabulations of age changes in enzyme activities, considered individually, showed that the changes were quantitative, variable according to population and species, and in general rather small.

One might still inquire about the efficiency of enzyme pathways in older animals. Subtle disorganizations of metabolism could possibly occur with age, perhaps due to small changes in the stoichiometry of multi-enzyme feedback loops, despite the persistence of each enzyme activity considered separately. However, it is clear that oxidative phosphorylation, anaerobic glycolysis and gluconeogenesis, fatty acid oxidation and fatty acid chain elongation in the liver (both known to occur within the mitochondrion), and active transport mechanisms in the kidney, intestine and nerve fibre are unimpaired in older animals. These metabolic activities depend on highly organized enzyme systems. Decreased cholesterol biosynthesis in rat liver, decreased oxidation of C2 fragments of caprylate via the Krebs cycle in liver mitochondria, decreased liver and kidney mitochondrial oxidation of β-hydroxybutyrate, and increased anaerobic fructolysis are exceptions to the general rule of metabolic constancy during ageing. The specificity of these changes is therefore noteworthy.

In sum, no dysfunction of complex metabolic pathways and highly organized multi-enzyme systems has been found. Ageing does not strike hard at intermediary metabolism in the mammal.

Table X

Age Changes in Activities of Anabolic Pathways

	% change	Change given per unit	Ages compared (months)	Reference
Fatty acid Biosynthesis				
1) rat liver 2-C^{14} acetate precursor				
slice	0	sp.act.fatty acid.	1.5 vs 24	Perry and Bowen, 1957
2) rat intestinal mesenteric fat 2-C^{14} acetate precursor				
whole tissue	-70%	"	"	" " "
Cholesterol biosynthesis				
1) rat liver slice	-55%	sp. act. cholesterol	4-5 vs 24	Perry and Bowen, 1957
Active transport				
1) rat kidney: PAH accumulation:				
outer slice	-50%	substrance transported/gm wet weight	12-14 vs 24-28	Beauchene et al., 1965
inner slice	-40%			
2) mouse small intestine:6-deoxyglucose accumulation				
whole	0	"		Calingaert and Zorzoli, 1965
Gluconeogenesis				
1) mouse kidney cortex: (oxalate subs)				
slice	0	glucose/tissue mass	6-7 vs 20-22	Zorzoli and Li, 1967
(succinate subs)				
slice	0	"	"	"
(fasted nor fed show age differences)				

Table XI

Age Changes in Activities of Catabolic Pathways

	% Change	Change given per unit	Ages compared	Reference
Anaerobic fructolysis (CO_2 evolved)				
rat liver slice	+35	tissue mass	5-6 vs 21 mos	Ross & Ely, 1954
Anaerobic glucolysis (CO_2 evolved)				
1. rat liver slice	-35%	tissue mass	5-6 vs 21 mos	Ross & Ely, 1954
2. rat liver slice	0	DNA	12-14 vs 24-27 mos	Barrows et al, 1960a
3. rat liver homog.	0	tissue mass	12-18 vs 24 mos	Reiner, 1947
4. rat brain, homog.	0	tissue mass	12-18 vs 24 mos	Reiner, 1947
5. bovine articular cartilage slice	0	DNA	1-7 vs 8-11 yr	Rosenthal et al, 1941
Fatty Acid Oxidation				
1. rat liver mito.	0	tissue mass	3 vs 24 mos	Weinbach & Garbus, 1959b
2. rat kidney mito. (hexanoic, octanoic, decanoic acids as substrates)	0	tissue mass	3 vs 24 mos	Weinbach & Garbus, 1959b
3. rat liver homog. (caprylate as substrate)				
a) total oxidation of caprylate	0	tissue mass	3 vs 24 mos	Pashkova, 1960
b) oxidation of C_2 fragments from caprylate via the Krebs cycle	-30%	tissue mass	3 vs 24 mos	Pashkova, 1960

5) Biosynthesis of macromolecules

The next aspect of the cell function to be considered in terms of ageing will be the biosynthesis of macromolecules. In the context of the information about cell composition and activities of intermediary metabolism, it would be surprising to find major alteration in the synthesis of macromolecules on which these other aspects of cell function directly depend. Such a change, in fact, does not occur.

a) Synthesis of RNA

In pursuit of the meaning of the age changes in RNA content of various cells (described in section IIIC1b) Quastler, Wulff, and co-workers have made an extensive study of the patterns of RNA synthesis in ageing rodents. A frequent finding, corroborated by Balis and Samarth (1962) and Gellhorn and Benjamin (1966), is an age-related increase in the incorporation of radioactive RNA precursors (dose adjusted for body weight) into RNA of certain tissues. A survey of these data is given in table XII. It can be seen that over a wide range of times of labelling, the incorporation of radioactive RNA precursors into polymerized RNA measured by autoradiographic techniques or by the specific activity of phenol purified RNA, is increased during ageing. The increase in RNA labelling appears to progress regularly with the age of the adult animal in liver, kidney, skeletal muscle, and heart ventricular muscle (Wulff et al, 1962 and 1964). Labelling of RNA does not appear to change with age in bone marrow cells or in loose connective tissue of the intestine. In the cerebral cortex (Wulff et al, 1961) and cerebellar Purkinje cells (Wulff et al, 1961, 1962 and 1965), however, the amount of labelling of RNA decreases. Simple categorization of dividing cells (showing increased RNA labelling with ageing) is not valid, because increased labelling occurs in heart ventricular muscle cells (Wulff et al, 1961, 1964 and 1965) and in dorsal root ganglion cells (Wulff et al, 1962 and 1964), and decreased labelling occurs in mature epithelial cells of the intestinal villi (Wulff et al, 1961). The tabulation of these changes in table XII indicates a generally consistent trend in most tissues, although the precise degree of the age change may vary a certain amount. These variations are probably related to differences in specific activity of the isotope injected, differences of strains and ages of animals used, and possibly age-differences in the circadian pattern of RNA synthesis.

Table XII

Age Changes in the Incorporation of Injected Radioactive Precursors into RNA of Various Tissues

(% change)

	Samis et al, 1964	Menzies et al, 1967	Detwiller and Draper 1965	Gellhorn and Benjamin 1966	Wulff et al, 1961	Wulff et al, 1965	Wulff et al, 1962	Wulff et al, 1964	Balis and Samart 1962
Labelling time	1 hr.	1 hr.	2 hrs.	3 hrs.	5 hrs.	6 hrs.	6 hrs.	6 hrs.	24 hrs.
Liver									
whole tissue								+45%	+40%
nuclei	+75%		-50%				+60%	+80%	
Spleen									
whole tissue									+70%
Connective tissue of intestine						0			
Epithelial cells on villus					-40%				
Kidney									
whole tissue									+130%
cortex (whole tissue)						+70%			
proximal tubule (nucleus)						+35%		+45%	
Separated bone marrow cells	0								
Skeletal muscle									
whole tissue			-20%		+20%	+45%		+50%	
nucleus			+100%			+70%		+30%	
cytoplasm			+180%						
Heart ventricle									
whole tissue					+115%	+35%		+10%	
nucleus					+35%	+60%		+40%	
cytoplasm					-13%				
Dorsal root ganglia									
whole tissue						+55%			
nucleus						+65%	+90%		
Cerebral cortex									
whole tissue					-10%				
nucleus					-25%				
Cerebellar Purkinje cells									
whole tissue					-35%	-30%			
nucleus					-40%	-35%	-35%		
cytoplasm					-40%				

1. values calculated from figures in text or estimated from graph
2. in studies employing autoradiography, values are given as percentage change of grain count (when possible, values estimated from RNA-se solublized grain counts)
3. in other studies employing autoradiography, values are given as percentage change of RNA specific activity

Table XII also demonstrates that there is no necessary correlation between the direction of the change in labelling and the changes in RNA content of a particular cell type during ageing. Thus cerebellar Purkinje cells, known to contain less basophillic material (Andrew, 1938), show decreased RNA labelling, whereas fat pad cells whose RNA content also decreases (Gellhorn and Benjamin, 1966) show increased RNA labelling.

In sum, quantitative age related changes in the incorporation of radioactive precursors into RNA regularly occur. The direction of change varies in a histotypic manner.

The observation that increased incorporation of precursors into RNA occurred in some tissues during ageing resulted in a new theory of ageing. In brief, Wulff, Quastler and Sherman (1962 and 1964) proposed that the increased incorporation of precursors represented a net increase in RNA synthesis. This increased RNA synthesis was postulated to be ultimately caused by damage to the genes during ageing. In these same cells, compensation for the ineffective enzymes coded for by these damaged genes results in a derepression of their transcription, thereby causing a general increase in RNA synthesis. However, the interpretation that changed incorporation of precursors represents a changed rate of synthesis of RNA is subject to certain criticisms.

The size of intracellular precursor pools has long been recognized to affect the incorporation of exogenous labelled precursor, which will mix with intracellular pools, into nascent macromolecules. Inasmuch as the pool size can vary with the physiological state (Yu and Feigelson, 1969), one must proceed cautiously before interpreting changes in the rate of precursor incorporation as indicating an actual change in the rate of biosynthesis. Direct measurements of nucleic acid precursor pools in ageing have only been made thus far in the rat liver (Bucher and Swafield, 1966): the total uridine triphosphate (UTP) pool of 24 month old rats is 25% smaller than in younger rats of unspecified age. Smaller pools of acid soluble nucleotides have also been found in mitochondria from livers of 24 vs 4 month old rats (Weinbach and Garbus, 1959a). Thus the exogenous precursor molecules would be diluted less in the older rat liver; a greater net labelling of newly synthesized RNA could result. Furthermore, following a single injection of C14-orotic acid, a UTP precursor, the specific

activity of the UTP pool tends to remain elevated longer than in younger animals (Bucher and Swafield, 1966), implying a slower clearance of blood orotic acid and/or a slower turnover of the liver's UTP pool. In either case, the net result would be a longer availability of labelled precursor which would result in a more intense labelling of newly synthesized RNA at certain times after injection.

The problem of evaluating intracellular precursor pool changes can also be approached indirectly by experimentally varying the specific activity of the injected precursor. If a smaller pool size was the basis for an increased incorporation of precursor into nascent macromolecules, injections of the same number of radioactive atoms of precursor at successively lower specific activities would tend to decrease the specific activity of a smaller pool more than a larger pool. Theoretically, as the specific activity of the exogenous radioactive precursor approaches zero, the differences in labelling of the originally different sized intracellular pools will tend to disappear. Thus, if age differences of incorporation of isotopically labelled precursors into newly synthesized macromolecules tend to decrease at successively lower specific activities, then an age related change in precursor pool size is implicated as the cause of the differences of incorporation.

The practical difficulty of this experiment in nature is that regulation of the passage of exogenous precursor molecules through the cell membrane, as well as the synthesis of precursors within the cell, is sensitive to the concentration of various precursors inside and outside of the cell. Thus, at certain precursor concentrations, the transport of the precursor into the cell and the enzymatic synthesis of precursor within the cell may be arrested through feedback inhibition, thereby confounding the intentions of the experiment (see Nierlich's excellent analysis of this problem (Nierlich, 1967; Nierlich and Vielmutter, 1968)).

Despite the previously described theoretical complications of an isotopic dilution experiment, a careful autoradiographic study (Wulff et al, 1965) has shown that dilution of the isotopic precursor progressively reduces age differences towards a common limit in the incorporation of cytidine into RNA of kidney, liver, skeletal and ventricular muscle, and dorsal root ganglia. These are cell types in which the incorporation of RNA

precursor increased with age. However, in two cell types for which the incorporation of precursors into RNA decreased with age, dilution of the isotopic precursor had different effects: in the motor neurons of the lower spinal chord, age differences in precursor incorporation were not significantly altered, whereas in the Purkinje cells of the cerebellum, age differences were diminished. (This later conclusion was reached from inspection of data given by Wulff et al (1965), despite statements in the text of that paper to the contrary.) The effect of precursor dilution was often complex; at certain specific activities, the amount of precursor incorporated was higher than expected from the dilution factor. The general trend toward the reduction of age differences in RNA synthesis with precursor dilution was also found in the epididymal fat pad of the rat (Gellhorn and Benjamin, 1966).

All of these measurements imply that the age differences in the incorporation of precursors into RNA may not represent increased RNA synthesis. Wulff and co-workers (1965) do not reach this conclusion from their analysis, but insist on their interpretation of an increased RNA synthesis with age in certain tissues because of the persistence of age differences in the amount of incorporation of isotopically labelled precursor at the lowest specific activity in certain tissues (dorsal root ganglia, liver, skeletal muscles, and lower spinal chord motor neurons). The practical difficulties of this experiment because of feedback inhibition, however, would seem to justify more emphasis on the fact that the age-related differences of precursor incorporation showed a progressive decline with increasing precursor dilution more than the fact that a final common non-zero limit to the incorporation of precursor was not always reached. The absence of differences in RNA synthesis of cultured bone-marrow cells from old and young rats (Menzies et al, 1967) is consistent with this conclusion.

The rate of loss of previously incorporated labelled RNA precursor is another index of the rate of synthesis of RNA, assuming steady state kinetics. The data of Wulff and co-workers (1964) indicates that the disappearance of radioactivity from the liver cytoplasm may be significantly faster in older mice (loss of 2.3 grains per hour) than in young (loss of 0.75 grains per hour) during the first 6 hours after the maximum labelling of cytoplasmic RNA occurs. Studies carried out for up to 24 hours in liver, brain and skeletal muscles of old rat nucleic acids (RNA and DNA combined) appear to show a slower turnover in senescent

rats than in young (Bulkanin and Parina, 1960). It is of interest that the initial specific activity of nucleic acids was found to be greater in old livers than in young, in agreement with data cited in table XII, but was less in old skeletal muscle. The specific activity of brain nucleic acids was found to be lower in old rats, in agreement with the trend observed for cerebral cortex with autoradiographic techniques (Wulff et al, 1961).

The molecular weight distribution of the RNA synthesized within one hour of precursor injection was found to be closely similar, if not identical, in the livers of one 3 month old and one 27 month old rat (Wulff et al, 1967). The RNA was extracted at successively higher temperatures (0°, 50°, 65°C) and was resolved by centrifugation on sucrose density gradients into the familiar molecular weight pattern of 45S RNA and other fractions heterogeneous in sedimentation velocity. No age differences were apparent.

Another set of observations which add weight to the general conclusion that major age changes in RNA synthesis do not occur in most tissues is derived from measurements of the volume of the liver cell nucleus. It has long been known to cytologists that the size of the nucleus can alter according to changes in the functional activity of that cell type. For instance, the liver nucleus increases in size during regeneration (Bucher and Glinos, 1950) when there is a major change in genomic activity (Church and McCarthy, 1967 a, b). The diameter of the liver cell nucleus decreases during a prolonged fast; upon refeeding, the nuclear diameter increases strikingly during the first 12 hours if a protein rich diet is given (Lagerstedt, 1949). A change in the genomic activity is implied during this period, when many enzymes involved in protein catabolism become induced, e.g., tyrosine aminotransferase (Watanabe et al, 1968). Similarly, the sizes of cell nuclei in the hypothalamus increase during the hypersecretion of ACTH which ensues after adrenalectomy (Szentagothai, 1962). If a major increase in genomic activity occurred in the liver cell nucleus during ageing, one might logically anticipate a change in nuclear volume. However, the volume of the interphase liver nucleus clearly does not change during senescence in rats (Bucher and Glinos, 1950; Falzone et al, 1959; Enesco, 1967) and in humans up to 80 years (Tauchi and Morikawa, 1954). Increased upper nuclear size limit and heterogeneity of sizes, however, do occur in human livers at ages greater than 80 (Tauchi and Sato, 1962).

Major quantitative changes in nuclear RNA synthesis do not appear to occur during ageing. The methods of analysis so far employed, however, are relatively crude, and would not elucidate subtle shifts in the pattern of genomic expression or in the turnover of particular RNA species.

b) Passage of RNA from the nucleus to the cytoplasm

The relationship between nucleus and cytoplasm is crucial to cellular life; upon it depends the delivery of transcribed RNA from nucleus to cytoplasm. This relationship can be monitored by following the passage of newly synthesized RNA from the cell nucleus to the cytoplasm. There is little knowledge concerning the effect of age on this process, although enough information exists to surmise that age changes do not occur in at least several cell types.

The rate of transit of RNA (measured autoradiographically as grains of RNAse sensitive, H^3-cytidine) from liver, skeletal muscle, kidney, and heart ventricular muscle nuclei is, if anything, slightly increased during senescence in the mouse. The loss of labelled nuclear RNA represents, at least in part, RNA transported to the cytoplasm. The increase in cytoplasmic radioactivity was described only for the hepatic parenchymal cell (Wulff et al, 1964). Although it is impossible to distinguish various RNA species in an autoradiographic study, major age changes in the rate of passage of RNA from the nucleus to the cytoplasm do not appear to occur. It is therefore unlikely that age changes in the expression of genomic information result from impaired transit of newly transcribed RNA.

c) Protein synthesis

(1) Intact tissue

Studies on the synthesis of whole tissue protein indicate that marked changes do not occur during senescence. Neuberger's precise early study showed that the specific activity of isolated, radioactive glycine from liver and muscle proteins declined during a 60 day period at the same rate in young and late mature rats (Neuberger et al, 1951). The loss of incorporated radioactive glycine (not subsequently isolated) over a four day period in liver and brain did not change strikingly in senescent rats, although some decrease in turnover rate is indicated (Bulkanin and Parina, 1960).

The specific activity of S^{35} - labelled methionine may have declined at a slightly greater rate during a 4 week long study of the senescent rat liver, kidney, brain, heart, and muscle, although the age differences were not considered statistically significant by the authors (Barrows and Roeder, 1961).

Studies of the turnover of total non-sclero proteins in uterus, tendon, and skin do not indicate a change with senescence in the rat, although the turnover rate of non-scleroproteins of the aorta may decline (Kao et al, 1961).

Tonna remarks that the metabolism of bone proteins, measured by studies employing H^3-histidine, is not changed during senescence in mice (see footnote 4 in Tonna, 1964).

Uptake of C^{14}-valine by brain slices may be significantly declined during late maturity in the rat (Orrego and Lipman, 1967).

Finally, uptake of radioactive leucine into liver slices in vitro of senescent rats proceeds at the same rate as in young animals (Beauchene et al, 1967). In sum, major age changes in total protein synthesis are not consistently indicated for most organs, although changes in turnover of particular proteins may occur in some tissues such as the non-scleroproteins of the aorta (Kao et al, 1961).

(2) Cell free protein synthesis

Protein synthesis in cell free fractions has not been systematically investigated during ageing. One interesting report mentions uptake of lysine by an in vitro system consisting of human microsomes and 105,000g/60' supernate from cerebral tissue of different aged humans. The fractions from a 62 year old man and a 70 year old woman were definitely less active than corresponding fractions from younger individuals (Suzuki et al, 1964). However, differences were not found during senescence in the rate of incorporation of leucine into rat microsomes (Beauchene et al, 1967). No conclusion can be drawn at this time.

(3) Individual components of cells

(a) Connective tissue

Study of the turnover of individual proteins of the connective tissue of various rat organs reveals a specificity of age changes. In aorta, uterus, tendon, and skin, the isotopically measured turnover of "soluble collagen" is the same for adult and senescent rats. The "insoluble collagen" could not be labelled in the senescent tendon, had an extremely slowly turning over component in the aorta (no change during senescence), and turned over most rapidly in the uterus with a clear decrease during senescence. Turnover of aortic elastin was very slow and did not change with senescence (Kao et al, 1961). It is of relevance that specificity in ageing of connective tissue of different body components is well known to histologists (e.g., Furstman, 1966).

(b) Liver mitochondria

A very careful examination of mitochondrial components from senescent rats has shown the absence of change in the turnover of mitochondrial saline soluble and saline insoluble proteins,and of purified cytochrome c (Fletcher and Sanadi, 1961a). Thus, the renewal of mitochondria in liver, which appear to turn over as a unit with a half-life of 10.5 days in the rat (Fletcher and Sanadi, 1961b), does not seem to be impaired or altered during senescence. The turnover of mitochondrial lipid appears to be about 40% faster during senescence and appears to include metabolically diverse components with a final turnover rate slower than other mitochondrial constituents in young animals. Fletcher and Sanadi suggest that the age difference in the mitochondrial lipid may represent an age difference in the turnover of the metabolic pool of lipids (Fletcher and Sanadi, 1961a).

Useful information about the rates of synthesis of other body proteins can be obtained from studies of various secretions.

(c) Salivary secretion

The basic ptyalin content of saliva from a group of senescent humans was only 3% of a group of young adults. Stimulation of salivation resulted in a flow of saliva 40% less in volume in the senescent group with approximately the same deficiency of ptyalin as in the basal measurements (Meyer et al, 1937). Further study revealed that the decline in ptyalin occurs after 60 years of

age (Meyer et al, 1940). These results might imply a major deficiency in starch digestion in the mouth during senescence. Correspondingly, atrophy of aged human salivary glands has been described in histological studies (Andrew, 1952a).

(d) Pancreatic secretion

In contrast to salivary ptyalin, the "fasting" content of pancreatic amylase in human digestive juice shows only a slight decrease during senescence (Meyer et al, 1940; Fikry, 1968). Thus, digestion of carbohydrates need not be seriously affected as the result of ageing.

Concentration of pancreatic lipase is not markedly changed during senescence (Meyer et al, 1942; Fikry, 1968).

Fasting pepsin and trypsin content fall sharply in humans during late maturity (40-50 years) and remain relatively constant throughout the rest of life. Fikry (1968) has also indicated reduced trypsin content in older human pancreatic secretion.

These precise and specific changes in enzyme secretion indicate that age changes in protein synthesis are not general, but are subject to differential regulation, with respect to the age at which the change occurs and the extent of the change, if any, in the production of a particular enzyme.

(3) Hormones of the pituitary

The output of the pituitary hormones in ageing is known from two lines of evidence: 1) the content of various hormones, which represents the balance between synthesis and secretion; and 2) the level of various pituitary hormones during ageing. No studies have been made of the turnover of pituitary hormones during ageing. On the basis of existing evidence, it appears that ageing has a differential effect on pituitary cell functions.

(i) Growth hormone

Biological assay of the growth hormone content of senescent rat pituitaries (Solomon and Greep, 1958; Bowman, 1961; Meites et al, 1962; Pecile et al, 1965) and senescent human pituitaries (Gershberg, 1957) do not show any decrease with age. The figures of Daughaday do not show any marked change in titres of growth hormone in human serum after 40 years of age (Daughaday, 1959).

Pecile et al (1965) have made the interesting observation that the content of growth hormone releasing factors in the hypothalamus is much less in rats aged 24 months than in rats 1 month old.

(ii) Gonadotrophins

It is generally observed that circulating gonadotrophin titres increase in human females during late maturity, especially after menopause (Witschi and Riley, 1940; Johnsen, 1959). Direct assay of pituitary in the post-menstrual rat also shows an increase (Lauson et al, 1939). In aged women, the original post-menopausal peak value for gonadotrophins may decline somewhat (Johnsen, 1959).

The existence of a male climateric has been much disputed. It is clear that testicular function wanes, but does not cease entirely. However, there is a definite trend towards increased gonadotrophin during ageing in men (Johnsen, 1959). Furthermore, the high post-castration gonadotrophin titres of eunuchs castrated before puberty are maintained through old age (Hamilton et al, 1945). This is a particularly severe test of cell function: the maintenance of a 6- to 20-fold, supra-normal rate of protein synthesis and secretion for 50 years by a group of cells, one component of which in the hypothalamus is non-dividing.

The general trend of increased gonadotrophin production during senescence is paralleled by a corresponding trend for a selectively increased number of chromophobic cells in the pituitary (the gonadotrophin producers) during ageing in rats, guinea pigs, and humans (reviewed in Bourne, 1967).

(iii) Thyrotrophin

Thorough investigation of the thyrotrophin content of the pituitary during ageing has not yet been made, although the one study (Blumenthal, 1954) implies a decrease after the age of 40 years in humans. A decrease in thyrotrophin output would be consistent with the often observed decline of thyroid gland activity (Wilansky et al, 1957; McGavack and Seegers, 1959).

(iv) Adrenocorticotrophin

Blumenthal's study indicates no change in ACTH content of human pituitary with age (Blumenthal, 1954).

In sum, there is no evidence for pituitary failure during senescence. The changes in hormone synthesis which may occur are selective (increased gonadotrophin, decreased thyrotrophin): synthesis of other pituitary hormones may not be much affected (adrenocorticotrophin, growth hormone).

(f) Hair

Careful measurements of the rate of hair growth reveal regional and sex differences in humans of any age (the longest hairs, in general, grow the most rapidly) and similarly specific changes in the rate of growth during ageing (Meyers and Hamilton, 1961). Thus, hairs on the crown of the scalp and in the eyebrow grow at the same rate in all ages. However, hairs of the chin (in men), axilla, and thigh grow at a progressively slower rate (in that order) during ageing.

Axillary hair, a secondary sexual characteristic, was found to be roughly proportional in amount to the output of urinary ketosteroids in men of any age (Hamilton, 1961). The mass of axillary hair declines sharply at the age of menopause and appears to be associated with the decline in women of ovarian secretions. In men, axillary hair declines more gradually (Hamilton, 1961).

Age was found to affect hair regrowth in mice. As in humans, there are regional differences in rate of hair growth (Whiteley and Horton, 1962). A delay of 3-54 days occurred in senescent mice before the new hair made its first appearance. The subsequent growth of the new hair was slower than in younger animals (Whiteley and Horton, 1962).

(g) Finger nail growth

Various studies describe the gradual decline with age in the rate of finger nail outgrowth in humans (Knobloch, 1951; Hamilton et al, 1955; Hillman, 1955; Orentreich and Sharp, 1967). During a 20 year period, ages 32-52, Bean made consecutive measurements on the rate of outgrowth of his own thumbnail and recorded a progressive decline (Bean, 1963).

(4) Conclusions

The effect of age on protein synthesis demonstrates once again specificity and selectivity. The production of a number of proteins is not altered during ageing (cytochrome c in the liver, elastin in the aorta, hairs of the eyebrow, etc.). Other proteins are produced at a lesser rate

(finger nail, collagen of the uterus, ptyalin of saliva, hair of the axilla, etc.). Still others may be produced in greater quantity during ageing (e.g., gonadotrophin of women). Protein synthesis is well known to be ultimately dependent on genomic activity. It thus seems exceedingly plausible to consider that the selective age changes in cell composition and production of proteins represent selective changes in the activity of genes, although the published data available at this time do not directly demonstrate this. General failure of the mechanisms through which genomic information is realized would have been manifested in these various data.

6) Growth processes not involving cell proliferation

a) Dietary shifts

Enzyme activities were followed in liver, kidney, and heart during a three week protein free dietary regime and during subsequent protein refeeding in rats of different ages (Barrows and Roeder, 1961). The content per cell of most enzymes (succinic dehydrogenase, pyrophosphatase, D-amino acid oxidase, and pseudocholinesterase of the liver; succinic dehydrogenase and alkaline phosphatase of the kidney) decreased markedly during protein depletion; after restoration of protein to the diet the levels of these enzymes rapidly returned to control values. Other enzyme activities (alkaline and acid phosphatase and cathepsin of the liver;succinic dehydrogenase, pyrophosphatase, and cathepsin of the kidney; succinic dehydrogenase of the heart) did not change markedly during the dietary shifts. The effect of dietary changes on enzyme activities may result from changes in either the rate of enzyme synthesis or degradation as was so elegantly shown by Schimke (1964) in his study of rat liver arginase. The differential effects of dietary change on enzyme activities studied by Barrows and Roeder (1961) are therefore the result of precisely regulated shifts in regulatory machinery which may be presumed to be ultimately under the control of the cell nucleus. This is a particularly favorable situation in which to study the effect of ageing on the regulation of cell activities.

No significant differences between young and senescent rats were found in the patterns of change of any enzymes during protein depletion and repletion, with the possible exception of liver cathepsin. The activity of this enzyme was generally higher in senescence during the period of change.

The results of Barrows and Roeder (1961) show that the control mechanisms governing enzyme levels do not appear to be lost during ageing. They also demonstrate that the ability of the liver and kidney to rapidly restore depleted enzyme activities is not altered. These events probably derive from changes in the rate of biosynthesis as well as in the rate of degradation. The exact role of synthesis and degradation in enzyme regulation must be determined for each enzyme. In any case, the differential control of enzyme levels does not appear to be lost during ageing.

b) Myocardial hypertrophy

Increased cardiac workload, e.g., through exercise or hypobaric stress, results in a hypertrophic growth of the heart, particularly manifested by increase in weight of the ventricular muscle. Such changes also result from mitral and aortic stenosis or from severe anemias. The increase in muscle is due to massive synthesis of muscle proteins directed by the myocardial cells.

Ventricular hypertrophy stimulated by hypobaric stress in mice (Swigart, 1969) or by a constricting aortic annulus in rats (Hügin and Verzár, 1956) was found to proceed on an identical time course and achieve the same final percentage increase of mass during senescence as in younger ages.

c) Neuromuscular relationships

Skeletal muscle cell activity depends on the neuromuscular relationship. Unlike the muscle of the heart, which shows no loss of function following denervation (Cannon, 1932; Donald and Shepherd, 1963), the skeletal muscles of the mammal depend on constant contact with their afferent neuronal connections, without which they quickly atrophy (Tower, 1935). So precise is this dependency, that switching motor nerves of slow and fast muscles results in a corresponding reversal of the muscle cell electrical (Buller et al, 1960; Close, 1965) and histochemical (Van der Meulen and Romanul, 1966) characteristics. Thus, the specific pattern of genomic activity which characterizes slow or fast skeletal muscle requires the constant support of the specific type of nerve cell. Studies also indicate that there may be an actual transport of molecules from the axon tip, across the motor end plate, and into the skeletal muscle cell itself (Korr et al, 1967).

Recent studies have provided some evidence indicating that age changes in skeletal muscle may be the result of a change in neuromuscular relationship. Following denervation of skeletal muscle, the course of reinnervation of intramuscular nerve bundles, the restoration of muscle protein mass, and the final recovery of motor function is delayed and reduced in 24 month old rats as compared with 1 month old rats (Drahota and Gutmann, 1961). Other evidence for a decreased vigour in neuromuscular relationships with ageing comes from experiments in which skeletal muscle is directly stimulated by electrical impulses. In intact young skeletal muscle there is a rapid increase in glycogen, protein, and RNA content within a few hours; no increase occurs in denervated young or in intact old skeletal muscle (Drahota and Gutmann, 1963).

Certain histological age changes in skeletal muscle resemble the effects of denervation (see table XIII). The incidence of such changes appears to be general in older populations of several species. Berg and associates (1962) consider that the changes which they observed are pathological and are not a true feature of the ageing process. This opinion must be given considerable weight because of the great knowledge of degenerative diseases of ageing rodents which Berg and Simms have acquired. Elderly rats of their colony became quite paralyzed; muscular dystrophy was considered a major, terminal cause of death (Berg, 1956). However, muscular paralysis is not a regular feature of ageing in mammals, whereas most of the changes in mechanical strength, psychomotor reactions, and histology are.

It should be recognized that these histological age changes do not occur uniformly in all cells of an old muscle, but appear to represent random degenerations of motor unit endings which affect only occasional muscle cells (Gutman and Hanzlikova, 1965). Support for this conclusion may also be derived from the distribution of myofibrillar sizes in voluntary eye muscles of elderly humans: there is a slight decrease in total number of myofibrils and an increase of average myofibrillar size. This latter change has been interpreted as due to compensatory hypertrophy of those myofibrils retaining complete function (Bucciante and Luria, 1934). A decrease in the number of myofibrils of the soleus muscle of old rats has also been observed (Gutman and Hanzlikova, 1965). It is not possible at

Table XIII

Comparison of Skeletal Muscle Atrophy Resulting from Denervation, Disuse, and Senescence

	Denervation atrophy	Senile atrophy	Disuse atrophy
loss of weight of muscular tissue	yes	yes	yes
altered structure of motor end plate (granular fragmentation, loss of	yes Savay and Szillik, 1956	yes Gutmann and Hanzlikova, 1965	no Gutmann and Zak, 1961
proliferation of nuclei in rows within the sarcolemma	yes Tower, 1935	yes Berg, 1956; Rubinstein, 1960 Gutmann and Hanzlikova, 1965	
reduced difference in end plate size between fast and slow muscles	yes Drahota and Gutmann, 1963	yes Gutmann and Hanzlikova, 1965	
increase of Cl and Ca decrease of K and acid sol. P	yes Hines and Knowlton, 1937	yes Lowry and Hastings, 1952	
loss of cross-striations in some myofibrills	yes Tower, 1935*	yes Bucciante and Luria, 1934 Berg, 1956 Rubinstein, 1960	

this time to weigh the contributions of actual nerve cell loss as distinguished from decreased neurotrophic support in the age changes in skeletal muscle. However, it is clear from table XIII that the age changes in muscle histology cannot be satisfactorily accounted for on the basis of disuse atrophy which might be a consequence of the decrease in locomotor activity with age. The fact that these changes are found in the voluntary muscles of the eye (Bucciante and Luria, 1933 and 1934; Rubinstein, 1960), an organ used constantly throughout life, would seem to minimize the possible contribution of disuse atrophy.

7) Cell proliferation and ageing

a) Introduction

(1) General comment

Decline of body size and weight of various organs has long been considered a usual aspect of ageing. Such changes have been surveyed in section IIIC2; it appears that they may occur more generally in humans than in various small mammals of apparently equivalent gerontological age. Nonetheless, loss of individual cells or groups of cells is a frequent event throughout the lifespan of any organism. The process of cell replacement is therefore of utmost importance to an ageing organism whose declining physiological vigour potentiates accidents and injuries which require immediate proliferative responses for the healing of wounds, etc.

(2) Circadian organization and ageing - a key technical consideration

Well conceived studies of cell proliferation for any age group must be attentive to the nychthemeral variations in the mitotic index (see Kiljunin, 1955, for early references; Bullough, 1949) and DNA synthesis index (Pilgrim et al, 1963) which occur in many tissues. However, in studies of ageing, strictly scheduled data sampling may not be sufficient: there is evidence that phase relationships in the circadian organization of certain activities become shifted during ageing.

In a beautifully designed study, Richter (1921) showed that rats become progressively more nocturnal in their pattern of spontaneous activity from early life through senescence. Rats of different ages were trained to eat their daily ration within 25 minutes at a standard time with a regular schedule of light and dark. The post-prandial peak of spontaneous

activity occurred later with increasing age; it finally disappeared altogether in senescent rats. Another aspect of this shift of phase relationships is the ratio of nocturnal to day-time activity which was found to increase throughout life in rats up to 24 months of age.

Bullough (1949) discovered that mitotic activity in the ear epidermis of mice is an inverse function of total spontaneous activity, occurring least in the quiescent immature and senescent mice of two strains and most vigorously in active, mature mice. Observations of the activity cycles of mice at any age revealed that the mitotic index is greatest during rest or sleep and least during periods of high activity. In agreement with the age changes in the nychthemeral distribution of spontaneous activity found by Richter (1921) (a study Bullough did not refer to), Bullough also noted that cycle of the mitotic index has a different timing in ageing mice of Strong's CBA strain: the peak of mitotic activity occurs at a progressively earlier hour during middle age. However, in Kreyberg's white label strain, the timing of the cycles of the mitotic index did not vary from maturity through senescence. In both strains of mice, the amplitude of the nychthemeral cycle of mitotic activity decreased after maturity. Falzone et al (1967) have also stated that daily pattern of mitotic activity in the ileal crypts of rats changes with age: in old rats, the peak activity occurs four hours later than in young rats. The patterns of mitotic activity have parallel contours and are otherwise superposable.

Various studies by Halberg and associates have included some groups of old mice. Phase shifts in the nychthemeral rhythm of serum corticosterone levels may possibly occur in old mice (Halberg et al, 1959). Temporal dissynchrony from animal to animal may also increase with age in the nychthemeral rhythm of rectal temperature (Halberg et al, 1955). The amplitude of the nychthemeral cycles of serum corticosterone (Halberg et al, 1959), circulating eosinophils (Halberg et al, 1953), and rectal temperature (Halberg et al, 1955) was found to decrease with ageing in mice. Studies of urinary steroid excretion also reveal decreased amplitude of nychthemeral variation; phase shifts may occur as well (Pincus et al, 1954).

It would be of considerable interest in itself if changes in circadian organization are generally found to occur during ageing. This matter could

also have great bearing on all studies intending to elucidate age differences in biosynthesis or any aspect of the organism which is subject to nychthemeral variation. Sampling such an activity at a single time point might well not represent homologous parts of a cycle of activity in animals of different ages.

b) Rates of cell renewal and the cell generation cycle

Certain cell types, in particular neurons, myocardial cells, and oocytes, as will be discussed in Section IIC9, never proliferate after a certain point in the growth of the mammal during the normal course of events. Other cell populations in skin, intestinal epithelia and liver, for example, are continuously replaced throughout the lifespan. Changes in rate of renewal of the intestinal epithelial cells during ageing is particularly well documented and will be considered next.

(1) The intestinal epithelium - model studies on a rapidly renewing cell population

The effect of ageing on cell renewal has been investigated with considerable detail in the epithelial cells of the duodenal villus of the mouse. Repeated measurements were made during at least one complete nychthemeron. Hence, the experimental observations are not subject to interpretive ambiguities arising from possible changes in circadian organization during ageing.

The intestinal epithelium undergoes continuous renewal (Leblond and Walker, 1956). Autoradiographic studies, from which most information about the renewal of the intestinal epithelium is derived, show that there is a procession of cells from the crypt of Lieberkühn past the crypto-villal junction towards the site of exfoliation at the tip of the villus in the small intestine (Hughes et al, 1958). The duration of the cell transit time from the crypt of Lieberkühn in the duodenum to the tip of the villus has been found to increase during ageing in mice: at three months, the process takes 41 hours; at twelve months, 48 hours; and at thirty-one months, 53 hours (Lesher et al, 1961a). No significant change in the length of the villus or its structure appears to occur with age. Both components of the duodenal transit time (crypt transit time and villus transit time) are lengthened during ageing. This suggests an age change in the progenitor cell cycle in the crypt of Lieberkühn of the duodenum. Definite age-wise increase of cell

transit time from the crypt of Lieberkühn to the tip of the villus has also been found in the jejunum (Fry et al, 1961), but not in the ileum (Fry et al, 1962).

Careful investigation of the kinetics of labelling of mitotic figures in the duodenal crypts over the span of two generation cycles after the injection of tritiated thymidine unequivocally showed an increased length of the cell generation cycle during senescence in mice (Lesher et al, 1961 b and c; Thrasher and Greulich, 1965 a and b). A recent study indicates that the lengthening of the generation cycle is not a linear function of age, but occurs in two stages, one at the onset of maturity (55-100 days) and the other at the onset of senescence (after 675 days): during most of adult life, the generation time is constant (Lesher, 1966).

Age changes in the generation cycle were also found in the colon (Thrasher, 1967) and in the ilium and jejunum (Lesher et al, 1961b) and in the epithelium of the descending colon (Thrasher, 1967). Furthermore, these same studies show (see Lesher, 1966, in particular) that the duration of the G2 phase and of mitosis appears to be closely similar in adult and senescent mice; the duration of the period of DNA synthesis was in general not found to change (Thrasher, 1967; Thrasher and Greulich, 1965 a, b), although a slight increase was found to occur in very old mice between 825 and 1050 days by Lesher (1966). Therefore, the major effect of ageing is a lengthening of the G1 phase (Thrasher, 1967; Thrasher and Greulich, 1965a; Lesher, 1966).

It is noteworthy that variations in the length of the generation cycle in other rapidly proliferating cell types have been primarily attributed to variations in the length of the G1 phase (Young, 1962; Cameron and Greulich, 1963). On the other hand, the duration of DNA synthesis appears to be closely similar in most rapidly proliferating cell types (Koburg and Mauerer, 1962; Cameron and Greulich, 1963), and as the studies of ageing in intestinal epithelia indicate, may be constant throughout most of adult life.

The mitotic index of the duodenal crypt was also observed to decrease by about 15% during senescence (Thrasher and Gruelich, 1965a). Changes of mitotic index have often been considered to indicate changes in the number of proliferating cells. However, the number and distribution of actively

proliferating cells within the crypts of Lieberkühn were found to be constant throughout adult life (Thrasher and Greulich, 1965b). Thus, the declining mitotic index would appear to be a consequence of the increase in cell generation time*, rather than a consequence of any hypothetical change in the percent of cells undergoing replication. This conclusion may in the future prove to be the proper account of the declining mitotic indices in other tissues of ageing organisms. Because of this problem in interpreting changes in the mitotic index, a tabulation of such age changes will not be given.

Another effect of ageing on cell proliferation of the intestinal epithelium was the increased heterogeneity in length of generation cycle of the labelled cohort of cells as it completed the first generation after labelling and entered the second generation (Lesher et al, 1961c; Thrasher and Gruelich, 1965a; Fry et al, 1966). Statistical analysis (Fry et al, 1966) indicates that one component of the increased variance is a greater variance in the distribution of the durations of the DNA synthesis phase; another component may result from increased interanimal differences during ageing, other examples of which were considered in section IIIB5.

(2) The oral epithelium

One study indicates, on the basis of the number of labelled cells found by autoradiography at a single time point after the injection of tritiated thymidine, that the number of progenitor cells per unit length decreases during senescence in the epithelia of the palate and tongue of the rat. However, increases in the fraction of cells which incorporated the tritiated thymidine during this period of time appear to compensate for the decreased number of progenitor cells, so that an approximately constant rate of cell turnover may be maintained in the tissue throughout life (Sharav and Massler, 1967).

*If the event of mitosis requires the same amount of time and the total cell cycle is longer, then the fractional part of the cell cycle occupied by the events of mitosis will be smaller. In a cell population of the same density, the number of mitosis observed per unit time would therefore be reduced.

(3) The alveolar epithelium

An autoradiographic study of alveolar epithelial renewal in three strains of mice indicated that the mitotic index, measured at a single standardized time, did not decrease with age in the A or Gr_b/A strains, whereas it decreased in old C57Bl mice (Simnett and Heppleston, 1966). The mean generation time, estimated from the mitotic index, increased in the C57 strain, but did not change in the A or Gr_b/A strains. The duration of DNA synthesis was calculated according to the labelling index method, a method subject to certain qualifications (Lesher et al, 1961c; Thrasher and Greulich, 1965a) which is notably less accurate than direct measurement of the kinetics of labelled metaphase cells throughout the entire cell generation cycle. The calculated duration of DNA synthesis did not significantly change in the A strain of mice, but increased in the C57Bl strain. The analysis also indicates sex differences in the duration of DNA synthesis at all ages, a conclusion which is in conflict with numerous studies showing the constancy of the duration of DNA synthesis in a wide variety of proliferating cell types.

(4) Antibody forming progenitor cells of the spleen

The injection of spleen cells into an isogenic, heavily irradiated host results in their rapid proliferation. The growth of this population of cells can be assayed by challenging them with antigens at various times after injection. Under precisely defined circumstances, the amount of antibody formed measures the size of the population of antibody forming progenitor cells from the original spleen (Perkins et al, 1961; Brown et al, 1966). Employing this technique, the rate of growth of antibody forming progenitor cells from spleens of 2- or 92-week old mice was found to increase logarithmically and at closely similar rates in a 2 week old host over a period of four weeks (Albright and Makinodan, 1966). Thus, the time required to complete the generation cycle of splenic antibody forming progenitor cells is not affected by age. This result is corroborated by the similar rates of DNA and RNA synthesis of short term, in vitro cultures of bone marrow cells from senescent or young rats (Menzies et al, 1967).

(5) Hemopoeitic tissue

The renewal of erythrocytes during ageing has been evaluated in studies of the rate of loss of injected Cr^{57} erythrocytes. No age changes were found in humans into their ninth decade (Miescher et al, 1958; Hurdle and Rosin, 1962) or in senescent rats (Mende, 1965). Assuming steady state kinetics, this absence of age change of erythrocyte turnover rate implies that the average rate of production of erythrocytes is unaltered during ageing in rat and man. This conclusion is consistent with observations that the rate of DNA synthesis in short term cultures of rat bone marrow cells does not change with the age of the donor (Menzies et al, 1967), and with observations that the rate of uptake of Fe^{59} into erythrocytes does not change between full and late maturity in mice (Yuhas and Storer, 1968).

Exposure of rats to low oxygen pressure (350mm Hg) for 10 days provoked the same increase in blood hemoglobin of 3-20 month old rats (Verzár and Flückiger, 1954). Thus, the hemopoietic response of the bone marrow is not diminished during the same period of life in the rat during which there was a dramatic loss of the ability to regulate body temperature during hypobaric stress by late maturity in rats (Verzár and Flückiger, 1954). This study also provides a good example of the differential impact of age on physiological regulatory mechanisms, as well as implying the absence of change in the ability to mobilize additional erythrocyte progenitor cells during ageing.

The increased uptake of tritiated thymidine in the absence of an increased mitotic index in senescent mice (Post and Hoffman, 1965; Falzone et al, 1967) could be interpreted as an increase with age in the size of the sub-population in an extended G2 phase.

The cells in extended G2 phase would be tabulated in any index of nuclear size or DNA content as being tetraploid. With a mitotic stimulus, these cells would return to their typical diploid DNA value; a true tetraploid or octaploid cell would not divide without first doubling its DNA content. The fact that octaploid cells are not found to incorporate tritiated thymidine after the age of 16 months in mice, although they do so up to at least 6 months of age in mice (Post and Hoffman, 1965) might imply an age change of the ability of octaploid cells to replicate themselves.

(6) The epidermis

The constant exfoliation of the epidermis implies a highly active rate of cell renewal. Ageing changes in this process have been known chiefly through the mitotic index which is said not to change during adult life in man (Thuringer and Katzberg, 1959) and in rat (Kiljunen, 1955). Age changes in the mitotic index of the epidermis of mice are currently in dispute: a decline has been observed by Bullough (1949) and Whitely and Horton (1963), whereas Bertalanffy and associates (1965) have measured an increase.

A single autoradiographic study on human skin found no relationship between renewal time of the entire germinative, prickle, and granular cell layers and age (22-83 years) (Epstein and Maibach, 1965). Thus, ageing may not affect the cell generation cycle in all tissues.

(7) The liver

The liver under normal circumstances contains a stable, slowly renewing population of cells, with a turnover time which has been estimated at 160 to 400 days in the rat (Swick et al, 1956; McDonald, 1961). The mitotic index appears to approach a minimum value of less than 0.2% in mice six months old and remains at this low level throughout the remainder of life (Post and Hoffman, 1964).

The spectrum of stages of the generation cycle in liver cells of the adult mouse are known to include a group of cells with an extremely long G2 phase of at least 60 hours duration (Perry and Swartz, 1967). Subpopulations of cells in an extenuated G2 phase have also been identified in tissues of the chicken (Cameron and Cleffmann, 1964) and may serve as a reservoir of cells capable of immediately entering mitosis. Thus, the length of the cell generation cycle cannot be determined in the resting liver because of the presence of at least two sub-populations of cells.

c) Conclusions

Ageing has been found to act on the cell generation cycle with the same selectivity as in previously considered cell activities. The generation cycle or rate of production of cells may not be effected in all tissues (e.g., epidermis, antibody forming progenitor cells, erythrocytes): in those affected cell types, not all aspects of the generation cycle may be altered

(e.g., intestinal epithelia). Ageing thus differentially affects proliferating cells.

The unchanged integrity of the cell's ability to proliferate at all phases of adult life provides an exceedingly powerful argument that the regulatory machinery of the cell is not damaged during ageing. It is well recognized that the progress of a cell through its generation cycle occurs as the result of a succession of interlocked events in the cell nucleus and cytoplasm which are dependent on the expression of genomic information. The fact that certain phases of the cell generation cycle of intestinal epithelial cells are affected during senescence, whereas other phases are not, indicates that the regulatory machinery concerned with the cell cycle has been precisely reset and not damaged indiscriminately during the course of ageing.

8) Proliferative potential of dividing cells

Weismann conjectured long ago that the loss of cells in the body during ageing is the result of a limitation of the number of generation cycles which a proliferating cell line can complete (Weismann, 1891). Such a limit has been observed during the culture of diploid fibroblasts in vitro (Hayflick and Moorhead, 1965). Evidence for this limit and its possible bearing on the senescence of cells in the organism will be considered next.

a) In vitro studies of cell proliferation

Long term cultures of mammalian cells have now been known for over a half-century. Carrel's celebrated culture of fibroblasts from an embryonic chick heart was begun in 1912 (Carrel, 1912, 1914) and maintained for 34 years without loss of vitality (personal communication of A. H. Ebeling to Parker, 1961). This culture became a highly popular example of the potential immortality of the mammalian cell, which was likened at that time to Woodruff's culture of Paramecium aurelia (Section IIA1). However, it is possible that Carrel's long term culture did not actually represent an unbroken lineage from the original embryonic cells during the entire span of years. Hayflick (Hayflick, 1965) writes that Carrel's culture may have been occasionally repopulated with new embryonic fibroblasts as a consequence of the addition of nutrient which was prepared daily from chick embryos under conditions which could permit the survival of embryonic cells. Despite shadows which may be cast on the claim that Carrel's culture has one cellular lineage (Ebeling, 1922), it seems reasonable to suppose that it was indeed

a bona fide serial culture of cells for intervals of sufficient length to justify its recognition as the first true long term cell line. Subsequently, other long term cell lines have been established (Gey and Gey, 1936; Earle, 1943), which provide incontrovertible proof that cells of mammalian origin can proliferate rapidly in vitro for an indefinite period. At present, several hundred such long term cell cultures have been described (Hayflick and Moorhead, 1962).

A property of most long term cell lines is their aneuploid karyotype. Unlike the diploid cells of the tissue from which they may have been derived, almost all established cell lines have been shown to be heteroploid (Levan, 1956; Hsu et al, 1957; Westwood and Titmuss, 1957; Chu and Giles, 1958; Ruddle et al, 1958).

Reports of rapidly proliferating long term diploid cell cultures have appeared (e.g., Tjio and Puck, 1958; Ferguson and Wansbrough, 1962; Capstick et al, 1962; Yeganian et al, 1965). However, there is always doubt that the karyotype was actually euploid or that the age of the culture was sufficient to establish its permanency. With regard to the evaluation of karotype, a culture of rabbit skin fibroblasts maintained by Puck for 500 generations proved to be pseudodiploid (Puck et al, 1966) as did the culture of baby hamster kidney fibroblasts (established by Capstick et al, 1962) upon examination after 110 generations by Hughes (1968). It is possible that rigidly controlled cell lines directly derived from the Chinese hamster, which proliferated for over two years ($\geq$150 doublings) without a detectable change of karyotype (Yerganian and Leonard, 1961; Yerganian et al, 1965) are true exceptions to this trend.

It has frequently been observed during attempts to establish long term cell lines that the cells cultured from the initial explant may proliferate steadily for a period of time. At some point, growth of the initial, "short term" culture begins to wane and eventually ceases. The time at which this occurs may be influenced by the tissue from which the cells were derived (Yerganian et al, 1965). At this critical point, the cell line may be lost. Alternatively, vigorously growing cells may appear in the cultures unifocally or multifocally. If this event, known as "transformation", occurs, then the newly arisen proliferating cells will rapidly dominate the cell population of the old short term culture and, in fact, some cells may be able to proliferate indefinitely on serial

culture. These cells and their descendents comprise a "long term" cell line (see the excellent review of Davidson, 1964) and are usually aneuploid.

The interval between the initiation of a short term culture and the first appearance of transformed cells reveals that the transformation generally occurs between 1 and 4 months after the culture was begun (tabulated in Davidson, 1964). This implies that the proliferative potential of the diploid cells of the original explant was finite. Hayflick has investigated the proliferative potential of human diploid cell lines in great detail (Hayflick and Moorehead, 1961; Hayflick, 1965). These fine studies show that onset of the phase of declining proliferation occurs after a characteristic number of serial passages in vitro. A definitive maximum number of passages, after which the proliferation of diploid cells diminishes, has also been found by other groups (Todaro et al, 1963; Yoshida and Makino, 1963; Hay, 1966; Uren et al, 1966; Levine et al, 1967). The number of divisions before proliferation in the culture begins to decline is given in table XIV. Among diploid cell strains isolated from human lung, a prominent feature is that the cells of fetal origin give rise to cells with a greater potential for proliferation than cells derived from adult tissues. Hayflick (1968) states that the potential for proliferation is 50 generations during fetal life, is reduced to 30 generations in children, and finally to 20 generations in the adult. Apparently, fibroblasts of all ages of adult humans (26-87) have the same proliferative potential (Hayflick, 1965). Hayflick has placed particular emphasis on the decrease in proliferative potential as providing an in vitro indication of the progress of ageing in the donor. Of paramount importance in evaluating Hayflick's argument is the fact that there is no further loss of proliferative potential after adulthood is reached: if the proliferative potential did mirror the process of senescence in the whole organism, one would have expected to find a marked diminution of proliferation in cells from the 80 and 87 year old donors.

Another feature revealed in table XIV is that the doubling potential does not appear to be related to the expected species lifespan. Thus, fibroblasts from the adult Chinese hamster can sustain more than 150 divisions, nearly 8 times that of the cells from adult humans. In contrast, the lifespan of the hamster is about 1/25th of the human. Similarly, the interval between the initiation of a culture and the occurrence of transformed cells

Table XIV

Selected Tabulation of the Doubling Potential of Diploid Cells

Species	Tissue of origin	Age of donor	Cell generations	Cell type	Species lifespan	Reference
human	lung	fetus	35-55	fibroblast	70 years	Hayflick & Moorhead, 1961
"	"	fetus	35-63	"	"	Hayflick, 1965
"	"	26 years	20	"	"	"
"	"	58 "	14-16	"	"	"
"	"	61-69 "	21-24	"	"	"
"	"	80 "	18	"	"	"
"	"	87 "	29	"	"	"
"	adrenal, bone, conjunctiva, skin, thymus	fetus	10-40 (average 20)	"	"	Levine et al, 1967
wallaby	buccal mucosa	adult	40-50	fibroblast	15 years	Uren et al, 1966
chinese hamster	lung	adult	150	"	3 years	Yerganian et al, 1965

is 3-4 months for rat eye connective tissue (Davidson, 1963), 2-3 months for horse liver (Umeda et al, 1961), 1-1.5 months for monkey kidney (Westwood et al, 1957) and about 1-3 months for various human tissues (Hayflick, 1961; Cruickshank et al, 1960; Chang, 1961). Although the number of cell cycles before the declining phase occurred cannot be accurately estimated from these values, it is clear that there is no strong correlation between the lifespan of a mammalian species and the proliferative potential in vitro of diploid cells derived from it.

The general characteristics of diploid cells during their declining phase prompted Hayflick (1965) to consider them as undergoing a senescent involution in vitro, which, he proposed, was related to senescence in vivo. Arguments have already been presented against Hayflick's presumption that the proliferative potential of diploid cells in vitro was related to the progress of senescence in the donor or to the length of life of the species. Experimental investigations of the properties of diploid cells during their decline in vitro shows that these changes are not paralleled by changes in cell function during ageing in vivo.

Autoradiographic study of exponentially growing diploid fibroblasts (WI-38, human fetal lung origin) showed that the cell generation cycle occupied 18 hours during the early phase of culture and over 25 hours during the declining phase (Macieira-Coelho et al, 1966). Analysis of the time course of labelling of metaphase figures indicated that the duration of the period of DNA synthesis was similar in declining and early cultures (8 and 6 hours, respectively). The increased length of the cell generation cycle was attributed mainly to a longer G2 phase (13 hours vs 5 hours, respectively). This marks one difference with senescence in vivo: in the rapidly proliferating cells of the intestinal epithelium, G1 was increased during senescence, not G2 (see section IIIC7b1).

The pattern of RNA synthesis of diploid cell cultures was observed to change drastically during their decline (Levine et al, 1967). RNA (20' labelling time) on the basis of autoradiography was found to be synthesized predominantly in the nucleus in early cultures, but predominantly in the cytoplasm in declining cultures. This singular change might represent an increase of RNA synthesis in mitochondria or the proliferation of a cytoplasmic virus. Analysis of lysates of whole cells showed that the RNA (synthesized in early diploid cultures (20' labelling time) had a major

45S component upon sucrose density gradient centrifugation, whereas the RNA synthesized (20' labelling time) in declining cultures had its major component at 18-20S. Only a slight amount of 45S RNA was found in declining cell cultures. Mixtures of extracts of early and declining cell cultures gave additive patterns on the gradient, indicating that degradation by nucleases is not a likely cause of the change in pattern of RNA synthesis. The total amount of incorporation of isotope after 20', 3 hours, or 24 hours labelling was markedly less in declining diploid cell cultures; this probably represents a true decrease in the amount of RNA synthesis. In agreement with the finding that little 45S RNA (the ribosomal RNA precursor) is labelled by a 20' pulse in declining diploid cell cultures, the total incorporation of labelled precursor into 20S ribosomal RNA was decreased by 80%. Furthermore, the total ribosomal RNA per cell was reduced by at least 50%. A major change in the pattern of RNA synthesis thus appears to occur during the decline of diploid cell cultures according to these workers. However, no alteration in the site of RNA synthesis was detected by Macieira-Coelho et al (1966), although the rate of passage of RNA from the nucleus to the cytoplasm was retarded and the total amount of RNA synthesis decreased.

Whatever the cause of these unusual changes, they do not appear to occur during the usual course of mammalian senescence. There is no evidence for alterations in the gross pattern, the rate, or the site of RNA synthesis, nor is there evidence that the rate of passage of RNA from the nucleus to the cytoplasm changes.

Another parallel frequently drawn between declining cultures of diploid cells and the cells in ageing mammals is based on the increase in chromosomal abnormality during ageing in vitro and in vivo (Hayflick, 1965). It is well known that heterploid cells frequently appear in or dominate declining diploid cell cultures (Westwood and Titmus, 1957; Saksela and Moorhead, 1963; Yoshida and Makino, 1963; Uren et al, 1966; Simons, 1967). Abnormal mitotic figures are also seen in recent (e.g., Ford and Yerganian, 1958; Levan and Biesele, 1958; Kleinfeld and Melnick, 1958) or longer standing primary cultures (Sax and Passano, 1961). The counterpart of this in vitro increase in deviations from euploidy and normal mitotic pattern may possibly be represented first by the increased aneuploidy of cells in cultures of peripheral leukocytes with increased age of sheep (Bruere, 1967) and humans (Jacobs et al, 1961, 1963; Court Brown et al, 1966) (although it has been

claimed that no such change occurs (Kleisner de Galán, 1961)), and increased aneuploidy in bone marrow cells of ageing guinea pigs (Hughes, 1968); second by the increased abnormal mitotic figures in regenerating liver of ageing mice (Stevenson and Curtis, 1961: Crowley and Curtis, 1963) and ageing dogs (Curtis et al, 1966; Curtis, 1966); third by the increasing incidence of certain types of malignancy (cells of malignant growths are nearly always aneuploid) with age (e.g., Nathan, 1924; Simms, 1945; Saxton, 1952; Jones, 1956); these considerations would seem to add renewed force to the argument that ageing of cells in vitro is a proper model for ageing of cells in vivo.

However, certain key facts do not permit this conclusion. In the first place, the increased aneuploidy of cultures of peripheral blood cells from ageing humans is now known to involve variations in a limited number of the 22 chromosome pairs: it is strongly suspected that all of the aneuploid cells are XO with a complete autosomal complement (Jacobs and Court Brown, 1966; Court Brown et al, 1966). This stands in contrast to the aneuploidy of long term cell cultures which include extremely diverse karyotypes. Moreover, Court Brown and his associates have shown that the onset of aneuploidy is 10 years earlier and the final extent of its increase about 3 times greater in women than men. Yet women are longer lived than men. Second, the same increase of mitotic abnormalities seen in regenerating livers of ageing mice can be produced by irradiation with doses of radiation which do not significantly shorten the lifespan of mice (Curtis, 1963). Thus, mitotic abnormalities per se have little to do with the residual length of life in vivo. Third, the incidence of many types of malignancy declines after a certain age (e.g., pituitary adenoma of rats (Simms and Berg, 1957); cancer of the gastrointestinal tract, cervix, and lungs of humans (Saxton, 1952). In sum, the changes in the activities of serially cultured diploid cells during the course of time bear no clear relation to those changes, more properly called senescent, which occur in the cells of a mammal during the course of its life. The proliferative potential of diploid fibroblasts in vitro does not change during adult life (Hayflick, 1968), in corroboration of the evidence already considered from the regeneration and hypertrophy experiments. The evidence concerning possible limitations on the number of cell generations of proliferating tissues in vivo will next be considered.

Two approaches have been used to investigate the proliferative potential of cells in an organism during the course of life. In the first category, the cell type is challenged to proliferate, either in animals of different ages or upon transplantation to a suitable host of another age. In the second category, attempts are made to exhaust the proliferative potential either by successive challenges in the animal throughout life or by transplantation through a series of hosts. Such experiments provide information about the proliferative potential of various tissues and about the size of the progenitor cell compartment as a function of age.

b) The liver

The liver is a tissue whose cells are apparently capable of regenerating an unlimited number of times. The differentiated parenchyma of the liver synchronously prepare for division if a sufficient stimulus is applied (Bucher and Swaffield, 1962). Repeated removal of 2/3 of the liver tissue has been performed once a month for twelve consecutive months in adult male rats, but no exhaustion of the potential for regeneration was found. The twelvefold regenerated livers were histologically normal and not neoplastic, although the liver weight was 80% of livers in sham operated rats one month after the twelfth and final hepatectomy (Ingle and Baker, 1957). There is no mention of any liver abnormality at the end of this period of strenuous proliferation. On the basis of the liver mass restored after each hepatectomy, a simple calculation shows that the original liver cells were caused to divide at least (1.5x 12) or 18 times. This is approximately the upper limit to the number of divisions which human lung fibroblasts can complete in the euploid state (Hayflick, 1965). If such a limit was being approached during the course of repeated regeneration, abnormalities of cell function similar to those which occur in declining diploid cell cultures in vitro, including neoplastic tendencies, would have been expected, but in fact did not appear to arise.

c) The intestinal epithelia

Daily exposure of rats to small doses of radiation is known to accelerate proliferation of the intestinal epithelia to a new, constantly maintained rate (Lamerton, 1966). This is a compensatory reaction to replace radiation damaged cells of the intestinal mucosa (Lesher, 1966) and occurs in other proliferating tissues as well, e.g., the bone marrow (Lamerton, 1966).

Irradiation of mice with 12R/day from a ^{60}Co source for 10 consecutive days was found to reduce the generation time, principally by contracting the G1 phase, to the same final number of hours in 3, 13 and 27.5 month old mice (Lesher, 1966). This is an important observation; it demonstrates that the ageing intestinal epithelia can produce a compensatory hyperplasia, but also that the response is greater in the older animals, whose cell cycle was originally the longest. Furthermore, mice continuously exposed to low doses of irradiation from 3 to 10 months of age have a generation cycle that is 20% faster than non-irradiated mice of the same age (Lesher et al, 1961d). Thus, the accelerated generation cycle can be maintained for extended periods of time. Therefore it seems highly unlikely that the reduced rate of proliferation of the rat's intestinal epithelia during ageing is the result of incipient exhaustion of the progenitor cells, or that such an exhaustion of progenitor cells would occur within the lifespan of the rat. This latter possibility is also rendered unlikely from the example of the European eel whose normal life limit can be extended at least six fold (Section IIB2c). The fact that the natural lifespan of the eel can be extended by at least 75 years, during which time the eel continues to feed actively (Lekholm, 1939), strongly implies that the progenitor cells of the eel's intestinal epithelia were not exhausted at the time of its natural life limit.

d) The epidermis

Strehler and co-workers (Strehler, 1967) have attempted to evaluate the proliferative potential of the cells in the human epidermis. Autoradiographic studies on the distribution of labelled cells in the basal and spinous layers of the epidermis at various times after local injection of tritiated thymidine support a model of proliferation in which the migration of basal cells after division is random (Epstein and Maibach, 1965; Strehler, 1967). As the germinative layer of human epidermis is renewed each 4-8 days (Epstein and Maiback, 1965), it must be duplicated 50 times each year and 3500 times during the course of human life. If post-divisional cells migrate randomly, then this means that the non-migrating cells will undergo an average of 3500 divisions during the human lifespan. This number is in almost 200 fold excess of the maximum number of divisions which diploid human fibroblasts can sustain in vitro according to Hayflick (1965).

e) Hemopoeitic tissue

Swigart (1969) has observed that hemopoeisis induced by hypobaria occurs in the liver as well as in the bone marrow and spleen of senescent mice. In fetal and immature mice, the liver is a site of active hemopoeisis. However, during and after maturation, hemopoeisis becomes restricted to the bone marrow and spleen, even with hypobaric stimulation. The reoccurrence of hepatic hemopoeisis during senescence could possibly imply a depletion of bone marrow progenitor cells.

The status of the progenitor cell population in the marrow of senescent mammals cannot be accurately inferred from histological studies. Progressively decreased marrow cellularity and increased fatty replacement of hemopoeitic tissue in bone marrow has been observed in the femora of ageing rats (Awaya et al, 1965) and ageing guinea pigs (Fand and Gordon, 1957), and in the rib and sternum (Custer and Ahlfeldt, 1931) and iliac crest (Hartsock et al, 1965) of ageing humans. These changes are said to develop less markedly with age in flat bones, e.g. ribs, as opposed to long bones, e.g. femora (Custer and Ahlfeldt, 1931). This is still another example of the differential effect of ageing on body cells. The loss of hemopoietic tissue may not necessarily be progressive during adult life (Yuhas and Storer, 1968). In fact, Plum (1941) found only slight decline in both the total cellularity and the distribution of cell types with advancing age after maturity in human sternal marrow (other references to such observations may be found in Plum, 1941, and Shapleigh et al, 1952).

Direct assay of size of the hemopoietic progenitor cell population can be made according to the technique of Till and McCulloch (1961), who discovered that bone marrow cells injected into lethally irradiated isogenic hosts grow as discrete nodules or colonies of hemopoietic tissue in the host spleen. The colonies, during their early growth phase, consist of pure erythroid cells (the predominant colony type), neutrophils, megakaryocytes, granulocytes, or eosinophils; lymphoid cell colonies are not found as discrete nodules in the red pulp (see tabulation of Curry and Trenton, 1967). The fact that single colonies can produce the entire spectrum of colony types upon injection into a second irradiated host (Lewis and Trobaugh, 1964; Curry and Trenton, 1967) is very good evidence that the progenitor cell of these colonies is pluripotent. The maintenance of the complete spectrum of blood cell types

throughout life (Shapleigh et al, 1952; Fand and Gordon, 1957; Grad and Kral, 1957; Talbot et al, 1965) again demonstrates the pertenacity of the complex mechanisms of cellular differentiation during ageing and senescence.

The number of progenitor cells (measured as splenic nodules without reference to cell type) was found to increase progressively from 3 to 18 months in mice and to decline thereafter in such a fashion that total number per femur was slightly greater at 24 than at 3 months (Yuhas and Storer, 1968). In agreement with this observation, the LD 50 (30) for these mice was found to be at its maximum at 16 months (Yuhas and Storer, 1968). As these authors point out, the correlation between the LD 50 (30) and the number of marrow progenitor cells of young mice (Porteous and Lajtha, 1966) is thus also validated for variations in progenitor cell number during ageing. The fact that the number of hemopoietic stem cells increases up to late maturity in these mice implies that the endowment of these cells is not fixed at birth, unlike the neuron, myocardial cell, or oocyte. On the other hand, the number of hemopoietic progenitor cells of the spleen, measured as total splenic nodules in the irradiated host, was found to decrease very gradually throughout life in mice (Albright and Makinodan, 1966).

Serial transplantation of bone marrow cells through lethally irradiated hosts can be accomplished up to a certain number of passages (usually about four) beyond which the hemopoietic recovery of the host does not occur (Barnes et al, 1959; Barnes et al, 1962; Koller and Doak, 1963, Cudcowicz et al, 1964; Siminovitch et al, 1964). Thus, under these circumstances, the hemopoietic progenitor cells are not capable of indefinite self-renewal. A parallel of serially passaged marrow cells to serially passaged diploid fibroblasts in vitro is thus suggested.

But is this a valid parallel? The fact that marrow cells passaged just once show a reduced ability to repopulate a second host spleen up to 6 months after the first transfer (Cudcowicz et al, 1964) means that the hemopoietic progenitor cell population probably cannot expand to its normal size under these conditions. The notion of a fixed endowment of hematopoietic progenitor cells which is drawn from steadily throughout life is not supported by the previously described measurements of Yuhas and Storer (1968). The fact that the number of progenitor cells increases until late maturity in intact mice (Yuhas and Storer, 1968) emphasizes the fact that exhaustion of the proliferative potential by serial passage may be due to altogether different causes

than those which result in the declining number of progenitor cells after late maturity. It may be found that the totally irradiated host does not provide the correct environment for the replenishment of the hematopoietic progenitor cell population, or that the act of grafting constitutes a harmful stress for these cells and may inactivate them. These possibilities arise from the fact that the kinetics of recovery of blood cell production are altogether different in auto-recolonizing experiments, in which part of a femur is shielded from radiation, than from exo-recolonizing experiments with grafted marrow. In auto-recolonizing experiments, there is a fast recovery and pronounced overshoot (Porteous and Lajtha, 1966), whereas in marrow grafts the recovery is greatly delayed and no overshoot is observed (McCulloch and Till, 1962; Lajtha, 1968). Until further information is available, e.g. from repeated autorepopulation experiments, no final statement can be made about the possibility of a neonatally fixed dimension of the hemopoietic progenitor cell population.

f) Lymphopoietic tissue

The vigour of immunological responses (e.g., response to foreign antigens, hypersensitivity reactions, homograft reactions, and recovery from immunological paralysis) has been generally found to decline with age in mammals (see notes of K. Landsteiner given by Cohen, 1942; reviews of Baumgartner, 1934; Albright and Makinodan, 1966; Stern, 1963; Ram, 1967; Walford, 1967a). General involution of lymphatic tissue throughout the body has long been noted, particularly in the case of the thymus gland (Hamar, 1921) to occur in advance of involution of other tissues, e.g., skeletal muscle (see reviews of Kurmbhaar, 1942; Andrew, 1952b; Cornes, 1965). In contrast, the unstimulated levels of γ -globulin have been found to be higher in senescent gerbils (Ringle and Dellenback, 1963), rats (Goullet and Kaufman, 1965), and humans (Das and Bhattacharya, 1961; Haferkamp et al, 1966; Cammarata et al, 1967). The size of the population of antibody producing cells is, however, not revealed by this contradictory evidence.

An outstanding series of investigations begun by Makinodan and his associates in 1959 has indicated that the declining ability to form antibodies during ageing in mice is closely paralleled by a decreased number of immunologically reactive spleen cells. Assay of antibody forming cells in spleens of different aged animals was made by testing the ability of spleen cells to produce agglutinins to heterologous erythrocytes in isogenic, lethally irradiated

host mice of one age (Perkins et al, 1961). This technique minimizes the effect of age changes in the physiological milieu, which might possibly alter the expression of antibody forming potential. Most important, there is a linear relationship between the number of injected spleen cells and the maximum level of antibody titre (Perkins et al, 1961). The actual fraction of non-primed spleen cells which react to the heterologous erythrocytes has been estimated from the distribution of all-or-none assay responses in successively smaller inocula to be about 10^{-6}, or 50-100 cells per young adult mouse spleen (Albright and Makinodan, 1966).

Employing these techniques, the reaction of spleen cells to a primary response was found to reach a maximum at 8 months in C31F1 mice and to subsequently decline exponentially throughout the remainder of life to 25% of its peak value by 30 months (Makinodan and Peterson, 1962, 1964). The efficiency of antibody production was judged to be unchanged with age, because the doubling time for antibody production was independent of age or peak titre. On the basis of the all-or-none assay it was concluded that the actual number of antibody forming cells, at least in 12 and 32 week old mice, is accurately represented by differences in agglutinin titres (Makinodan and Peterson, 1964). Direct assay of antibody forming cells in the spleens of different aged mice with the Jerne plaque hemolysin test in CBA mice has confirmed the exponential decline in number of antibody forming cells after a maximum at 7 months (Stjernswärd, 1966; Wigzell and Stjernswärd, 1966). Changes of the number of antibody forming cells in the spleen may be related to the decreasing mitotic index of phytohemagglutinin stimulated cultures of peripheral lymphocytes from humans of increasing age (Pisciotta et al; 1967).

The elimination of Pseudomonas and Salmonella from the mouse populations resulted in greater production of antibody per spleen cell which was revealed more clearly when rat rather than sheep erythrocytes were used as a test antigen. The improvement in antibody production with better health was small (15%) and did not alter the time course of the age change (Makinodan and Peterson, 1964), a fact which supports the validity of many earlier studies of ageing which employed animals whose health, in retrospect, is necessarily questionable.

Conventional stimulation of the antibody forming cells with injections of foreign erythrocytes produced a primary response which changed with age

in parallel to the antibody forming potential of spleen cells injected into an irradiated host in C3lF1 mice (Makinodan and Peterson, 1964) and in BC3F1 mice (Makinodan and Peterson, 1966a). It is of much interest that splenectomy was found to reduce hemagglutinin production to a greater degree in old than in young BC3F1 mice, especially with small inocula (Hanna et al, 1967). Thus, an increasingly greater fraction of the antibody forming capacity of ageing mice originates in the spleen. Evidently, age differentially affects lymphopoeisis in the various lymphatic compartments of the mouse.

Lifelong studies of the hemagglutinin titres in BC3F1 mice injected at the age of one month revealed a multiphasic response curve of four cycles, each lasting 16-24 weeks and with progressively decreasing amplitude. With increasing age, the number of mice synthesizing detectable hemagglutinins decreased; after 2 years, only 25% of the group had detectable hemagglutinin. (Makinodan and Peterson, 1966a). The absence of hemagglutinins in some mice provides another example of increased heterogeneity in an ageing population, although its meaning is obscure. The absence of cyclical age changes in the antibody forming potential of spleen cells injected into an irradiated young host implies that there may be long term physiological rhythms, approximately two per year, whose amplitude, like that of nychthemerally varying processes (discussed in Section IIIC7a2) decreases with age. The importance of the physiological environment is thus indicated in interpreting age changes in cell activities.

The type of antibody synthesized in response to heterologous erythrocytes has been found to change with age, based on observations of the sensitivity of hemagglutinins found in either the primary or secondary response to β-mercaptoethanol. Under controlled conditions, this agent will preferentially inactivate 7Sγ-blobulins, without substantially affecting the 19Sγ-globulins (Uhr and Finkelstein, 1963). The hemagglutinins formed by a conventionally elicited primary or secondary response in intact BC3F1 mice injected at the age of 6 mohths were far more sensitive to β-mercaptoethanol in senescent than in young mice (Makinodan and Peterson, 1966b). This implies that a different type of antibody producing cells may be involved in immunological responses of older BC3F1 mice. However, in CBA mice, Wigzell and Stjernswärd (1966) did not find any differential effect of age on 19S and 7S γ-globulins, which decreased in parallel.

Another aspect of age change in the character of the antibody forming cells is revealed by experiments in which unprimed spleen cells from donors of different ages are cultured for various times in irradiated isogenic 2 and 92 week old hosts with heterologous erythrocytes. It was found that the antibody forming capacity of spleen cells from 2 and 92 week old mice increased exponentially over a 4 week period in 2 week old hosts, thus establishing the ability of immunological progenitor cells from young or old mice to proliferate freely. However, in the corresponding experiment with 92 week old hosts, the spleen cells from 2 week old donors supported an exponentially increasing amount of hemagglutinin formation during culture, whereas the spleen cells from 92 week old donors did not increase in their capacity to produce hemagglutinins during culture. In short, old progenitor cells do not appear to grow well in an old environment, although they may grow in a young environment (Albright and Makinodan, 1966). This represents a type of age change which may be of great importance: an effect of ageing on cells in old mammals which is only revealed in the environment of the old animal. Thus, complete reciprocal transplants may be needed to demonstrate reversibility of age changes. Other examples of reversible age changes in cell activities will be discussed in Section IIIE.

In sum, the antibody forming progenitor cells of the spleen are subject to regulation by unknown mechanisms which elevate their numbers to a postnatal maximum by one-third the lifespan of the mouse. The fact that spleen cells from senescent mice proliferate at the same rate as spleen cells from neonatal mice in an irradiated isogenic host implies strongly that there is no in vivo limitation on the number of divisions the lymphoid progenitor cell can perform. Thus, the numbers of both hemopoietic and lymphopoietic progenitor cells appear to be regulated by endogenous physiological mechanisms, rather than by an intrinsic cellular limit beyond which their potential for further proliferation will be exhausted, as appears to occur uniquely for diploid cells in vitro. The regulatory agencies which control the decline of the antibody forming and hemopoietic progenitor cells are entirely obscure.

g) An interpretation of age changes in the population size of proliferating cell types

The preceding examples of proliferating cell populations indicate that the capacity for cell proliferation of at least some cell types persists in full strength throughout life. Replacement of these cell types should therefore

normally follow cell death. In organs composed of cells capable of proliferating (e.g., liver and spleen), loss of weight and cell number during senescence, observed in human livers particularly after the age of 80 (Tauchi, 1961), cannot be ascribed to the loss of potential for cell division. Such cases imply an age change in the regulation of the number of cells in the liver and other tissues, as is known to occur in young mammals. For example, an increase in the number of liver cells occurs during the hyperphagia in the rat which results from surgical lesions of the ventromedial nucleus in the hypothalamus (Kennedy and Pearce, 1958) or which follows the onset of lactation (Kennedy, 1958). It is possible that during senescence, when voluntary physical activity (Slonaker, 1912; Richter, 1922) and food intake (Everitt, 1958) are waning, a decreased demand for the metabolic functions of the liver could result in a resetting of the machinery regulating liver cellularity opposite to that caused by hyperphagia. In any case, the size of the cell populations in liver and lymphopoeitic tissue are clearly subject to differential regulation during post-natal growth and during adult life. Such is not the case for certain cell types and organizations of cells whose number is fixed during early development. Evidence for this will be considered next.

9) Non-dividing cells

a) The neuron

(1) Mitosis and DNA synthesis

Mitotic figures have never been certified in the nerve cells of adult mammals (e.g., Allen, 1912). The developmental stage at which genesis of new nerve cells ceases differs from species to species (Altman and Das, 1967). In all known mammalian species, proliferation of nerve cells ends before the termination of growth. It is of interest to note that regeneration of the optic tectum of adult goldfish and other teleosts can be accomplished by the differentiation of new neurons and glial cells from a reserve, germinative region which becomes completely depleted in older fish (Kirsche, 1960).

Autoradiorgdiographic evidence for DNA synthesis in nuclei of normal adult (Altman, 1962; Pelc, 1965) or injured (Watson, 1965) nerve cells has been claimed in recent years. It is possible that synthesis of DNA in neuronal nuclei does occasionally occur in adult animals. There is a long standing series of microscopical observations of binucleate neurons (see

Andrew, 1939, for references to the older literature), each of which appears to contain a prominent nucleolus (particularly clear examples are shown by Andrew, 1955 and 1956). The frequency of binucleate neurons is said to increase progressively during ageing in the mouse cerebral cortex (Andrew and Andrew, 1940) and cerebellum (Andrew, 1955). Multinucleated cortical nerve cells have also been found at the periphery of a malignant glioma; these occurred only in cells which were undergoing regressive changes and probably do not represent a neoplastic tendency of the nerve itself (see Courville, 1956, for such a case and references to other examples). No spectrophotometric determinations of the DNA content of the nuclei of any multinucleated nerve cell has been made as yet. It might be conjectured, however, that the claims of DNA synthesis in adult neurons represent either a stage in the formation of a multinucleated nerve cell or possibly an increase of ploidy (tetraploidy is the predominant ploidy class in adult human Purkinje nuerons) (Lapham, 1968). In any case, if DNA synthesis in adult neurons does occur, it is not followed by nerve cell proliferation on the basis of evidence known to me at present.

(2) Loss of neurons

The loss of nerve cells, first described in old bees by Hodge in 1894 has often been declared a process of great moment during ageing. However, accurate enumeration of the loss of nerve cells is difficult to achieve because of (1) great individual variability in the number of nerve cells of specific nerve tracts in rats (Corbin and Gardner, 1937), rabbits (Ferdnand and Young, 1951), and humans (Hoffman and Schnitzlein, 1961); (2) age changes in the compactness of nervous tissue which complicates calculations of neuronal density per unit area (Kety, 1956); and (3) difficulties in staining all nerve fibers, irrespective of their degree of fineness of mylenization (Moyer and Kalizewski, 1958). Thoughtful appraisal of various studies of nerve cell loss in man and other mammals is given in Moyer and Kalizewski (1958) and Wright and Spink (1959). Because of the attention which nerve cell loss has drawn over the years, a tabulation of published studies giving quantitative data is given in table XV.

One conclusion which can be drawn from this tabulation is that approximately the same percentage loss of nerve cells may occur during the life of men or mice. This implies that nerve cell loss is regulated in some way according to the lifespan of the species. If a man were to lose the same

Table XV

Loss of Neurones During Ageing

	% loss	Age range	Reference
Mouse			
spinal roots (T6-L4)	-15-20%	12-26 mos	Wright & Spink, 1959
Rat			
sciatic nerve	0	2-28 "	Birren & Wall, 1956
spinal roots (T8, L4, L5)	-40%	1-26 "	Duncan, 1934
cerebellum (Purkinje cells)	-18%	6-36 "	Inukai, 1928
spinal roots (L1-L3)	-20%	3-18 "	Sant'ambrogio et al, 1961
Guinea pig			
cerebellum (Purkinje cells)	-20%	0-50 "	Spiegel, 1928
cerebellum (Purkinje cells)	0	5-92 "	Wilcox, 1956
Cat			
spinal roots (C8, T1)	0	1-18 yrs	Moyer & Kalizewski, 1958
Dog			
cerebellum (Purkinje cells)	-70%	2-17 "	Harms, 1927
Human			
all areas examined revealed a loss of nerve cells with age: superior temporal gyrus, precentral gyrus, area striata; average loss about 30% from 20-80 years			Brody, 1955
dorsal spinal roots (T8, T9)	-20%	10-94 "	Corbin & Gardner, 1937
cerebellum (Purkinje cells)	0	6-88 "	Delorenzi, 1931
cerebellum (Purkinje cells)	-24%	22-81 "	Ellis, 1920
vagus	0	10-82 "	Hoffman & Schnitzlein, 1961
dentate nucleus	0	6-99 "	Höpker, 1951
facial nucleus	-14%	22-86 "	Maleci, 1934
cochlear nerve	-7%		Rasmussen, 1940
olefactory nerve & olefactory glomeruli	-70%	20-80 "	Smith, 1942
dorsal spinal roots (T8, T9)	-30%	40-90 "	Gardner, 1940

percent of neurons per year as may occur in the rat (7.5% loss/year), simple calculations show that his central nervous system would be depleted of 50% of its neurons in about 8 years. A similar adjustment of the rate of accumulation of cardiac ageing pigment to the species' lifespan is indicated (Munnel and Getty, 1968).

It is very difficult from reading the original papers to weigh the merit of their conclusions. Studies which enumerate neurons in the cross-section of well defined nerve tracts (sciatic nerve, spinal roots, etc.) might appear to be the most reliable. Yet, observations of nerve cell loss in spinal roots of ageing humans (Corbin and Gardner, 1937; Gardner, 1940), rats (Duncan, 1934), and mice (Wright and Spink, 1959) are in obvious conflict with other observations that no nerve cell loss occurs with age in the human vagus (Hoffman and Schnitzlein, 1961), cat spinal roots (Moyer and Kalizewski, 1958), and rat sciatic nerve (Birren and Wall, 1956). Unless nerve cell loss follows a pattern so different from species to species that being a cat confers protection against loss of the spinal root nerves, whereas being a man or a rat does not, then nerve cell loss may only be an irregular and occasional feature of mammalian ageing. There is no doubt that nerve cell loss can be particularly marked after certain environmental stresses such as heat stroke (Krainer, 1949) or in areas of the brain in which vascular degeneration has occurred (Corsellis, 1962). Such "accidental" events may ultimately account for most of the nerve cell loss.

It might be supposed that age changes in the weight of the brain could be accounted for by the loss of neurons, if indeed this loss is a regular feature of ageing in mammals. However, the weight of the human brain is nearly constant through the fifth decade before declining at an ever increasing rate in the following years (Himwich and Himwich, 1959). In contrast, nerve cell loss, as it has been described, is progressive throughout adult life. Brody (1955) indicates that the time of greatest nerve cell loss may occur before maturity in humans. Thus, the precipitous loss of brain weight after the seventh decade does not seem to be paralleled by a corresponding loss of nerve cells.

b) The myocardial cell

(1) Mitosis and DNA synthesis

Opinion about the inability of myocardial cells in the adult to proliferate

is traditionally based on the absence of mitotic figures in myocardial cells either in the resting or hypertrophying adult heart (Karsner et al, 1925; see Robledo, 1956, for other references to the older literature). Mitotic figures can be found in myocardial cells for a short time after birth (Robledo, 1956). Recent studies of hypertrophying heart muscle which show that there is a relative increase of RNA with respect to DNA are also in support of this view. No change in DNA per myocardial nucleus was found in hypertrophied heart (Capers, 1964). However, the total quantity of DNA does increase somewhat in the hypertrophied portion of the heart (Norman & Carter, 1960; Meerson et al, 1964) and the number of myocardial nuclei is also said to be increased (Arutyunov, 1966; Meerson et al, 1964). It is therefore probable that myocardial cells do not proliferate during adult life, although the degree of multinucleation may increase during hypertrophy. The observations of occasional myocardial nuclei which appeared to be synthesizing DNA on the basis of autoradiographic evidence (Messier and Leblond, 1960; Pelc, 1965; Falzone et al, 1967) may indicate a turnover of nuclei within the syncitium of the myocardial cell. In any case, the proliferation of myocardial cells appears to be unlikely at any point in adult life.

(2) Loss of myocardial cells

There is little information relating to the possible loss of myocardial cells during life, although one report shows that the number of myofibrills per $cm.^2$ is constant during the life of the rat (Rakusan and Poupa, 1964). The fact that hypertrophy of the myocardium occurs with the same kinetics and achieves the same final end point throughout the lifespan of mice (Swigart, 1969) and rats (Hügin and Verzar, 1956) strongly implies that little loss of myocardial cells occurs during life. However, hypertrophied myocardial cells, the number of which increase with age in the rat (Korenchevsky et al, 1950; 1953) may represent a compensation for the dysfunction or death of other myocardial cells.

c) The oocyte

Formation of new oocytes in the ovary of post-partem mammals has been debated for many years (see review by Zuckerman, 1960). Recent evidence makes abundantly clear that the number of oocytes is fixed at birth. Autoradiography after injections of tritiated DNA percursors reveals that DNA is synthesized in oogonia only before birth (Sirlin and Edwards, 1959; Borum, 1966). Label

incorporated at that time is retained during subsequent maturation of the oocyte (Rudkin and Greich, 1962; Borum, 1966). With repeated pre-natal injections of H^3-thymidine, 95% or more of the oocytes in the ovaries retained their label until at least 6-7 weeks after birth, the longest post-partum interval examined (Borum, 1966). To date, labelling of oogonia with DNA precursors has not been achieved at any time after birth (Sirlin and Edwards, 1959; Borum, 1966).

Additional support for this view is derived from studies of the ovarian hypertrophy which follows unilateral spaying: the surviving hypertrophied ovary does not contain additional oocytes (Mandl and Zuckerman, 1951a). This conclusion can also be inferred from the observations of John Hunter, reported in 1787, that a unilaterally ovariectomized sow produced one half as many total offspring as a control (the average litter size was unchanged). Hunter states, "the ovaria are from the beginning destined to produce a fixed number beyond which they cannot go" (Hunter, 1787). Halving of the reproductive potential after unilateral spaying has been subsequently documented for rats (Jones and Krohn, 1960; Biggers et al, 1962).

Diminution of oocyte numbers throughout life was first described in rats by Arai (1920). The period of greatest numerical loss occurred between birth and puberty. Mandl and Zuckerman (1951b) have confirmed this and established from their own and Arai's data that the number of oocytes varies as a negative exponential function of age. It has subsequently been found that the rate constant of this exponential function is specific for each strain of mouse, as is the number of oocytes in the ovaries at birth (Jones and Krohn, 1961a). The rate of loss of oocytes in (CBAxA) F1 mice was found to be less than in females of either parental stock (Jones and Krohn, 1961a).

The exponential nature of the loss of oocytes implies that, unlike other aspects of physiological ageing, the likelihood of oocyte loss is constant throughout life. Reproduction ceases in females at a characteristic time for each strain, in all cases while the ovary still contains detectible oocytes. No relationships have been found between the number of offspring, the number of oocytes at birth, and the rate of loss of oocytes (Jones and Krohn, 1961a).

The rate constant of oocyte loss is known to be decreased by hypophysectomy of young rats (Ingram, 1953; Jones and Krohn, 1961b). Normally the percent of oocytes lost each 20 days for A, CBA, RII and (RIIxA) F1 is 13, 28, 12, and

11% respectively. After hypophysectomy, the percent lost each 20 days became 8, 11, 6, and 3% respectively (Jones and Krohn, 1961b). This is an extremely important result. It shows that ageing of the ovary is not autonomous but is sensitive to the physiological status of the organism. The fact that loss of oocytes is markedly less in hypophysectomized animals implies that the pituitary secretions are important in the ageing of the ovary, directly or indirectly. As Jones and Krohn (1961b) point out, the greatest loss of oocytes occurs near to birth and before puberty, when gonadotrophin secretion is low. Thus, gonadotrophins are probably not the only hormonal influence on the loss of oocytes. Reproduction itself does not appear to influence the loss of oocytes, which occurs at the same rate in virgin rats or in multiparous rats with a modal number of seven litters (Shelton, 1959). Evidently, the rate of loss of oocytes varies according to the lifespan of the species: if women lost oocytes at the same rate as females of the CBA strain of mouse (28% each 20 days), then only 10^{-220}% of those oocytes present at birth would survive to the age of 25 years. However, published data show survival of at least 10% (Block, 1953).

d) Conclusion

The non-dividing character of the neuron, the myocardial cell, and the oocyte is established soon after birth in the mammal and is not subject to further change with age during adult life. However, there is some indication of occasional synthesis of DNA in the nucleus of the neuron or myocardial cell. If true, this is consistent with the postulate that differentiation does not involve an absolute loss of the potential for expression of any information in the genomic magazine. Evidence for the potential ability of neuronal nuclei to respond to signals for DNA synthesis is given by experiments in which nuclei from adult mouse brain were injected into unfertilized enucleate frog oocytes. Active synthesis of DNA was promptly initiated in 75% of the nuclei (Graham et al, 1966). Although care was not taken to distinguish neuronal nuclei from the normally proliferating glial cells, it is clear that at least some neuronal nuclei began to synthesize DNA. Thus, given the proper stimulus, nuclei in non-dividing neuronal cells display a latent potentiality. The fact that the incidence of multinucleated nerve cells increases with age implies that this potentiality has not been lost during ageing.

10) Irreplaceable organizations of cells

There is evidence that in certain tissues the endowment of multicellular functional units is set before or soon after birth. Loss of these structures is therefore permanent, although compensatory hypertrophy usually takes place in the remaining tissue. The neuron, the myocardial cell, and the oocyte have been considered as examples of irreplaceable cell types. Examples of tissues which contain dividing cells, but whose functional units cannot be replaced, will be considered next.

a) The nephron

Formation of new nephrons ceases shortly after birth at the age of 1 week in rats (Jackson and Shiels, 1927) and within 36 weeks in humans (Potter and Osathandoth, 1966). The subsequent growth of the kidney is entirely due to elongation of tubules and the elaboration of connective tissue and vasculature. Even the stimulus of unilateral nephrectomy within a few weeks of birth in the rat does not increase the number of nephrons (Jackson and Shiels, 1927; Moore, 1929). Recent studies employing fluorescent antibody techniques provide the best evidence that compensatory hypertrophy of the renoprival kidney does not involve formation of new glomeruli in the adult rat (Hiramoto et al, 1962).

Loss of nephrons is known to occur from histological studies and appears to be progressive throughout life (Arataki, 1926; Moore and Hellman, 1930). The rate of loss does not appear to be influenced by unilateral nephrectomy at birth (Moore and Hellman, 1930). It is of interest that glomerular filtration rate and tubular excretory capacity decline during ageing in humans in such a manner that the ratio of these quantities is constant at all ages (Davies and Shock, 1950). The ratio of glomerular filtration rate and tubular resorption rate of glucose was also found to be constant (Miller et al, 1952). This is consistent with a concurrent loss of both glomeruli and tubules during ageing. Apparently, the nephron looses its function as a unit (Davies and Shock, 1950).

b) The alveoli of the lung

The ability to form new alveoli is lost at some point before maturity in cats (Bremer, 1937) and dogs (Longacre and Johansmann, 1940). Unilateral pneumonectomy results in the formation of new alveoli in the remaining lung in immature animals only. Thereafter, compensation is made by the hypertrophy of pre-existing alveoli.

There is no information on the possible decline in the numbers of alveoli during ageing. However, the vital capacity of the lung (the maximum volume of air which can be inhaled and expelled per breath) declines progressively during human life (Norris et al, 1956). The decline of vital capacity may prove to be due in part to a loss of alveoli during ageing.

c) Fat pad

No regeneration of partially excised fat pads appears to occur in adult rats (Cameron and Seneviratne, 1947). Discretely organized fat tissue may thus be fixed in its cellular content at birth.

d) Conculsion

The examples of those cells (e.g., nerve) and organizations of cells (e.g., nephron) which are not replaced in the mammal after the earliest period of development stand in contrast to the readiness with which many cellular structures can be regenerated in adult mammals. Evidence for the maintenance of the processes of regeneration, wound healing, and compensatory hypertrophy during ageing will be considered next.

11) Regeneration, hypertrophy, and wound healing

Regeneration of complex cellular structures and organs becomes progressively limited either during the development of any species or according to the phylogenetic position of the adult. In adult mammals, intricate cellular mechanisms exist for the repair of local damage (e.g., healing of skin wounds, fractured bones, etc.). Regeneration of damaged or extirpated portions of certain organs, e.g., liver, uterus, gall bladder, urinary bladder, intestinal villi, spleen, pancreas, thyroid gland, and adrenal gland is also possible in the adult (see reviews by Johnson and McMinn, 1960; Goss, 1964).

Limited regeneration of striated skeletal muscle is known to be possible from work of the last 20 years in adult rabbits (Le Gros Clark, 1946; Godman, 1957) and adult mice (Lash et al, 1957). Traditionally, regeneration of skeletal muscle in the adult has been regarded as so restricted as to be insignificant (see references in Cameron, 1952). Regeneration of lost or damaged parenchymal tissue in other organs (e.g., heart, kidney, lung) cannot be accomplished in the adult mammal. In these tissues, compensatory hypertrophy occurs, a process distinct from regeneration.

a) Regeneration of liver

Surgical ablation of a portion of liver tissue or damage from such agents as carbon tetrachloride, initiates a remarkable hyperplasia of the remaining or healthy tissue. Recent studies have shown that this hyperplasia involves a major change in the pattern of genomic expression which can be detected within minutes after hepatectomy and which includes the activation of genes not expressed since the fetal period (Church and McCarthy, 1967a, b). During the next hours, DNA synthesis is begun in the parenchymal cells, cell division ensues, and within 7 or 8 days the original mass of the liver tissue is nearly restored (see the excellent review of Bucher, 1963). Regeneration of the liver is therefore an extreme test of the functional capacities of the liver cells and requires a cascade of rapidly succeeding changes in genomic activity and cellular metabolism. The fact that the mass of liver tissue is restored with identical kinetics and to the same extent in adult and senescent rats (Bucher and Glinos, 1950; Bourlière and Molimard, 1957) provides an extremely forceful argument against the supposition that senescence engenders basic changes in the potential for cell activity. However, there are significant age differences in the process of liver regeneration which serve more to indicate shifts in the physiology of the animal in which the regeneration is occurring than to modify the key fact that full liver regeneration is possible in senescence.

The common experimental procedure for studying liver regeneration is the surgical removal of a large portion of tissue, usually the large left lateral and median lobes (Higgins and Anderson, 1931), which in rats comprise 68% of the original liver mass. Careful investigation has revealed various thresholds for provoking hyperplasia in the remaining tissue: when less than 35% of the original mass of the liver is removed from a young adult rat (4 months old), the ensuing cell proliferation is randomly distributed throughout the liver and is not synchronized (Bucher and Swaffield, 1962). However, if 43% or more of the liver tissue is removed, there is then an intense proliferation of cells in the peripheral zones of the liver lobule, tending to spread centripetally with excision of increasing amounts of tissue. In these cases, there is a very well defined and synchronous wave of mitosis. In rats approaching late maturity (12-15 months), the threshold mass of tissue for synchronous regeneration is decreased to about 9% (Bucher and Swaffield, 1962). An age change in the setting of the unknown physiological mechanisms which activate liver regeneration has obviously occurred.

One of the early events in regeneration is the rapid loss of liver glycogen (Harkness, 1957). A preliminary study, comparing 3 and 22 month old mice, indicates that the older animals may lose 50% more glycogen than the younger group (Hollander and Thung, 1966).

A long standing observation is the increase with age of the interval after hepatectomy before the first peak of mitosis (Marshak and Byron, 1945). Comparison of 1 month, 4 month, and 12-16 month old rats disclosed that the post-hepatectomy peak of DNA synthesis which precedes mitosis is delayed progressively by a few hours with age (Marshak and Byron, 1945; Bucher et al, 1964). There is also a decreased synchrony in the temporal pattern of DNA synthesis with age which is reminiscent of the decreased synchrony of DNA synthesizing cell cohorts in intestinal epithelia of older animals (Section IIIC7b1).

The temporal pattern of mitosis in truely senescent mice has been examined in a study which employed carbon tetrachloride damage of liver tissue as a stimulus for regeneration (Post et al, 1960). No age difference in the increase of DNA in the residual liver was evident among mice aged 6-24 months, although the rate of healing, by histologic criteria, was said to be progressively retarded with increasing age.

An interesting age difference was noted by Post et al (1960) during carbon tetrachloride stimulated regeneration of a mouse liver: hepatocytes of senescent mice (24 months old) only include a 16n class of nuclei. Thus the absence of synthesis in 4n and 8n class of nuclei of resting liver in mice aged 6 months or more (see Section IIIC7b6) does not indicate that the nuclei of higher ploidy never divide (Post and Hoffman, 1965).

If the restitution of the hepatectomized liver is considered in terms of the number of cells rather than the total mass of tissue or DNA content, senescent rats lag somewhat. At 7 days post hepatectomy, 97% of the original number of cells are restored in 1.5 month old rats, 75% in 6 months old rats, and 63% in 25 month old rats (these fitures are corrected for binucleate cells) (Bucher and Glinos, 1950). The significance of the difference between regeneration evaluated in terms of mass or cell number is not clear.

A final consideration underlines the integrity of regenerative processes in the liver throughout life. Measurements of nuclear diameter at various

times in liver tissue show parallel and equal increases from 1 to 3 days post hepatectomy (Bucher and Glinos, 1950). The association of changes in nuclear diameter with changes in genomic activity, previously noted (Section IIIC5a) implies that the shifting patterns of genomic expression during liver regeneration occur in the same schedule in at least most of the nuclei of old animals as well as young. The fundamental mechanics of regeneration do not appear to be impaired in senescent mammals.

b) Cicatrization

Healing of wounds is the result of an extraordinarily complex series of cellular phenomena which are directed, in turn, towards provisional closure of the lesion, protection of the body against infection at the site of the wound, removal of damaged tissue, subsequent revascularization, and regeneration of the wounded tissue. The famous studies of Du Nöuy (1916) demonstrated the retardative effect of age in the healing of wounds, measured as changes in the area of the wound. Observations were made on the healing of battlefield wounds in a French field hospital under the constant sound of gunfire during World War I (Du Nöuy, 1937). The patients were from 20 to 40 years old, with the exception of two patients aged 50 years and one aged 60 years. An index of cicatrization, computed from the rate of wound healing at different ages, was found to be inversely proportional to age (Du Nöuy, 1937). There is no reason to doubt that under the given circumstances that the healing process in older individuals required more time. Subsequent investigations have in general born out the fact that wounds of rats (Howes and Harvey, 1933; Paul et al, 1948; Bourlière, 1950; Gourevitch, 1951; Butcher and Klingsberg, 1963) and of a hairless strain of mice (Montagna et al, 1954) heal more slowly in older animals, particularly during the initial phases of the process. Closure of the wound occurred and normal healing occurred in all cases except in the palatal wounds of senescent rats described by Butcher and Klingsberg (1963), which suffered incomplete closure of the wound surface and incomplete replacement of granulation tissue with connective tissue fibres. In general, age differences in the rate of healing become progressively diminished during the final stages of healing (Paul et al, 1948; Bourlière, 1950). The size of the wound appears also to influence the extent of age differences (Orentreich, 1969, personal communication).

A recent, excellent histological study of the healing of tongue wounds in well kept conventional and germ free mice proves that cicatrization under the best laboratory conditions may not be affected by age at all (Rovin and Gordon, 1968). To this extent, cicatrization is like hepatic regeneration: there may be an early delay in the mobilization of the cellular response, but normally no age difference prevails which could indicate any fundamental age change in the ability of the body to elicit the full repairative response from its cells.

c) Renal hypertrophy

Following unilateral nephrectomy in adult rodents, the remaining kidney begins a long term increase in weight (Arataki, 1926) and DNA, RNA and protein content (Barrows et al, 1962b). The synthesis of RNA in the organ increases rapidly within the first hours post-nephrectomy; the incorporation of isotopically labelled RNA precursors into free and bound ribosomes is greatly enhanced by 3 hours (Malt and Stoddard, 1966). A peak of mitotic activity subsequently ensues between 3 and 10 days (Sulkin, 1949). In many respects these changes are similar to those which occur during liver regeneration. However, there is an essential difference: no new nephrons are generated; only hypertrophy of the remaining nephrons occurs. Renal hypertrophy can also be stimulated by enriching the protein content of the diet (MacKay et al, 1928).

Early studies on the effect of age on post-nephrectomy hypertrophy indicated that the process was completed in all ages within 40 days although the percentage increase in kidney weight declined slightly between the ages of 6 and 18 months in the white rat (MacKay et al, 1932). The smaller percentage increase in weight with age has recently been confirmed (Konishi, 1962; Barrows et al, 1962b), although other workers (Verzár and Hügin, 1957) find no diminution with age. With protein enriched diet as a stimulus for hypertrophy, the percentage and net increase in renal weight decreased from 2 through 13 months. The linear relationship between renal weight and daily protein intake is valid, however, in rats from the age of 2 months through 13 months (MacKay et al, 1928). This is obviously change of maturation rather than senescence.

If the post-nephrectomy change in kidney mass is expressed in terms of per cent of the original mass, an agewise decrease in hypertrophy is indicated.

However, if the increase is expressed as the total increase in cells, measured as net change in DNA, then no age difference is found (Barrows et al, 1962b). The different results from these two methods of calculation appear to stem from the fact that the increase in DNA per gram tissue may be greater in the old hypertrophied kidney than the young, although the net increase in mass is less in the old hypertrophied kidney (Barrows et al, 1962b). The composition of the new tissue of the hypertrophied kidney was also found to be identical in 12 and 21 month old rats, with respect to the RNA, protein, succinic dehydrogenase,and alkaline phosphatase concentrations per cell (Barrows et al, 1962b). The process of renal hypertrophy does not reveal any essential change in cell function with age.

The details of the cell proliferation during renal hypertrophy have been subjected to only cursory examination as a function of age. There is no information about the effect of age on the timing of the post nephrectomy wave of mitosis. In weanling rats, for instance, the peak mitotic index occurs at about 2 days (Rollason, 1949); in 3 month old rats, the peak occurs somewhat later, between 3 and 10 days (Sulkin, 1949). It would not be surprising if the peak of mitotic index became progressively retarded with increasing age, as it is for regenerating liver. However, until the peak mitotic index is defined, measurements of the mitotic index of young and old rats at a single time point after nephrectomy cannot provide a definitive comparison of the proliferative ability of kidney tissue as a function of age. This particular uncertainty may account for divergence of the data of McCreight and Sulkin (1959), which shows that the mitotic index 3 days post-nephrectomy is one fifth as large at 37 months vs. 4 months, from Konishi's data (1962), which shows that the mitotic index 7 days post-nephrectomy is more than twice as large at 22 months than 5 months in the rat, as Konishi himself points out. Konishi's study also demonstrated that nephrectomy plus a high protein diet causes a greater increase in mitotic index than nephrectomy alone in 5 and 22 month old rats. Moreover, the mitotic index was found to be increased to an equal degree during feeding of a protein rich diet following sham nephrectomy in 5 and 22 month old rats. These are additional demonstrations that the integrity of mechanisms of growth and hypertrophy is maintained during ageing.

d) Decidual reaction of the uterus

Traumatic stimulation of the pseudo-pregnant rodent uterus by mechanical damage to the endometrium (e.g. Selye and McKeown, 1935) or by the injection of oil into the uterine lumen (Finn and Hinchcliffe, 1964) causes the formation of a mass of decidual cells in the endometrial stroma which is attended by an intense increase of alkaline phosphatase (Finn and Hinchcliffe, 1965). A comparison of the response of pseudo-pregnant 4 and 15 month old DF1 mice to the crushing of one uterine horn revealed that the increased uterine mass, due at least in part to the formation of decidual tissue, was about twice as great in young mice. Injection of oil did not provoke the formation of deciduomata or the increase in alkaline phosphatase in old mice (Finn, 1966). The fact that deciduomata are formed with the stimulus of crushing indicates that the endometrial stromal cells can proliferate in a menopausal mouse. The absence of a response to oil may be due to a change in the threshold for cell proliferation.

e) Annual regeneration of antlers

Senescence is dramatically manifested in male Cervidae through changes in antler size. After a progressive annual increase in the size of antlers during successive cycles of regeneration and shedding, an upper size limit is attained. During subsequent years, the size of the antlers diminishes; in old age the annual cycle is abolished. The small, residual antlers are then frequently deformed and become permanently covered with velvet, a soft, richly vascularized tegument characteristic of the early stages of antler formation (Wislocki, 1956). An excellent illustrative annual series of antlers throughout the life of an axis deer is given by Anthony (1929).

The annual cycle of antler regeneration in male Cervidae results from the intense activity of bone forming cells and is known to be dependent on androgens. The annual cycle does not occur in castrated Cervidae, but can be induced by testosterone. Wislocki (1956) attributes age changes in the antler cycle to diminished secretion of hormones by the gonads and anterior pituitary.

f) Healing of bone fractures

The healing of bone fractures in humans does not appear to be affected by age in human adults. No published studies of this aspect of ageing exist to my knowledge. According to Bick (1960) and Bauer (personal communication), both

wide experience, repair of fractures occurs at an

undiminished rate in older humans, including in post-menopausal women during the phase of marked resorption of bone. On the other hand, Tonna (1959) reports that the rate of repair of fractures in rats (estimated from x-ray and histochemical studies) is slowed during the transition from puberty to early maturity. It is difficult to evaluate the extent of any subsequent age changes from the data presented; it is clear, however, that evidence of active healing was found in 24 month old rats. The pattern of age change in healing of bone fractures thus stands in obvious contrast to the pattern of age change in the regeneration cycle of antlers.

12) Serial transplantation experiments - a demonstration of potential immortality

If the cells of the body have no intrinsic limit to the time which they can function normally or proliferate, then transplantation through a sequence of younger hosts should theoretically perpetuate their immortality or at least demonstrate the ability of these cell types to survive beyond the absolute potential longevity characteristic of the species. Such prolonged experiments are beset by practical difficulties concerning failure of the graft due to accidental trauma, etc.

Various attempts of this type of experiment indicated that the upper limit to the lifespan of transplanted (mouse) tissues was close to the absolute potential longevity of the mouse in skin (Krohn, 1962) and mammary gland (DeOme, 1965). Degenerative changes were observed in these transplanted tissues within the maximum lifespan of the mouse. However, it is not clear that these degenerative changes are in any way comparable to senile changes. Fortunately, other experiments have given more encouraging results.

The current maximum recorded lifespans of various tissues maintained through sequential transplantations in mice is 41 months in thyroid tissue (Loeb, 1945), 46 months in mammary gland (Hoshino and Gardner, 1967),and 55 months (Horton, 1967) and 80 months in skin (Krohn, 1966). There is little doubt that in all of these experiments, the cells have survived significantly longer than the current absolute potential lifespan of the mouse (about 40 months): in the case of Krohn's skin transplant experiments (Krohn, 1966) the natural limit to longevity is exceeded by a factor of two. There was no evidence of degeneration or neoplasia in the 80 month old skin transplants (Krohn, 1966). This fact adds credence to the suspicion that serially transplanted grafts of euploid cells possess immortality, without first undergoing transformation as in the case of

ssible, of course, that a transformational event occurred

in Krohn's old skin transplants without giving rise to noticeably neoplastic tissue.

13) Conclusion about cell proliferation and cell loss during ageing

The foregoing analysis indicates that there is no change in the proliferative potential of cells in the mammal after maturity has been reached, with the sole exception of the annual regeneration of antlers. Thus, the liver regenerates as effectively, the rate of proliferation of antibody-forming cells from the spleen is undiminished, and the passage limit of lung fibroblasts in vitro does not differ among adult human donors of any age. Other cells (e.g. neurons) and cell organizations (e.g. nephrons) are not replaceable during adult life, but this is not a change of senescence. On the contrary, the number of these elements is determined during early development.

Loss of body tissues may be conjectured to have a twofold origin, the result of (1) an "active" regulation of cell number (e.g. in the liver, in hematopoietic, in lymphopoietic tissue (see discussion in Section IIIC8g)) and of skeletal muscle mass (determined jointly by the demands of voluntary activity and the strength of the neuromuscular trophism); (2) a "passive" loss of cells through accident (e.g. neurons, nephrons). The relative contribution of these two processes is probably different from tissue to tissue and cannot be evaluated from currently available information.

14) Temporal aspects of genomic response

Successful physiological adaptation to changes of environmental conditions, diet, etc., frequently depend on the realization of a new pattern of gene activity. Slowed physiological reactions are anecdotally held to be a hallmark of the ageing process. To what extent this feature of ageing is the result of changes in the rapidity with which genomic information is expressed will be considered next.

Several types of cellular activities have been discussed which indicate that no major change in the time course of genomic expression occurs with age, e.g., regeneration of liver and other processes involving cell proliferation occur on roughly the same time course as does the myocardial hypertrophy, the primary or secondary immunological responses, etc. These cellular responses are aggregate parameters representing many activities within the cell, yet the trend they describe will be found significant for the rate of induction of individual enzymes.

There are relatively few studies of enzyme induction during ageing. Zorzoli and Li (1967) have found that the percent increase of phosphoenolpyruvate

kinase in kidneys of mice fasted for two days was not reduced in senescence. Singhal (1967) found that treatment with dexamethsone produced a progressively smaller increase per cell of liver glucose 6-phosphatase or fructose 1, 6-diphosphatase during the ageing of mature rats, 9-15 months. This is clearly a change of late maturation rather than senescence.

Examination of enzyme induction in the liver revealed no difference in the extent of induction of tryptophan pyrrolase by tryptophan or hydrocortisone, or of tyrosine aminotransferase by hydrocortisone in young and senescent rats at a single time point 4 hours after injection (Gregerman, 1959). This has been subsequently corroborated in another study of tryptophan pyrrolase induction in rats up to the age of 1 year (Correll et al, 1965). Detailed examination of the first 2 hours of tyrosine aminotransferase induction by insulin or corticosterone has likewise revealed no age difference in the kinetics of induction between young or senescent mice (Finch et al, 1969, see Section IIIF3c10b for details). In conclusion, there is no evidence that the expression of genomic information in response to the appropriate stimulae is delayed as a consequence of ageing.

D. General interpretation of age changes in the activities of individual cells

Many aspects of cellular activities have been found to change with age. A synopsis is given in table XVI. From this table it may be concluded that all characteristics and activities of cells persist qualitatively throughout adult life in mammals. There is no evidence for an absolute loss of the potential for any cell function during senescence. In the case of female reproductive failure, there is excellent evidence that the surviving oocytes possess viability (Talbert and Krohn, 1966), as will be discussed in section IIIE1c.

An essential feature of ageing is the histotypic selectivity of age changes in cell activities. The profile of histotypic ageing changes in cell activities is conjectured to represent differential changes in gene activity. The best evidence for this derives from observations of the selective changes in the phases of the cell generation cycle in the intestinal epithelium. In other words, the physiological transitions from youth through old age are conjectured to result from changes in the pattern of genomic expression. The physiological transition which occurs during puberty is also well known to result from a hormonally-mediated change in the pattern of genomic expression. Although the forces which drive the mammal through the sequence of post-maturational changes are entirely unknown at this time, it is clear each differentiated cell type

Table XVI

Synopsis of Age Changes in Cell Activities

1) Weight changes of organs
   a) frequently affects liver, skeletal muscle, brain
   b) no weight loss in heart ever found

2) Cell nucleus: no change in DNA content

3) Enzyme profiles
   a) slight changes in enzyme activities, usually less than 25%
   b) enzyme activities may increase, decrease, or not change at all
   c) the same enzyme can be affected in opposite directions in different tissues

4) Ageing pigments
   a) differences in rate of accumulation according to cell type and region of brain
   b) important species differences in regions of pigment accumulation

5) Respiration in vitro
   a) most tissues show decrease during ageing (e.g., liver, brain), but ear epithelium does not
   b) regional differences in decrease of respiration exist in the brain
   c) liver and kidney mitochondria, but not brain mitochondria, have decreased ability to metabolize β-hydroxybutyrate; no age difference found with other substrates

6) Metabolic pathways
   a) no change in active transport in kidney or intestine
   b) no change in most pathways of intermediary metabolism (e.g.,gluconeogenesis or anaerobic glycolysis)
   c) synthesis of RNA: tissue specific changes of incorporation of isotopic RNA precursors, e.g., increase in liver, kidney, skeletal muscle, dorsal root ganglia; decrease in cerebral cortex, cerebellar Purkinje cells
   d) synthesis of proteins: differential changes, e.g., decreased content of trypsin and pepsin in pancreatic secretion; lipase secretion, however, is unchanged; turnover of cytochrome C in liver is unchanged.

7) Cell proliferation
   a) cell generation cycle: in intestinal epithelium, slowed rate of proliferation is attributable specifically to an increase in G1 length; no change in length of S or G2; no change in rate of proliferation of antibody forming cells or of epidermal cells.
   b) no post-maturational changes of the proliferative potential of diploid fibroblasts in vitro
   c) no post-maturational changes in ability of liver to regenerate completely, in healing of skin wounds, or in repair of bone fractures

reacts in a characteristic way. From this point of view, the changes of old age are further events in the developmental pattern of mammals which, like events of earlier post-natal development stages, are derived from changes in the pattern of genomic activity.

E. Reversible age changes of cell activities

The classical experiments of Stone (1950) on the regeneration of neural retina cells from pigmented iris cells of the adult newt (Wolffian regeneration) and of Gurdon (Gurdon, 1962; Gurdon and Uehlinger, 1966), on the ability of nuclei from differentiated intestinal epithelial cells of the tadpole to direct the full development of an unfertilized egg, have demonstrated the reversible nature of the differentiated state of cells in certain situations. If cell differentiation is reversible and if ageing is a process involving cell differentiation, then it should be possible to reverse the changes of old age. In fact, a certain number of examples of reversible age changes in cell activities have been described.

1) Reversible age changes related to changes in sex hormones

One group of reversible age changes includes those changes of cell activities which probably result from the diminishing output of ovarian and testicular steroids.

a) Gonadotrophin production

At the time of menopause, when the output of estrogenic steroids markedly decreases, the output of pituitary gonadotrophins markedly increases in rats (Lausen et al, 1939) and humans (Witschi and Riley, 1940; Johnsen, 1959). This is the result of the release of the negative feedback inhibition by sex steroids on the output of pituitary gonadotrophins. Injection of estrogenic steroids to post-menopausal women was found to reduce the output of pituitary gonadotrophins to normal levels within a period of 4 days (Frank and Salmon, 1935). This example also demonstrates that various systems of the body may make compensatory reactions to ageing changes in other organ systems.

b) The prostate

The prostate begins a characteristic involution during late maturity, which involves a decrease in secretory activity of the glandular epithelium in both mice (Franks, 1959) and men (Moore, 1936, 1952). In vitro organ culture of prostates from 24 month old mice (Franks, 1959) and especially transplantation to younger hosts (Franks and Chesterman, 1964) have been found to reverse the atrophy of the secretory epithelium and to increase the frequency

of mitosis. Atrophy of the secretory epithelium is known to follow castration in rats and can also be reversed by testosterone (Moore et al, 1930).

Acid phosphatase, an enzyme of the prostatic fluid, produced in great concentration by the secretory epithelium of the prostate (Gomori, 1941) rises to a high level during puberty (Gutman and Gutman, 1939; Hansen, 1945), and declines precipitously in normal, hypertrophied, or infected prostates during senescence in humans (Kirk, 1948). The reduced alkaline phosphatase content of the prostatic fluid in eunuchs (Watkinson et al, 1944) parallels the histological appearance of the secretory epithelium mentioned above. Treatment of elderly men with testosterone over a period of 2 to 7 months was found to gradually increase the activities of prostatic alkaline phosphatase an average of twofold (still one third the values of young men) in 9 out of 12 subjects (Kirk, 1949). This increase is less than found in testosterone treatment of young eunuchs (Hansen, 1946). The reactivity of the prostate to testosterone has been found to decrease with time after castration in rats (Price, 1944; Singhal, 1967b). This may be due in part to the loss of responsive epithelial cells in the prostate (measured as the net loss of DNA following castration (Kochakian and Harrison, 1962)). Thus, there may be a loss of responsive prostate cells in surgical castrates or in very old men whose decreasing production of androgens may approach a hypogonadal condition. In any case, it is clear that the ageing changes of the prostate are, in part, reversible.

c) Oocyte viability

The decline in litter size and the cessation of reproduction in female mammals is known to occur long before the store of oocytes is completely exhausted (Jones and Krohn, 1961a). An impressive series of transplantation experiments, performed by Krohn and associates (see review of Krohn, 1962) has provided evidence on the importance of systemic factors in the decline of litter size in the mouse. The culmination of these experiments has been the demonstration that ova, morulae and blastulae from female mice during their reproductive decline (up to 2 years old) can develop into full term fetuses upon transplantation to a young female; the number surviving (about 50%) is the same as transplants from young donors into young hosts (Talbert and Krohn, 1966). In marked contrast, only about 15% of young ova survived to become full term fetuses in old mice (Talbert and Krohn, 1966). This result shows that alteration of the uterine environment is the major factor in the

reproductive decline of old mice and that ageing changes of the mouse oocyte itself do not occur. Similar experiments, performed with ova from old hamsters, indicate that old hamster ova transplanted into young hosts do not develop into full term fetuses (Blaha, 1964), implying a major species difference in the effect of ageing on oocyte fertility.

Marked age changes of the decidual cell reaction of the uterus have been demonstrated by Finn (1966) (See Section IIIC11d). As implantation of the blastocyst is dependent on the elaboration of decidual tissue, Finn's study provides a rationale for the decreased frequency of implantation of young ova transplanted to old uteri observed by Talbert and Krohn (1966).

2) Reversible age changes in cell activities which are probably not related to changes in sex hormones

a) The intestinal epithelia

The slowed cell generation cycle in the intestinal epithelia of old rats can be accelerated with certain doses of irradiation to new, steady state values which are the same as in young irradiated rats (Lesher, 1966) (see Section IIIC8c). These age changes in the cell generation cycle are therefore reversible.

b) Ageing pigment

Administration of the agent centrophenoxine for one to two months has been found to virtually eliminate accumulation of ageing pigment in neurons of senescent guinea pigs (Nandy and Bourne, 1966; Nandy, 1968). This observation adds to the experimental manipulations of ageing pigment deposition which can be performed (see Section IIIC3). The consequence of centrophenoxine treatment on the lifespan or on other aspects of senescence in the guinea pig has not been described.

c) Hair regrowth cycle

The pelage of the adult mouse, like the hair of the human scalp, eyebrows, and eyelashes, is not dependent on the support of sex steroids. It is known that in senescent mice, initiation of the hair regrowth cycle may be delayed up to four weeks in a dipilated hair follicle. The rate of hair growth, once initiated, tends to be slower (Whitely and Horton, 1962). Horton has subsequently reported that transplantation of skin from a senescent mouse to a young mouse abolished the age difference in the hair regrowth

cycle: hairs plucked from the old graft are replaced at the same rate as hairs plucked from adjacent areas of the young host. Furthermore, this process can be repeated many times without revealing an age difference between the graft and the host (Horton, 1967). Although no numerical data are included in this report (the only evidence presented is a picture of an old graft at the same stages of hair regrowth as the younger host), there is little reason to doubt its conclusion. This example gives an important insight into the nature of age changes: at least some age changes appear to reflect reactions of local cells and tissues to a new physiological milieu in senescent animals. Age changes in the hair regrowth cycle, like the rate of loss of oocytes described in Section IIIC9c, appear to be under the control of circulating factors.

d) Circulating factors

The importance of circulating factors in causing age changes has long been suspected. Carrel performed a fantastic experiment which is described by Du Noüy (1937):

> "....There was at the Rockefeller Institute, before the war, a dog nearly eighteen years old. This poor animal never stirred from its corner and could hardly get up to eat. He slept all day, his coat was coming out, his eyes were dim, and his eyelids stuck together.
>
> This animal was anaesthetized, put on the operating table and treated as follows. Carrel bled him by the carotid artery and removed nearly two-thirds of his blood. This blood was collected aseptically and immediately centrifuged, so as to separate the red cells from the serum. The red cells were washed in Ringer solution, recentrifuged and mixed with fresh Ringer solution to re-establish the initial volume of the blood. This was then re-injected to the dog. The circulation was restored by massaging the heart, and the skin was sewn up. A prince of royal blood, heir to the throne, on whom the peace of the world depended, could not have been the object of more attentive care than this old animal. After several days he had regained strength and appetite. The same operation was repeated so as to eliminate practically all the serum of his blood.... The animal lived. Not only did he live, but, once over the operative shock, he was a different dog. He ran and barked, a thing he had not done for years. His eyes were clear, his eyelids normal. His coat started to come in; he was gay, active, and most important of all, he was no longer indifferent to the charms of the other sex. He was regenerated."

We will never know how long the rejuvenated dog survived, or what its true ailment was. Nonetheless, this experiment has considerable historical interest, as it provided an early indication of the possible importance of circulating factors in ageing.

Another approach, also begun by Carrel, has been to study the rate of cell growth in sera from animals of different ages. In all cases, the rate of fibroblastic outgrowth was slowest in serum from old chickens, dogs, or cats (Carrel and Ebeling, 1921, 1922). The serum of nine year old chickens appeared to contain factors adverse to the cell proliferation; further growth of the culture did not occur unless the serum was diluted (Carrel and Ebeling, 1921). Fractions of the serum from old and young chickens (obtained by precipitation with CO_2) were found to differ in their content of "growth activating" substances (Carrel and Ebeling, 1923). A detailed discussion of these observations may be found in Strehler (1962).

Recent studies of the growth of alveolar wall cells in adult mouse lung have shown that sex differences of mitotic index in vivo were progressively eliminated during in vitro culture and that age differences (3 vs 18 months) were reduced below the level of statistical significance (Simnett and Heppleston, 1966). Similarly, the increase of mitotic index during short term organ culture of the prostatic epithelium eliminated the differences of mitotic index in vivo between 3.5 and 10.5 month old mice (Simnett and Morley, 1967). Therefore circulating factors may be an important cause of age and sex differences of the mitotic index in vivo.

The role of circulating factors in other age changes of cell activities is also strongly implied in the skin transplant experiments described in the preceding section. It would appear likely that many changes of senescence are under the control of circulating factors, as are the changes of an earlier, post-natal developmental transition, viz. puberty.

3) Conclusions

Although these preceding examples indeed encourage the notion of reversible age changes, the experiment of Albright and Makinodan (1966) discussed on page 83 introduces a cautionary restraint: the piecemeal rejuvenation of cell activities outside of the milieu of the ageing organism may not reveal the true extent of the ageing change and may not indicate the proper course to its amelioration. Furthermore, certain age changes may be reversible but may be unrelated to the true seat of the change in the physiological milieu, i.e., it may be impossible to reverse the increase in death rate by manipulating individual components of many single control loops in the homeostatic machinery. For instance, restoration of normal

gonadotrophin levels in post-menopausal women by estrogen injections may be easily accomplished (Frank and Salmon, 1935), but it is doubtful that this would significantly increase longevity, as spayed female rats (Talbert and Hamilton, 1965) and cats (Hamilton, 1965) have an average longevity at least equal to, if not longer than, that of intact animals. It appears that the seat of ageing changes in the Pacific salmon is the gonadal-adrenal-pituitary axis (see Section IIB2d). However, comparisons of ageing changes in humans and the Pacific salmon (Robertson and Wexler, 1960) show that senescence in humans and other mammals is not due to a single glandular change as in the adrenal hyperplasia of the Pacific salmon. If anything, the adrenal cortex tends to become slightly atrophied in ageing mammals (Korenchevsky, 1961).

F. Age changes in the integration of individual cell activities at the physiologic level (an experimental analysis)

1) Introduction

The preceding examples of reversible age changes in the activities of individual cells suggests that the cells of the body might live indefinitely if the body which supports them did not die. There is no evidence that the increasing mortality rate during ageing results from a diminution of the potential for cell activity, although changes in many cell activities, in fact, do occur.

The vital functions of the body are the result of the integration of activities of many cell types throughout the body. The age changes in circadian organization of cell activities (see Section IIIC7a2) hint that integrated physiological processes may be subject to change during ageing. In the present investigation, the integration of physiological processes known to play a major role in determining survival to environmental stress, was examined in an experimental analysis of age changes in the response of mice to cold.

2) Experimental animals

a) Source of mice

The mice used in this study were C57Bl/6J males, obtained at the age of 8 months as retired breeders from the Jackson Laboratory, Bar Harbour, Maine. A total of eight shipments of 100 mice each were kept as separate groups.

b) Maintenance and care

The mice were housed five per cage in double cages of the same specifications (12" x 5" x 6" high) used in the breeding colony at the Jackson Laboratory. The double mouse cages were obtained from Amesbury Metal Products Co., Amesbury, Mass. The ears of the mice in a cage were marked and the cages were marked according to group and cage number. Only mice of one age group were kept in the same shelf in the colony.

All mice used in this study were kept in a single room (D6 in the North Animal House) with a locked door. No other strains of mice or mice used by persons other than myself were kept in the colony room. The room temperature was maintained at 74 - 78°F. A program of light and darkness was scheduled on a Tork Timer at 7 p.m. (dark) and 7 a.m. (light).

The mice were cared for exclusively by Mr. Joe Shaw, a man who has had considerable experience in this job and whose handling of the mice was gentle and thoughtful. No other person besides Mr. Shaw and myself usually entered the room or ever handled the mice.

The water bottles were sterilized and changed twice per week. The cages were sterilized and changed once per week with fresh Lab Litter as a nesting material.

The diet given was the standard "Lab Chow" produced by Purina. Purina Lab Chow is notably lower in fat (4.3%) than most mouse chows (10-15%) which are designed for pregnant and nursing females. This fact may be of some consequence in determining longevity and lifelong weight patterns, and will be discussed in detail in Section IIIF2c5.

c) Long term observations of the colony of ageing mice

(1) General health

The colony appeared to consist almost entirely of healthy animals at all times. Mice, randomly sampled for enteric parasites, for bacteria of the genus Salmonella (with SS agar (Difco) and Selenite broth (Difco))and for surveys of lung, kidney, liver and heart histopathology yielded negative results on several occasions. I am much indebted to Dr. R. W. Leader, Dr. S. H. Weisbroth, Dr. A. Hurvitz, Dr. M. A. Rizack, Dr. R. W. Schaedler, Dr. J. B. Nelson, and Mr. S. Scher for assistance in these spot checks of animal health.

(2) Deaths due to fighting and other causes

A certain number of deaths initially resulted from fighting. A great deal of fighting occurred after the mice of group I and of certain other groups were transferred from the large cartons in which they had been shipped to the cages to which they were randomly apportioned in groups of five. In general, the fighting subsided within three weeks, perhaps indicating the establishment of a stable social hierarchy. In certain cages a particularly dominant mouse was identified by a complete absence of wounds, whereas the other mice in the cage had areas of exposed flesh. In such a case, the aggressive mouse was removed to a separate cage to spare the lives of the other mice in the cage.

Occasional instances of new outbreaks of fighting, indicated by fresh wounds, occurred in mice which had lived together in apparent peace for many months. The provocation for this is unknown. Perhaps it is related to the observation that in primate groups, an ageing leader who is no longer able to assert his position in the social hierarchy may begin to show changed behavior characteristics of his new relative social rank (Green, personal communication). If a reshuffling of social hierarchy occurred due to the weakening of an ageing, dominant mouse, contests of aggressiveness might arise within the group. The C57Bl derived strains of mice have been noted for a high incidence of fighting, particularly in groups of adults which are strangers to one another (Ginsburg and Allee, 1942).

After the initial period of mortality due to fighting, the mortality rate remained low in all groups (1% of any group died per month) until after the 20th month of age. The cause of incidental deaths in young or middle aged mice was not apparent. Occasionally all mice in one cage died because the stopper of the water bottle became loose and allowed its contents to spill into the cage. When this happened the mice usually died shortly thereafter unless the accident was discovered and the mice were immediately transferred to a cage with dry Lab Litter. Apart from such episodes, there were few incidents of death in which more than one mouse in the same cage died in the same 2 week period.

(3) Selective hair loss and regionalized wounding

In mice of certain cages (about 15% of the total number of cages) there was a pronounced regional loss of hair. The precise region so affected (snout,

top of head, flank, etc.) was characteristic of that particular cage of mice. Conspicuously, in any such group, one mouse was entirely unaffected. Because the hair of a partly depilated mouse began to grow back if the mouse was removed from the cage, I concluded that the unaffected mouse was probably nibbling at the others. There were no indications of mites or other ecto-parasites. Spontaneous, localized alopecia has been described in a C57Bl derived strain of mice and was found to be prevented by gonadectomy (Houssay and Higgins, 1949). Therefore, it is possible that hair nibbling resulting in alopecia is an aspect of social dominance in male mice. In wild rats (Rattus norvegicus and Rattus rattus) gentle nibbling of the fur on one rat by another has been observed, particularly in situations of conflict (Barnett, 1958).

One of the most bizarre aspects of the hair loss and of many instances of wounds in general is that all mice in that cage except one are affected. Furthermore, the loss of hair or the wound occur in exactly the same region of all affected mice. This was particularly striking in mice wounded repeatedly on their anal region or on the tip of the tail. No other region of their body was affected. These particular lesions persisted for well over a year in many cases. It might be concluded that the attacking, and presumably dominant, mouse had a long term preference or monomania for nibbling at other, presumably subdominant, mice in just one anatomical region.

(4) Absence of mixing of age groups

The detailed records of physical appearance and the ear marking system enabled me to recognize any mice which were accidently put in the wrong cage or with another age group. Such accidents are a nightmarish possibility to an experimenter who desires a uniform group of ageing animals. However, only two or three instances were recognized in over 18 months time, during which a total of 15,000 cage changing operations were accomplished in the colony. Therefore, any heterogeneity in the senescent mice is not due, to any significant degree, to misplacement in the colony.

(5) Body weight trends

The studies of Everett (Everett, 1957; Everett and Webb, 1957) demonstrated that a marked and prolonged loss of body weight (extending over a 3-4 month period) occurs before death in an Australian strain of white rat. It was evident from concurrent measurements showing a decreased food intake (Everett, 1958) that the rats Everett observed to be losing weight were in a very different physiological state than animals of the same chronological

age which were not. It was therefore anticipated that the metabolic patterns in the liver (the object of this study) might diverge considerably between animals of the same chronological age which were or were not losing body weight. The breeders of my mice at the Jackson Laboratory, Bar Harbour, Maine, informed me that a loss of body weight did occur during senescence in the C57Bl/6J strain of mice. A characterization of the body weight trend of each animal in the colony (about 750 mice) was therefore begun in August, 1967. However, weight charts of the mice have not revealed any general loss of body weight before death up to the age of 30 months. Table XVII shows the distribution of body weight before death in group I. Only 25% of the deaths of senescent mice were preceded by a weight loss of more than six weeks duration. It can be concluded that mice living in the conditions of this colony did not suffer weight loss as a general feature of their ageing up to the age of 30 months.

Table XVII

Weight Changes Before Death in Group I

Class of change	#	% total
Mice without weight change	23	59.0%
Mice with weight gain (usually tumour bearing)	6	15.4%
Mice losing weight for more than 6 weeks before death	10	25.6%

(The mice initially killed by fighting were not included in this sample)

Dr. John M. Yuhas of the Jackson Laboratory, Bar Harbour, Maine, has subsequently informed me that his colony of ageing C57Bl/6J male mice (also stocked with retired breeders from the main colony at Jackson Laboratory) tend to have a greater maximum body weight, show a marked and prolonged loss of body weight during senescence, and die at an average age which is 4 months younger than mice from my colony (28 vs 24 months respectively). The only difference in conditions which can be ascertained is the fat content of the diet: his mice eat a 12% fat diet, whereas my mice ate the 4.3% fat diet regularly supplied to mice in The Animal House of the Rockefeller University. This difference in diet is consistent with the higher average body weight of Yuhas' mice. The relationship of body weight to average longevity in mice is obscure.

It is apparent that mice from my colony had an unusually long average lifespan (28 months) for this strain in comparison with Yuhas' colony and

other colonies maintained at the Jackson Laboratory (Russell, 1966). The average lifespan of the C57Bl/6J mouse in colonies elsewhere (Goodrick, 1967b; Lang, 1969) is comparable to the values I have observed. Conditions at the Jackson Laboratory may be less than optimal.

The many measurements of body weight did serve the useful purpose of monitoring the condition of each mouse in the colony during most of its lifespan. Any unusual feature (wounds, weakness, etc.) was noted. These records comprise an impressive certificate of good health in my colony and underline the suitability and uniformity of these mice for use in experiments on ageing.

(6) Rate of mortality

One of the most reliable indicators of the progress of the ageing process in mammals is the rate of increase in mortality (Comfort, 1966; Jones, 1956). Detailed records were therefore kept of deaths in the colony. The details of the mortality curves were derived in particular from a group of mice denoted group I (95 animals) which was kept as a control group; no experimental mice were drawn from group I. The survival of group I, and the mortality rate of survivors as a function of age are given in fig. 2 and fig. 3 respectively.

(7) Ageing and the incidence of disease

I have observed (see fig. 3) that the mortality rate remained very low until sometime after the age of 20 months. According to Simms and Berg (1957), there is an increase in pathology which precedes in parallel the logarithmic increase in mortality rate. Although I cannot demonstrate this fact in detail for my mouse colony (a project of major dimensions), I can unequivocally state that the incidence of pathology (tumours, lung disease, liver and kidney abnormalities, etc.) did increase dramatically during the period of increasing mortality: abnormal features were almost never found in young mice; at autopsy, abnormalities were found in only 2 or 3% of middle aged mice, whereas obvious pathological abnormalities occurred in 10 to 20% of mice 24 months or older.

The most frequently found pathologic abnormalities (which inevitably lead to death) were tumours of mesenteric lymph nodes which sometimes appeared to be concurrent with hypertrophy of the liver. In such cases, the grossly enlarged liver was discolored and fibrotic. Mice so afflicted were easily identified because of atypical increases of body weight and obviously

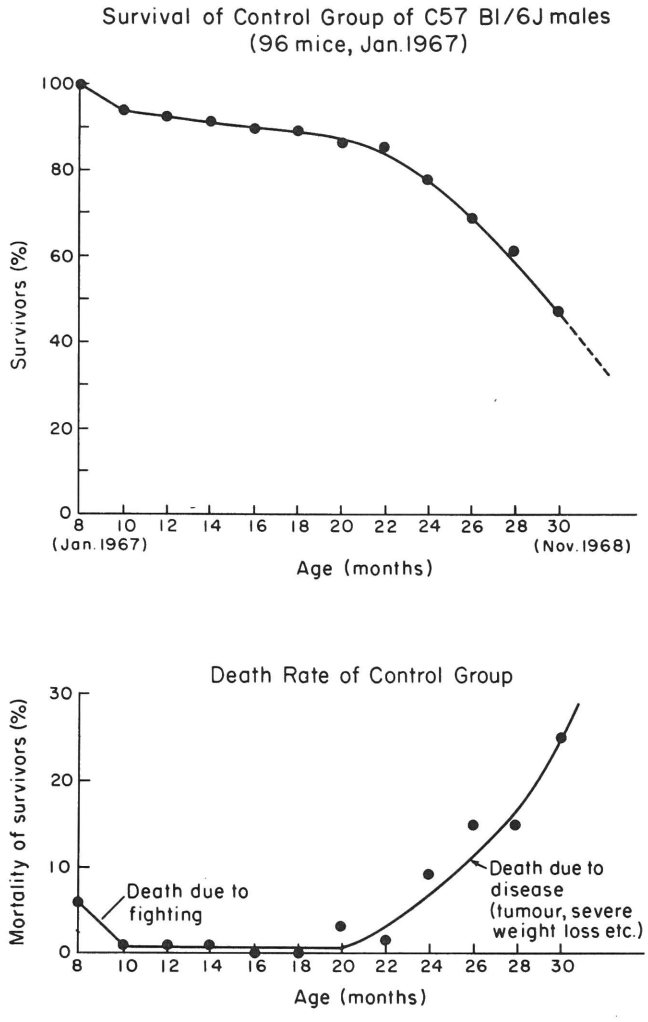


Figure 2(top): Survival of the control group as a function of age. The data were determined from colony records of a group of 96 mice.

Figure 3(bottom): The death rate of the mice in figure 2 as a function of age. This graph represents the percent of survivors at any age which died during the next two month interval. Calculated from figure 2.

enlarged abdomens. About 15% of all deaths in mice aged 20 months or more occurred as the result of this condition (see table XVII). From records of autopsy of experimental animals, 5 out of 133, or 3.8%, had obvious tumors of mesenteric lymph nodes and 7 out of 133, or 5.2%, had obviously abnormal livers of diverse descriptions. Various C57Bl derived strains have been noted for the prevalence of reticular cell neoplasia which may involve the liver as well (Russell, 1966; Murphy, 1966). Histological study of liver tissue from randomly chosen healthy appearing older mice from my colony did not disclose any abnormality, although slight hyperplasia of bile duct endothelium was occasionally observed in mice of all age groups (for description of this study, see pg. 160).

The mortality rate of groups of mice in the same room caged only a few feet apart differed strictly according to age (fig. 4). This indicates that no ordinary episode of communicable disease can account for the increasing mortality with age in the mice of this colony.

3) Experimental studies of cold stress

a) Age differences in survival during cold stress

Cold stress quickly reveals the condition of senescence in mice. Mice of different age groups were fasted for 20 hours and then put into individual, clear glass jars (2 quart size), which had been pre-chilled to 9-10°C. A brief fast of 20 hours preceeded most experiments in order to minimize individual differences in the pattern of feeding. A 20 hour fast will eliminate stores of liver glycogen which would vary according to the recentness of feeding (Ekman and Holmgren, 1949). Large liver glycogen stores would be expected to increase survival during cold stress. No food or water was given subsequently. Death resulting from starvation occurs after a 5-6 day fast in young C57Bl/6J male mice (Finch, unpublished). The mean survival time during fasting does not change in rats between the ages of 6-26 months (Jakubczak, 1967). Age differences in survival during cold stress therefore cannot be attributed to changes in the ability to survive a brief (< 60 hours) fast.

The length of time the mice were able to withstand the stress of cold was an inverse function of age. Senescent mice die, on the average, before young mice, as is demonstrated in figure 5. The data of several such experiments are given in figure 6. These results are in agreement with other

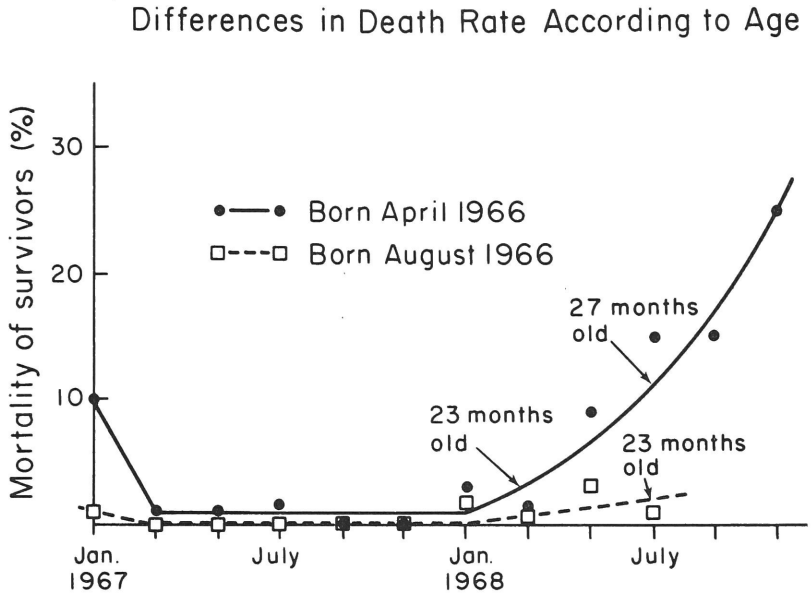


Figure 4: Death rate as a function of age in two groups of mice.

The data have been calculated as described in the legend of figure 3 for two groups of mice. The mice born April, 1966, were previously described in figures 2 and 3. The mice born August, 1966, numbered 99 on January, 1967. It is evident from this graph that the death rate remains low until about the 23rd month of life and increases noticeably thereafter.

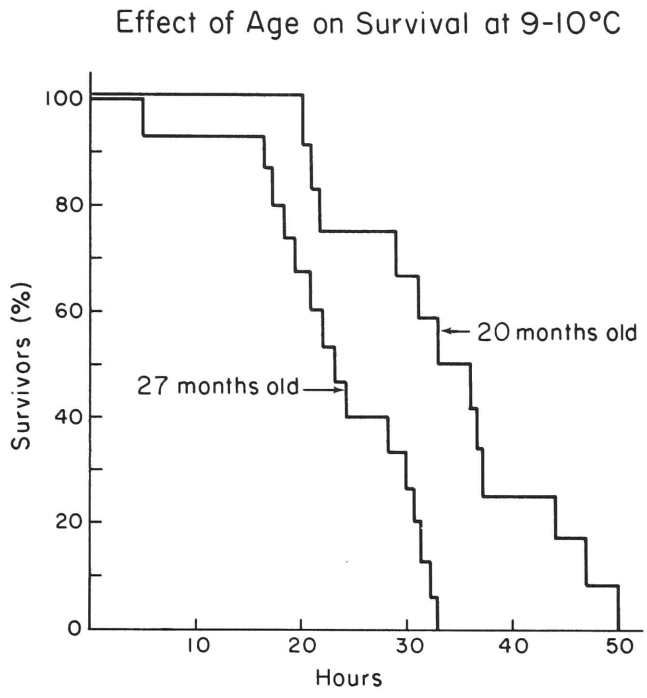


Figure 5: The effect of age on survival at 9-10°C.

Mice, previously fasted for 20 hours, were placed singly in two quart glass jars without food, water, or nesting materials. The number of mice surviving at any time was recorded as the number of mice which had not lost their balance (lying on their sides, etc.). 16 mice aged 27 months, 13 mice aged 20 months.

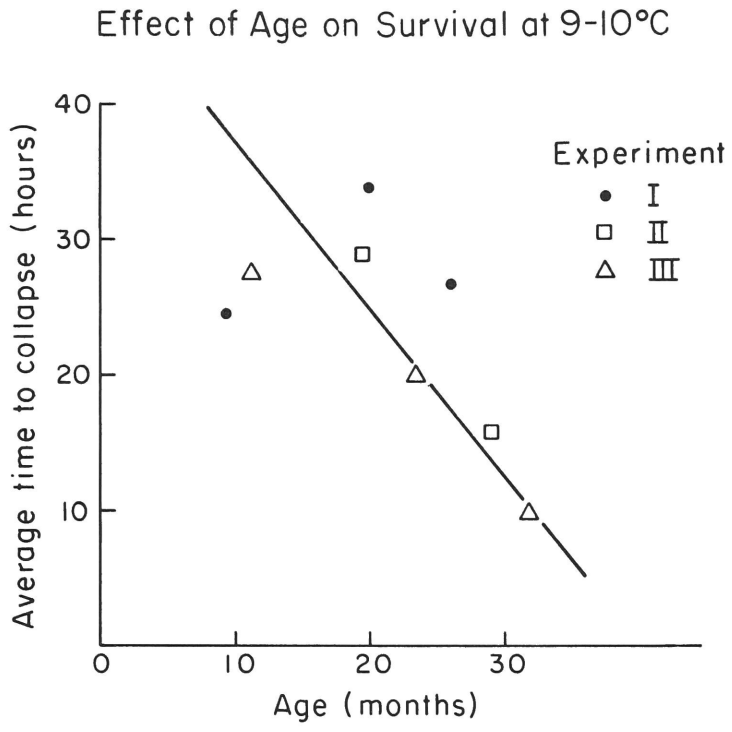


Figure 6: The effect of age on average survival time at 9-10°C.

Data from several experiments conducted as described in the legend of figure 5. Average time to collapse denotes the average length of time which mice were able to maintain their balance. 10-15 mice in each age group in any experiment.

observations on the greater mortality rate of senescent mice in the cold (Grad and Kral, 1957; Trujillo et al, 1962). In these studies, food and water were available ad libitum, but nesting materials were not provided.

I have found no significant age differences in mortality if the cold exposure is 5°C or lower (see figure 7). This is in agreement with previous observations (Grad and Kral, 1957).

It should be remembered that mice and rats can survive and breed remarkably well at below zero temperatures if nesting materials and adequate food and water are provided (Barnett, 1965). The importance of separating the mice so as to prevent mutual conservation of body heat by huddling during experiments involving cold exposure has been noted (Grad and Kral, 1957). Huddling appears to be a key factor in thermoregulation and survival of small mammals, particularly during their immediate post-natal period (King and Connon, 1955).

b) Age differences in behaviour during cold stress

Although age differences in mortality rate become significant only after many hours at 9-10°C, age differences in the response to cold are almost immediately evident. Thus, it was always observed that senescent and middle aged mice (16-28 months old) made more jumping movements toward the top of the jar during the first 30' at 5-11°C than did mice 6 months or younger. These jumps appeared to be attempts to escape from the jar. During this same period of time, the young mice sat on their haunches and appeared to be quite undaunted by the cold. Eventually, the younger mice, too, began to jump, as shown in figure 8. (The number of jumps in an episode of jumping and the number of episodes of jumping varied considerably from mouse to mouse. It was not possible to record these details by simple observation; any age differences in these parameters remain to be elucidated.) These behavioural changes are the reflection of underlying age changes of the response to cold. This is directly revealed by measurements of body temperature during cold stress.

c) Age differences in the ability to maintain body temperature during cold stress

The internal temperature of the mice before and during cold stress was measured by gently inserting a thermocouple 4-5 cm. through the anus into the colon, after first causing defecation by abdominal massage as cautioned by Barnett (1956). The thermocouple was carefully calibrated at 25°C and

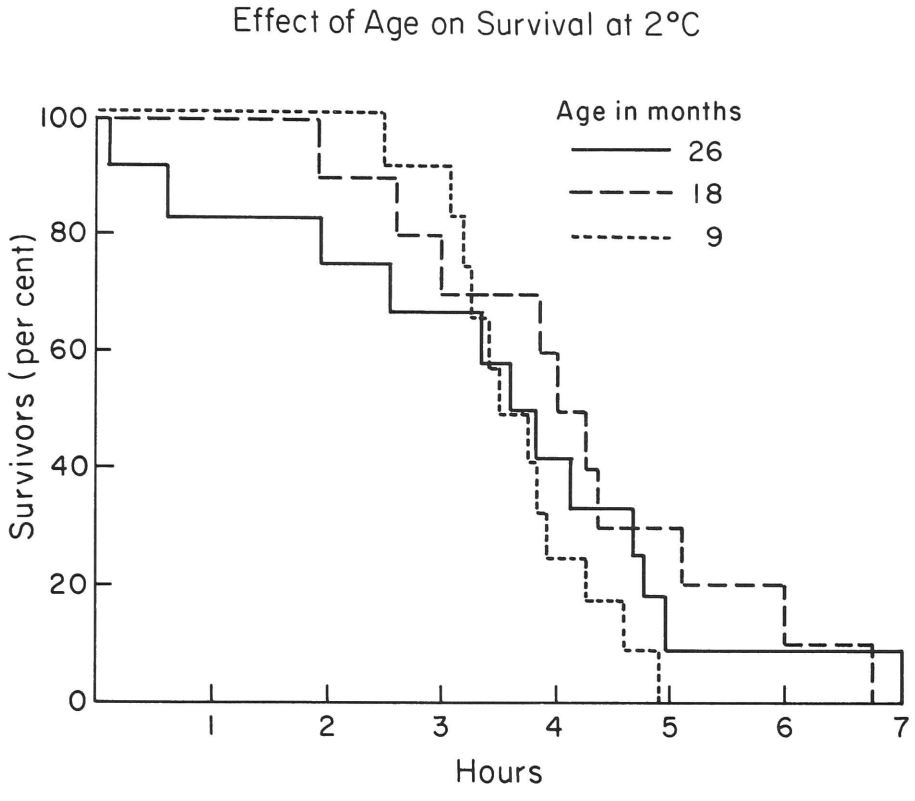


Figure 7: The effect of age on survival at 2°C.
Experiment conducted as described in the legend of figure 5.

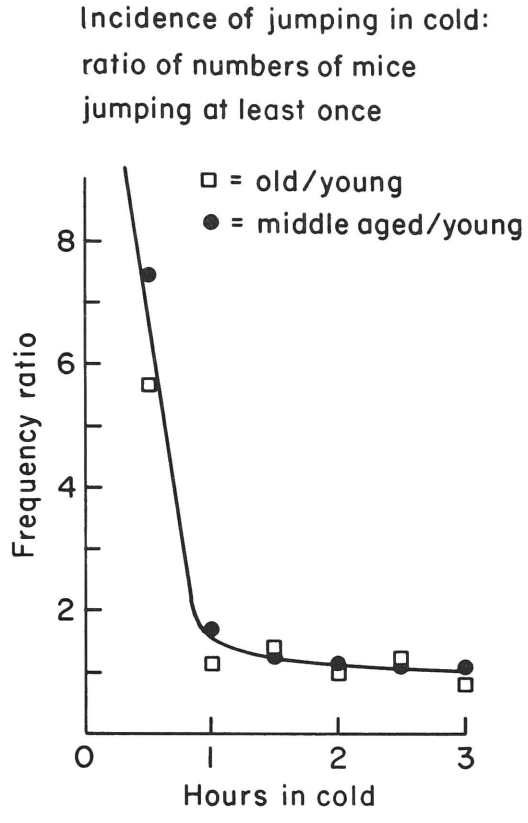


Figure 8: The effect of age on behavior in the cold.

Experiment conducted as described in the legend of figure 5. The ratio of the total number of mice at any time which had exhibited jumping behavior (middle aged and senescent) to the total number of younger mice which had exhibited jumping behavior during cold stress at 5°C. 12 mice 9 months old, 10 mice 18 months old, 12 mice 26 months old.

37°C. A statistically significantly age difference ($P < .01$) in colon temperature was found at an ambient temperature of 20°C: in mice 10 months old, the colon temperature was 35.9 ± .05°C; at 30 months, the temperature was 34.8 ± .3°C (± S.E.M., 7 mice per age group). The values for young mice are lower than usual because of the 20 hour fast before the experiment. (The colon temperature of non-fasted young mice at the same time of day was measured to be 36.7 ± .05°C (n=8), in agreement with internal temperatures recorded for another C57Bl mouse strain (McLaren, 1961) and other strains of mice as well (Weir, 1947).)

A series of measurements made on these same mice during cold stress at 9-10°C revealed a marked and highly significant age difference in the ability to maintain body temperature as shown in figures 9 and 10. There were large decreases in body temperature in all senescent mice within 0.75 hours of cold exposure; the loss of body temperature was greatest in senescent mice whose initial body temperature was less than 35°C. In contrast, the average body temperature of young mice did not decrease significantly until after 2 hours of cold exposure. Ageing has clearly resulted in a change in the regulation of body temperature.

The ability of senescent mice to maintain body temperature was investigated further by studies of cell activities in 2 organs concerned with thermogenesis in the cold exposed mammal, viz. the liver and the adrenal cortex. Cell activities in these organs were examined with the hope of detecting age changes in the regulation of hormonal and nutritional factors at a supra-cellular, physiological level which might be reflected in some aspects of cell function.

d) Studies on tyrosine aminotransferase (TAT) regulation in the liver

1) Introduction

Pre-eminent among the responses of a homeothermic mammal to cold is the effort to maintain body temperature. Within a very short time in young mice, the neuroendocrine system initiates the well known changes in metabolism in which caloric reserves are mobilized throughout the body according to their lability (e.g., liver glycogen before proteins). The liver is an important site of the increased metabolic activity which results from cold exposure, manifested for instance as hepatic hypertrophy (Chevillard and Mayer, 1939). An indication of age changes in thermogenesis was sought in the regulation of a liver enzyme which plays a role in amino acid catabolism, tyrosine aminotransferase.

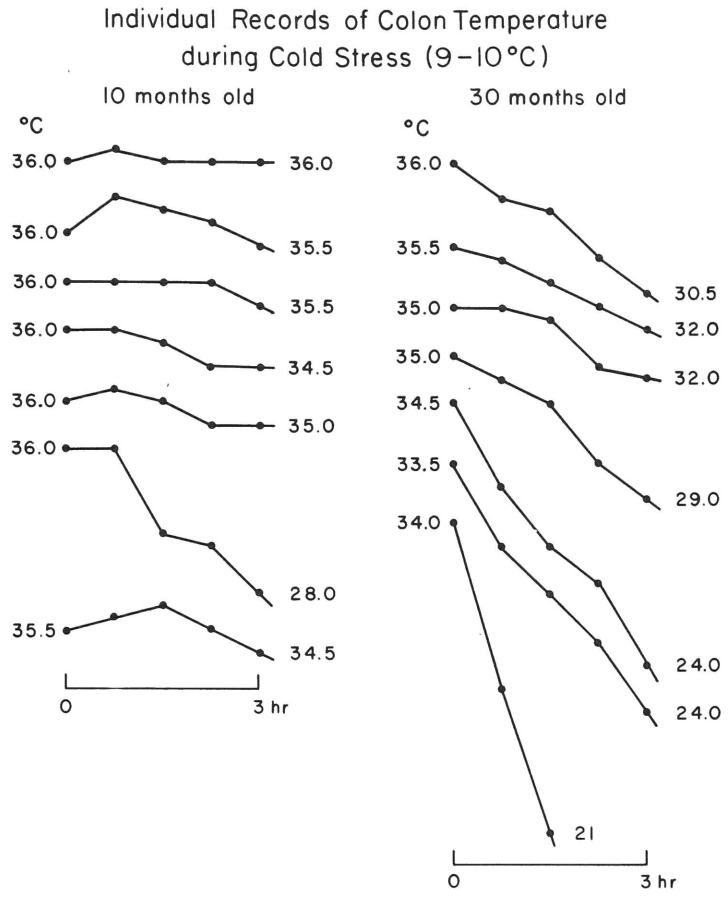


Figure 9: The effect of age on colon temperature during cold stress (individual records).

Experiment conducted as described in legend of figure 5. Colon temperatures were measured by inserting a thermocouple 4-5 cm. through the anus of mice before and during cold stress.

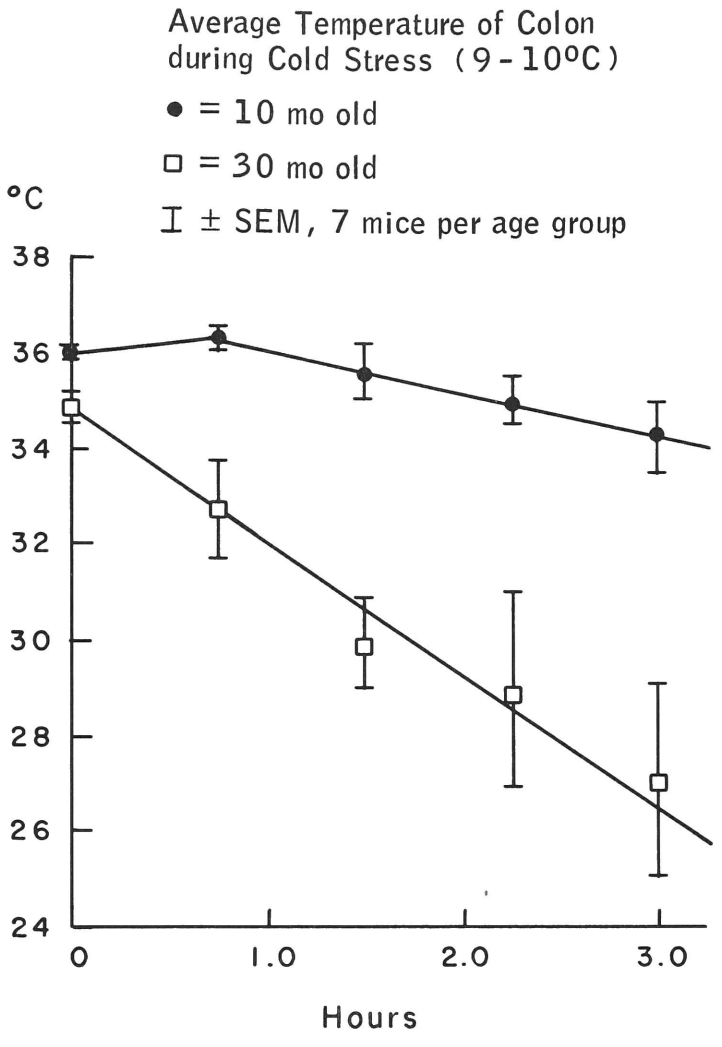


Figure 10: The effect of age on colon temperature during cold stress (average values).

The data are calculated from figure 9.

Tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, E.C.2.1.6.5) is known to catalyze the first stage of the catabolism of tyrosine in the liver (Knox and LeMay-Knox, 1951; Schepertz, 1951). One study indicates that phenylalanine and tryptophan may also be transaminated by this same enzyme (Jacoby and La Du, 1962). The most interesting feature of TAT (tyrosine aminotransferase) is the rapidity with which the activity is adjusted according to hormonal and nutritional stimulae. It was discovered by Lin and Knox (1957) that intra-peritoneal injections of hydrocortisone or large amounts of tyrosine result in a rapid, 8 fold increase of TAT activity within 5 hours. Induction of TAT has been found to occur only in the liver, although TAT activity is present in many tissues (Lin and Knox, 1958). Subsequently, in numerous investigations, a broad spectrum of agents and physiological circumstances have been recognized to result in rapid changes in TAT activity in the liver of the rat, the animal almost exclusively employed in studies of TAT. For instance, a rapid induction of TAT results from injections not only of hydrocortisone (Lin and Knox, 1957), but also of insulin (Holten and Kenney, 1967) and glucagon (Holten and Kenney, 1967). The stress of laparotomy (Tsukada et al, 1968) and the stress resulting from the injection of large amounts of peritoneal irritants, such as suspensions of diatomaceous earth (Celite), tyrosine, or tryptophan (Kenney and Flora, 1961), also result in a rapid induction of TAT. A striking 24 hour cycle of activity, in which TAT increases coincide with the onset of the nychthemeral cycle of feeding and locomotor activity, has also been observed (Potter et al, 1966; Wurtman and Axelrod, 1967; Watanabe et al, 1968). On the other hand, injections of growth hormone have been observed to result in a rapid decrease in TAT levels (Kenney, 1967). Experiments have shown that perfusion of the isolated rat liver with hydrocortisone, insulin, or glucagon results in an immediate increase of TAT activity, indicating a direct action of these hormones on the liver (Goldstein et al, 1963; Hager and Kenney, 1968). However, no change in TAT activity occurs during the perfusion of growth hormone through the isolated liver, implying that this hormone acts indirectly (Hager and Kenney, 1968).

Rapid increases of enzyme activity in liver may be the result of (1) activation of an inactive precursor, as in the case of the phosphorylase and glucose-6-phosphatase increase which result from stimulation of the splanchnic nerve (Shimazu and Amakawa, 1968); (2) decreased rate of degradation,

as in the tryptophan mediated increases of tryptophan pyrrolase (Schimke et al, 1960); (3) increased rate of synthesis, as in the case of hydrocortisone mediated increases of tryptophan pyrrolase activity (Schimke et al, 1960). The studies of Kenney (1962) have unequivocally demonstrated that the hydrocortisone mediated induction of tyrosine aminotransferase is due to an increased rate of synthesis of the enzyme. In these elegant studies, radioactively labelled TAT was isolated by the use of specific antibodies prepared against the highly purified enzyme. During the time of increased incorporation of radioactive amino acids into TAT after hormonal injection, there was no effect on the labelling of total, soluble liver proteins.

The induction of TAT is known to be inhibited by Actinomycin D administered either prior to (Greengard and Acs, 1962) or simultaneously with (Csány et al, 1967) hydrocortisone. This fact implies that the induction of TAT is dependent on gene activity.

(2) Assay

TAT was assayed by the method of Lin and Knox (1957) under experimentally verified conditions of substrate and cofactor excess. Each assay consisted of the following components.

1) 2.5 ml of 0.5 M Na_4BO_4(pH.8.0), which contained 0.004M l-tyrosine (Merck) and 0.025M 2-oxoglutaric acid (Mann).

2) 0.5 ml of 0.002M pyridoxal phosphate (Sigma)

3) 0.05 ml of 0.15M diethyldithiocarbamate (Matheson, Coleman, and Bell).

4) 0.15 ml of 1:10 dilution of tautomerase (p-hydroxyphenylpyruvate enol-borate tautomerase) (beef kidney, grade I; Sigma). All batches of tautomerase were found to be free of detectable TAT activity when tested in this assay by omitting the liver extract.

5) 0.1 ml liver extract (described below)

The assay was conducted in covered, 1 $cm.^2$ cuvettes at 34°C in a water-jacketed cuvette holder whose temperature was regulated by a Haake circulating water bath. The spectrum of the enol-borate complex of p-hydroxyphenyl pyruvic acid, the final product of the enzyme reactions, was found to have its maximum absorbance at 310mμ, as expected (Knox and Pitt, 1957). A linear rate of product formation was established after a pre-incubation of 3 minutes. The proportionality of the rate of reaction to the concentration

of liver extract was verified during each set of measurements. The activities of mixtures of extracts from mice at different ages were additive.

As a rule, a small piece of liver tissue (0.2 gm wet weight), which had been rapidly frozen with dry ice, was homogenized at 2-4°C in 8.0 ml of 0.14M KCl in a loosely fitting, motor driven Ten Broeck homogenizer (6 double strokes). After centrifugation (5,000 g for 10'), the supernatant extract was decanted and analyzed for TAT activity. A second cycle of homogenization and extraction of the centrifugal pellet did not liberate more than 5% of the previously extracted TAT activity. There was no age difference in the extractability of liver tissue during this procedure..

No loss of TAT activity was observed upon freezing and thawing of liver tissue prior to extraction, in accordance with Kenney and Flora (1961).

The amount of TAT activity was expressed per unit DNA as in the experiments of Grossman and Mavrides (1967). This convention was adopted instead of the usual convention of expressing enzyme activity per unit protein in the extract, because it was anticipated that the stress experiments would result in a change of liver protein content, whereas the DNA content, reflecting the number of cells, would be unaltered during the same time period. In some preliminary experiments, TAT activity was expressed in units of liver mass normalized for body mass (TAT/liver of 100 gm body weight).

The pellet of the centrifuged liver homogenate was found to contain 98% of the DNA of the homogenate as measured by the Burton modification of the diphenylamine reaction (Burton, 1956).

(3) Nychthemeral variations

Studies of TAT must take into account the large variations of activity which occur during a 24 hour period (Potter et al, 1966; Wurtman and Axelrod, 1967; Watanabe et al, 1968). I therefore investigated the schedule of these variations so that changes in enzyme activity observed during the course of an experiment could be properly attributed to the experimental stimulus as opposed to changes occuring as part of the nychthemeral cycle of activity.

A preliminary experiment with mice aged 6 months is shown in figure 11. The mice, caged in groups of 5, were randomly sampled during a 24 hour period. The peak of TAT activity occurs before 11 p.m., the levels gradually declining during the night and reaching a low level which was maintained for several hours during the morning. The onset of the increase of TAT activity

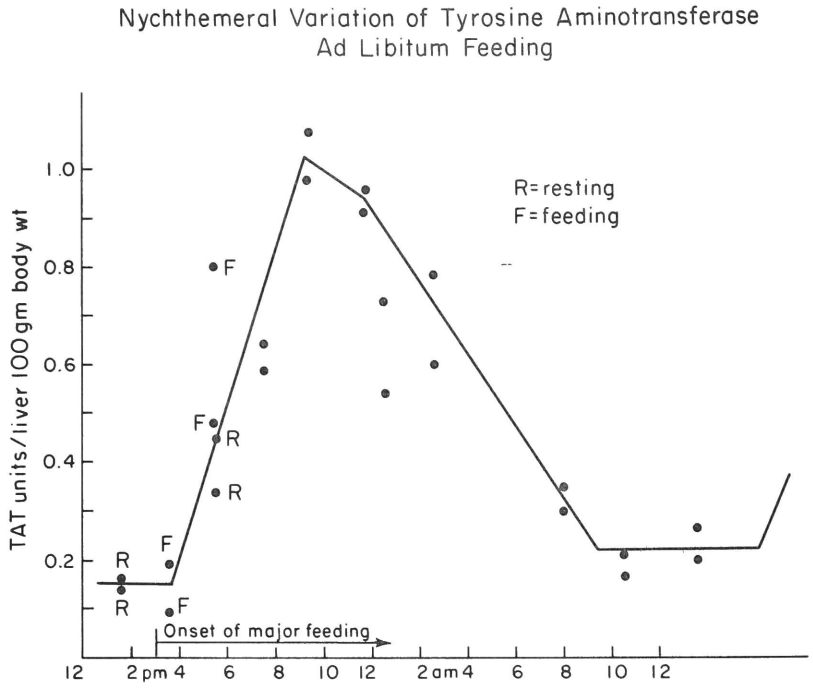


Figure 11: Nychthemeral variation of TAT during ad libitum feeding.

TAT levels in young mice were determined in mice randomly sampled during a 24 hour period. Food and water were available ad libitum. The lights were scheduled to go on at 7 a.m. and off at 7 p.m. Each point represents the TAT activity in the liver of a single mouse.

was concurrent with the onset of feeding and locomotor activity in the late afternoon. There were marked differences from cage to cage in the time at which awakening occurred, even if the cages were adjacent. This appears to be the origin of some of the heterogeneity observed. In accordance with other observations on mice (Anliker and Mayer, 1956), the feeding activities appeared to end by the early morning.

To reduce the variations in time of feeding, mice were trained for 5 days to eat on a rigid schedule; food was withdrawn after 3 hours. It can be seen in figure 12 that the individual variation is somewhat reduced.

Experiments henceforth employed mice which had been fasted overnight (20 hours) to eliminate irregularities in feeding or mice which had been trained to eat on a rigid schedule. Experiments were conducted, with certain exceptions, between the hours of 9 a.m. and 2 p.m. when the levels of TAT are subject to only gradual changes.

(4) Sources of variation in TAT levels

Despite close control over the feeding schedule, some variation of basal levels persisted. Certain possible causes were considered.

(a) Error in assay

All measurements of TAT activities were based on duplicate assays of each liver extract. Assays were repeated if the two measurements of enzyme rate varied more than $\pm$ 5% of their average. In general, the TAT assay was highly repeatable.

Measurements of DNA were also based on duplicate assays. The variation was less than $\pm$ 2% of the average.

(b) Regional hepatic differences in TAT content

To avoid possible inter-lobular differences in TAT activities, hepatic tissue was sampled only from the distal portion (0.2gm) of the left lateral lobe.

Therefore, the individual variations in TAT activity, which were found to occur even in singly caged mice on a rigid feeding schedule, cannot be readily controlled or accounted for.

(5) Preliminary experiments on the induction of TAT

Preliminary experiments to test the responsiveness of the C57Bl/6J male

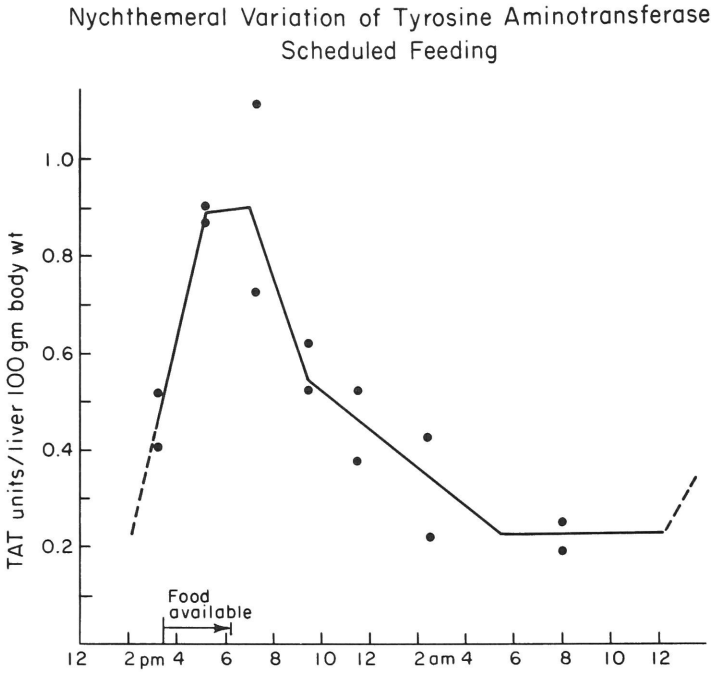


Figure 12: Nychthemeral variation of TAT during scheduled feeding.

Young mice. For five days preceeding the experiment, food was available only three hours a day (3 - 6 p.m.). Water was available throughout. The mice were kept in individual cages. Lights were scheduled to go on at 7 a.m. and off at 7 p.m. Each point represents the TAT activity in the liver of a single mouse.

mouse liver to inducers of TAT (previously published experiments were performed almost exclusively with rats) resulted in the usual rapid increase to 5-6 times the basal level with 6-8 hours, followed by a rapid decline towards basal levels (figure 13). In this experiment, the inducing agents (administered i.p. with a 23Gl needle) were corticosterone suspensions (3 mg/100 gm body weight, in 0.9% NaCl at 5 mg/ml) and casein hydrolysate solutions (Difco Casamino Acids, an acid hydrolyzed casein product; 0.25 gm/100 gm body weight, in 0.9% NaCl at .250 gm/ml). The course of changes in TAT activity was much the same with these two agents.

(6) The induction of TAT by cold stress

(a) The exploratory experiments

In published experiments, the stress of laparotomy and of the injection of various peritoneal irritants had been observed to cause a rapid increase of TAT. It was considered plausible that the stress of cold might also result in an increase in TAT, particularly because of the role TAT plays in catabolic processes which are of vital importance in the maintenance of body temperature during cold stress. This expectation was amply confirmed in numerous experiments.

An exploratory experiment with adult (9 month old) mice placed singly in pre-chilled jars is given in figure 14. It is clear that large increases in TAT occurred upon exposure to cold; all mice fasted for 20 hours before the cold exposure showed a large increase in TAT activity. Ad libitum fed mice tended to have a smaller increase in TAT, although the difference between the means of the 2 groups was not statistically significant (P=0.9). It was noted in this experiment that fasted mice succumbed more rapidly to the cold than did fed mice; this is probably the result of smaller metabolic reserves at the beginning of the experiment beacuse of the overnight fast.

As exposure to 2°C resulted in a rapid rate of collapse after 3 hours, an experiment was performed in which fasted mice were exposed to 2°C for 0.75 hr. or 1.5 hr. The mice were then removed from the cold jars and returned to a cage at room temperature. As seen in figure 15, there is a rapid increase in TAT activity which continues after the mice have been removed to room temperature. The maximum level of activity is related to the length of cold exposure. Subsequent decline of TAT toward basal levels occurred as in the hormonal inductions of TAT. It is clear that the TAT levels are sensitively regulated by physiological conditions during and after cold stress.

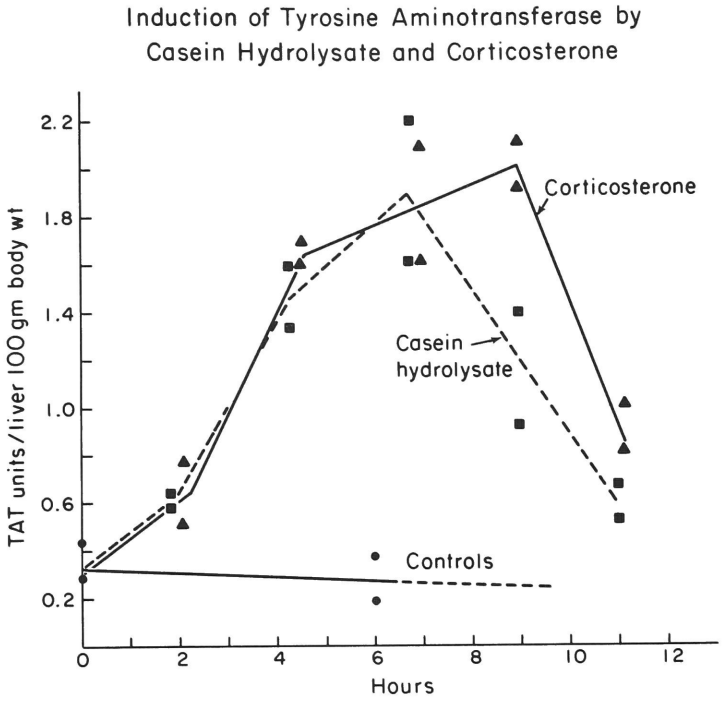


Figure 13: Induction of TAT by corticosterone and casein hydrolysate.

Young mice were fasted for 20 hours and then injected i.p. with corticosterone suspensions (3 mg/100 gm. body wt, in 0.9% NaCl, 5 mg/ml.) and casein hydrolysate solutions (Difco Casamino Acids; 0.25 gm/100 gm. body wt., in 0.9% NaCl, .25 gm/ml.) at 9 a.m.

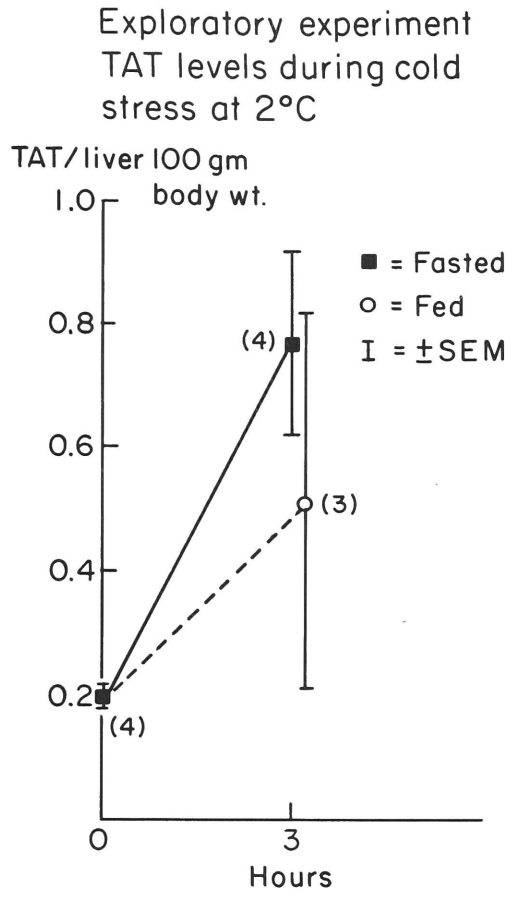


Figure 14: Exploratory experiment on the influence of cold stress on TAT levels.

Young mice, fed ad libitum or fasted for 20 hours, were exposed to cold as described in the legend of figure 5. The number of mice is given in parenthesis.

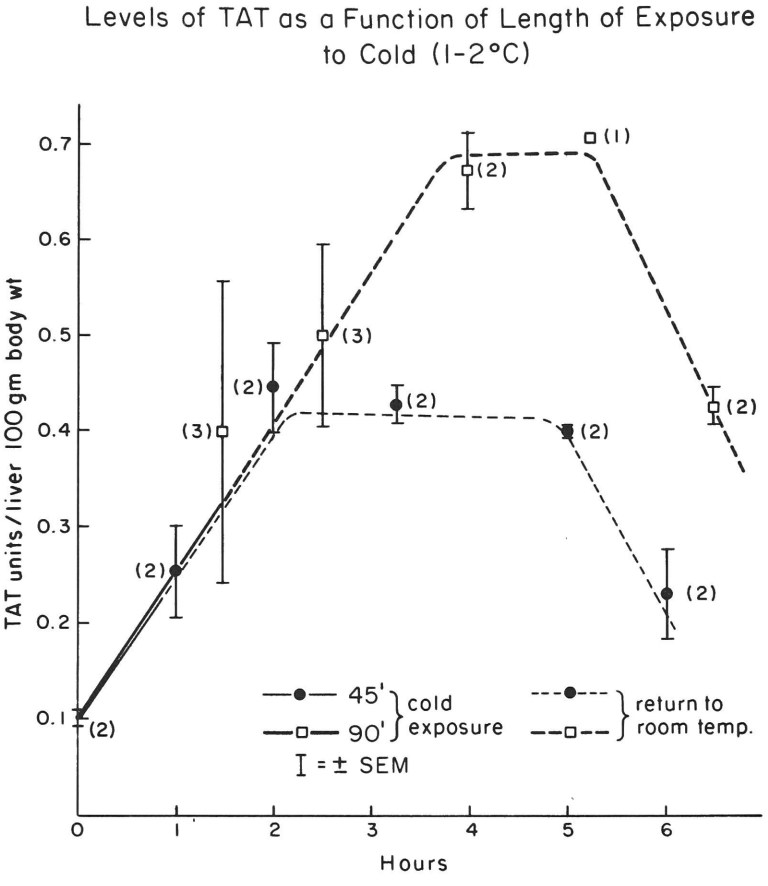


Figure 15: Levels of TAT as a function of the length of exposure to cold.

Young mice, fasted for 20 hours, were exposed to cold as described in the legend of figure 5. At 45' and 90' mice were removed from the cold jars and returned to a cage at room temperature. The number of mice is given in parenthesis.

(b) Rapidity of induction

The rapidity of the induction of TAT during cold stress is shown in figure 16. Statistically significant increases were evident in almost all experiments with young fasted mice within 1 hour of exposure to cold.

(c) Inhibition of TAT induction by cold stress with Actinomycin D

Actinomycin D (generously provided by W. B. Gall of Merck, Sharp, and Dohme) injected intra-peritoneally (2mg/100 gm. body weight) 15' before exposure to cold, abolished the increase of TAT in fasted, young mice (see figure 16). Studies, not described here in further detail, had shown that this dose of Actinomycin D was sufficient to halt the incorporation of injected H^3-orotic acid into liver RNA within 15'. The induction of TAT by cold stress is, therefore, dependent on gene activity.

(d) The influence of the post-prandial interval

In the exploratory experiment of this series (figure 14), it was noted that the induction of TAT by cold stress appeared to be more vigorous in mice which were fasted overnight as opposed to non-fasted mice.

The influence of the post-prandial interval on the induction of TAT by cold stress was investigated by testing the response of mice which had been trained to eat their daily food ration in 4 hours time. A feeding schedule was rigidly adhered to for 7 days, in which food pellets were available only from 1-5 p.m. The room was lighted from 7 a.m. to 7 p.m. The mice were singly caged and quickly appeared to become synchronized in their patterns of feeding and spontaneous activity. In these experiments, the post-prandial interval is measured as the length of time from the beginning of the most recent period of feeding to the beginning of the experiment.

In a preliminary experiment, not given in further detail, mice were exposed to cold (9-10°C) at different times post-prandium. No statistically significant increase was found 12 hours post-prandium, whereas a vigorous increase occurred 20 hours post-prandium. This result posed the question: is the liver itself at 12 hours post-prandium refractory to the physiological stimulae resulting from cold exposure (unknown at this time) which act on the liver to induce TAT within 8 or more hours later? On the other hand, the liver may be capable of responding, although the usual physiological stimulae to the liver may not be elicited in a recently fed mouse which is replete

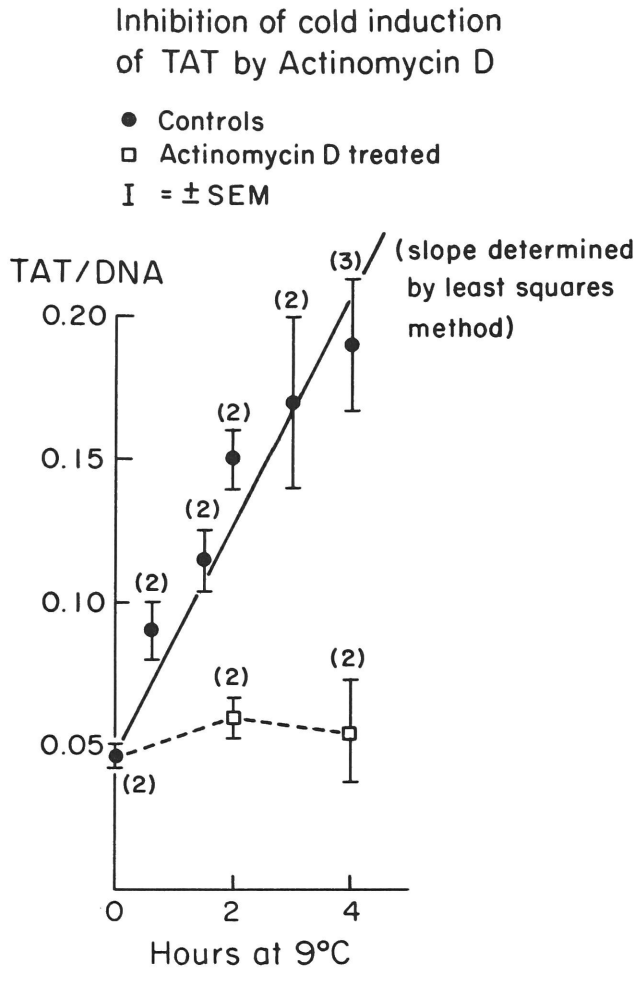


Figure 16: Inhibition of cold induction of TAT by Actinomycin D.

Young mice, fasted for 20 hours, were exposed to cold as described in the legend of figure 5. Four mice were given i.p. injections of Actinomycin D (2 mg/100 gm body wt.) 15' before the beginning of cold exposure. The mice injected with Actinomycin D did not appear less able to bear the cold stress than the untreated controls. The number of mice is given in parenthesis.

with metabolic reserves. This point was clarified by challenging the ability of the liver in recently fed mice to respond to injected hormones.

The results of such an experiment are shown in figure 17. The absence of a response to cold exposure (9-10°C) is clearly shown 12 hours post-prandium, whereas i.p. injections of insulin (0.75 i.u. Iletin/100 gm body weight) resulted in a large TAT increase during the same period. The usual increase of TAT was observed at a later time, in this experiment 20 hours post-prandium. It is concluded that the biosynthetic machinery of the liver cell can function in the production of new TAT 12 hours post-prandium, although the physiological stimulae elicited by cold exposure 20 or more hours post-prandium which would induce TAT, are either not elicited or their action is inhibited at shorter post-prandial intervals. The results of this experiment demonstrate the sensitivity of the stress-mediated enzyme induction to the physiological condition of the mouse.

The fact that the liver cells are capable of renewed synthesis of TAT in response to insulin immediately after the major TAT increase which follows feeding corroborates similar results of repeated injections of hydrocortisone which cause a "super-induction" of TAT without the usually ensuing decrease in activity (Grossman and Mavrides, 1967). The ability of the liver to make TAT is evidently not exhausted by rapidly succeeding stimulae.

(7) The induction of TAT by shaking stress

It was found in each of 4 experiments that 30' of shaking on a linear shaker (180 excursions/min., 4" stroke length) resulted in a rapid, transitory increase in enzyme activity, in which the basal level was nearly regained by 3 hours (figure 18). In these experiments, the open double cage containing the mice was fixed to the shaker so that the motion was perpendicular to length of the cage. Within a few minutes, the mice arranged themselves in the cage so that they did not collide either with each other or with the walls of the cage. The mice were fasted for 20 hours as usual before the experiment.

The maintenance of balance during this stress obviously required enormous muscular effort and severely challenged the metabolic resources of the mouse. Measurements of internal temperature were made by inserting a thermocouple into the colon as described in Section IIIF3c. As seen in table XVIII, mice stressed by shaking had a significant and transient decline of body temperature.

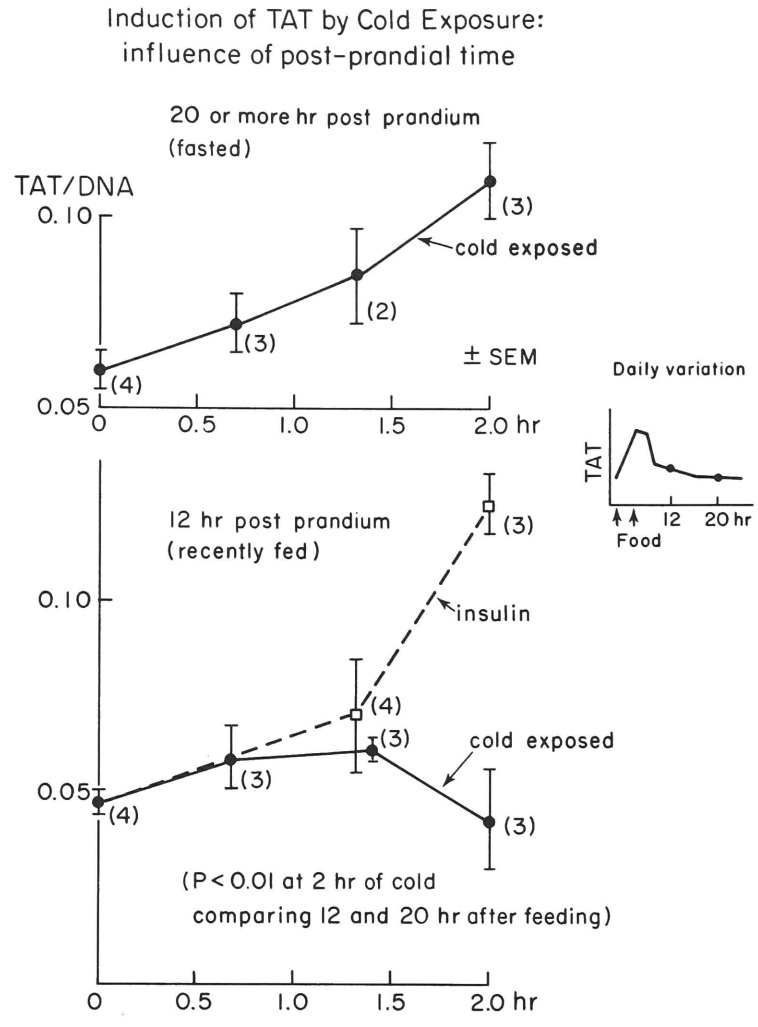


Figure 17: The influence of the post-prandial time on the induction of TAT by cold exposure.

Young mice were trained to eat their daily rations between 1 and 5 p.m. Other details of training as given in legend of figure 12. Mice were exposed to cold as described in the legend of figure 5 at 12 hours or 20 hours post-prandium (recently fed vs. fasted). At 12 hours post-prandium some mice (interrupted line in lower graph) were injected with insulin (0.75 i.u. Iletin/ 100 gm body wt.) but were not exposed to cold. The small graph represents the nychthemeral variation of TAT for reference (data of figure 12); the two points on the line of TAT activity indicate when groups of mice were removed from the colony and exposed to cold. The number of mice is given in parenthesis.

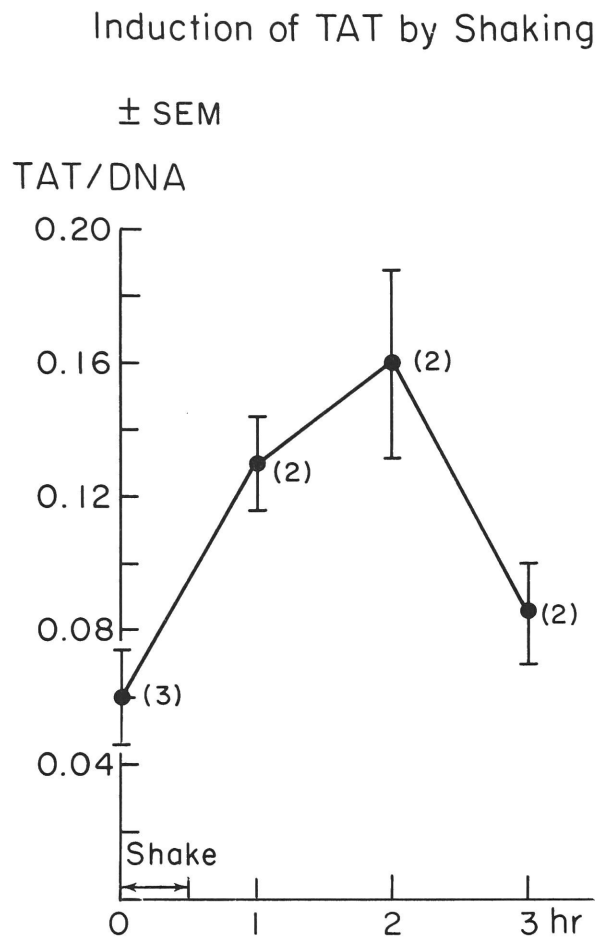


Figure 18: The induction of TAT by shaking stress.

Young mice, fasted for 20 hours, were stressed in their cages (3 mice per cage; cage tops removed) on a reciprocating shaker (180 excursions/min.; 4" stroke length) for 30'. The mice remained in their cages (room temperature 22°C) until sacrifice. The number of mice is given in parenthesis.

Table XVIII

Body Temperature and Shaking Stress

(1) before shaking	(2) after 30' shaking	(3) 30' after end of shaking
36.0°C.	34.0	36.0
36.5	34.0	37.0
37.0	35.0	37.0
37.5	35.0	37.0
36.75 ± .3	34.5 ± .3	36.75 ± .3

P of (1) vs (2) < .001

Both shaking and cold stresses have, therefore, similar effects on reducing body temperature and on inducing TAT in young mice. It is possible that the physiological stimulus which acted on the liver to induce TAT is the same in both types of stresses.

The effect of the post-prandial interval on the extent of TAT increase was not investigated in much detail. In an experiment in which the mice were not fasted overnight before shaking, the increase of TAT was equal to that in other experiments in which the mice had been fasted overnight. It would be of interest to know if the induction of TAT by shaking stress is influenced by the post-prandial interval as is the induction of TAT by cold exposure.

A series of studies have been made by Schapiro, Yuwiler, and Geller on changes in the levels of various rat liver enzymes following shaking stress (Schapiro et al, 1964 and 1966; Geller et al, 1964). Although the conditions of shaking stress were similar to those employed in my experiments, no increase of TAT was found, whereas a marked increase in tryptophan pyrrolase did occur. This lack of agreement seems clearly due to the fact that TAT levels were measured at a single time point 3.5 or 4.0 hours after the end of the stress. TAT levels in the experiment cited in figure 18, as well as in several other experiments, have nearly returned to basal levels by 3 hours after the end of the stress.

In general, the rapidity with which TAT levels and, in fact, the whole pattern of genomic activity can be altered is not well appreciated. Although measurements have been made only on TAT, the circumstances which result in

changes of its level without doubt also affect the activity of a great number of other gene loci during the same brief period of time.

(8) The effect of mild stresses on TAT levels

The stresses of cold exposure and of shaking are severe and have a great impact on the mouse. The effect of mild stresses was investigated to ascertain, if possible, the minimum stressful conditions which would result in the induction of TAT.

TAT levels were measured at various times after mice were transfered to a new cage in a strange room, put in bottles at room temperature, or given intra-peritoneal injections of 0.3ml of 0.9% NaCl with a 23Gl needle. The results, together with behavioral observations, are given in table XIX. No significant changes of TAT occurred in any of these circumstances. It is very likely that serum corticosterone levels increased in all these circumstances, as the mild stress of handling, or being placed in a strange environment generally results in the secretion of corticosterone (Ader et al, 1967). It is therefore probable that mild stresses which may result in adrenal cortical activity nonetheless may not sufficiently tax the metabolic reserves to elicit the induction of TAT.

(9) Evidence that physiological doses of corticosterone are not a sufficient cause for the induction of TAT

The observations (in the preceding section) that mildly stressful circumstances which are known to result in the secretion of corticosterone (Ader et al, 1967) nonetheless did not affect TAT levels, imply that corticosterone, the principle glucocorticoid released during stress in mice (Cortés et al, 1963) is not the only factor active in the induction of TAT during severe stress. This was directly tested by injection of physiological doses of corticosterone. The hormone was injected intra-peritoneum with a Hamilton microsyringe calibrated in units of .005 ml. The intra-peritoneal route of injection delivers most of the hormone to the liver (McEwen, personal communication Corticosterone solutions for injection were prepared by rapidly diluting stock solutions (2 mg/ml or less in absolute ethanol) into an appropriate volume of warm, 0.9% NaCl. Because preliminary experiments indicated that small amounts of ethanol (0.005 ml) may depress TAT levels slightly, the control mice were injected with an equal volume of saline-ethanol solution in the same proportions and killed 1.5 hours later with the corticosterone injected mice. The results of these experiments are given in figure 19.

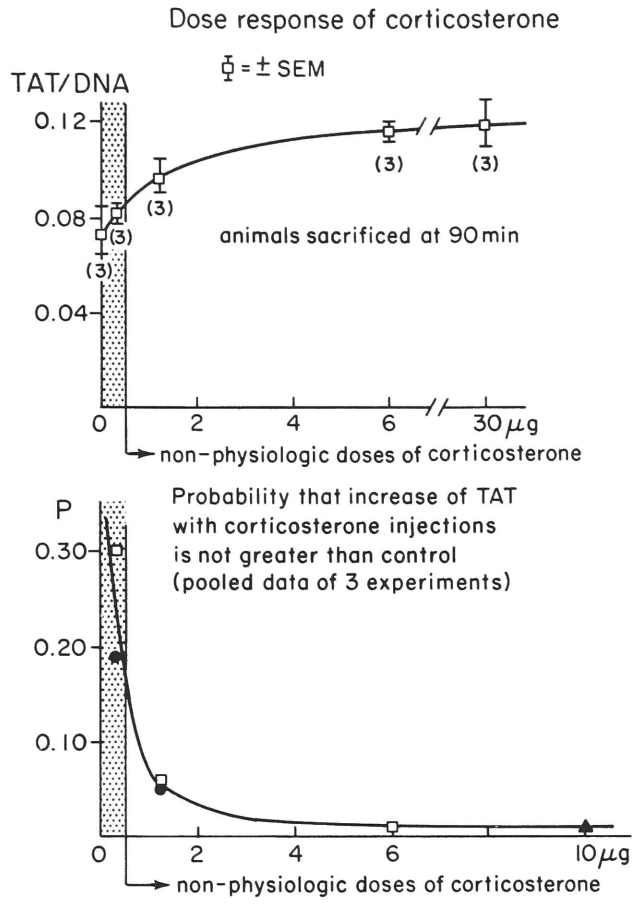


Figure 19: The influence of the dose of corticosterone on the increase of TAT.

Young mice were fasted 20 hours and were injected with various doses of corticosterone as described on page 141. The mice remained in their cages until sacrifice. The number of mice is given in parenthesis.

Table XIX

Mild Stresses and the Regulation of TAT

	TAT/DNA ± S.E.M.		
1) Put in new cage in strange room:	Control	2 hr.	3.5 hr.
Mice were active (exploring, etc.), but became quiet within 0.5 hr.	(3) 0.050 ± .006	(2) 0.035 ± .001 P = 1.0	(2) .043 ± .025 P = 1.0
2) Put singly in 2 qt. glass jars at room temperature:	Control	2 hr.	
Mice were active (exploring, standing up on hind feet, etc.) for at least 2.0 hrs. The bottoms of the jars were large enough to allow complete freedom of movement.	(3) 0.060 ± .012	(3) 0.065 ± .017 P = 0.8	
3) Saline injections in a familiar cage:	Control	1 hr.	2 hr.
During the injection the mice struggled to escape, urinated, defecated, and attempted to bite, as usual. However, the disturbance did not persist and the mice soon returned to sleeping, the normal "activity" for the morning hours. So soundly did they sleep prior to sacrifice, that they awoke only on having their ears tickled!	(2) 0.030 ± .01	(2) 0.034 ± .005 P = 0.90	(2) 0.027 ± .003 P = 1.0

P is probability that the experimental values are the same as the control values.

Number of mice are given in parentheses.

Conclusion: mild stresses do not result in induction of TAT.

Physiological doses were calculated to be 0.3 μg for a 30 gm mouse as described in Section IIIF10e. It can be concluded that up to 4 times the amount of corticosterone present in the circulation during cold stress does not cause a significant ($P < .05$) increase in TAT. During the same length of cold stress (1.5 hours), TAT levels were regularly observed to increase at least 2 times.

The induction of TAT which occurs with 20 or more times the total quantity of corticosterone in circulation during stress may therefore be regarded as resulting from a non-physiological stimulus. This point has also been discussed in reference to TAT induction and corticosteroids by Geller et al (1964). It should be noted that the induction of TAT in the perfused liver with 17-hydroxycorticosterone-21, hemisuccinate (Solucortef) employed about 100 times the concentration of serum corticosteroids present during stress (Hager and Kenney, 1968).

(9) Regulation of TAT during ageing

(a) Induction of TAT by cold stress

(i) General description of results

The rapid adjustment of TAT levels during the physiological changes which occur after feeding and during the stress of cold or shaking provide an extremely sensitive way of detecting changes in physiological regulation during ageing. The effect of age on the induction of TAT by cold stress was investigated in a series of experiments conducted at $10 \pm 1^{\circ}C$, a temperature at which there is a pronounced differential mortality according to age (see figure 6).

Examples of experiments in which the cold stress-mediated induction is compared in mice of different ages are given in figures 20 and 21. In 5 such experiments, TAT levels of young and middle aged mice appeared to increase at the earliest time sampled, whereas there appeared to be a marked delay before an increase occurred in senescent mice.

(ii) Determination of the best fitting function

The function describing the changes in TAT during the course of each experiment in figures 18 and 19 was determined by an analysis of the data on a digital computer. A program in Fortran, kindly provided by Mr. Bruce W. Knight, Jr., of the Rockefeller University, was employed to determine the best fit of the data according to a broken line function as described in figure 22. For any lag time (≥ 0), the computer determined the starting

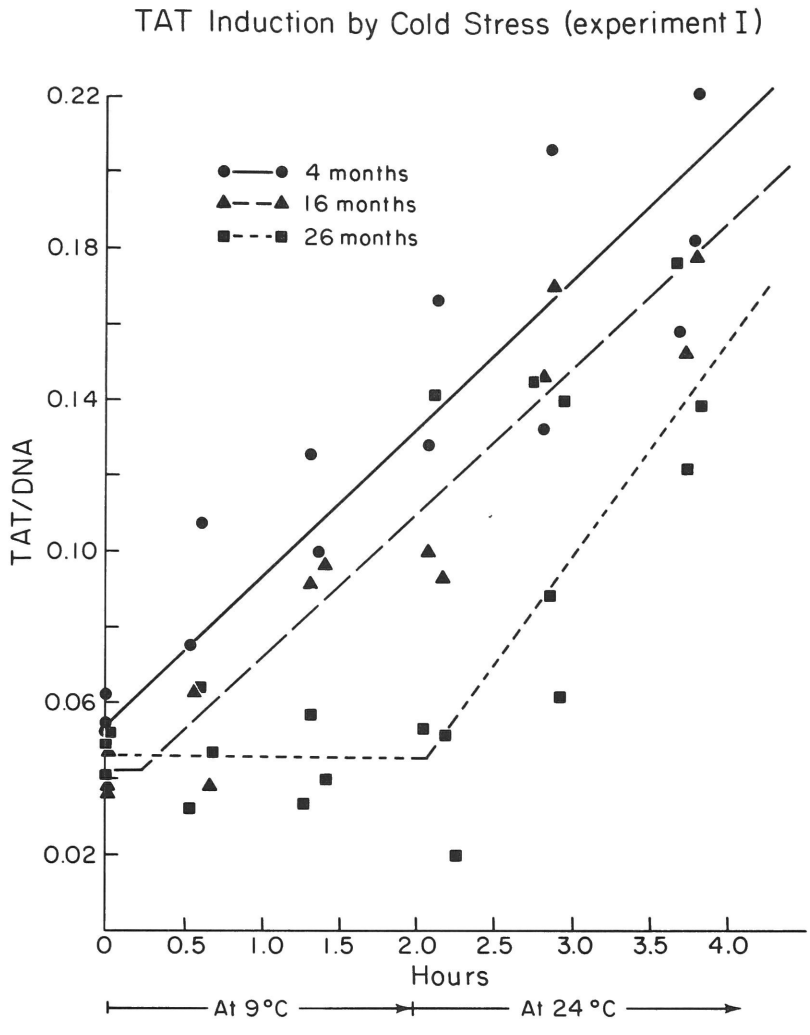


Figure 20: The effect of ageing on the induction of TAT by cold. Experiment I.

Mice fasted 20 hours. Experiment begun at 9 a.m. At 2.0 hours of cold exposure, all jars containing mice were removed to a room at 24°C.

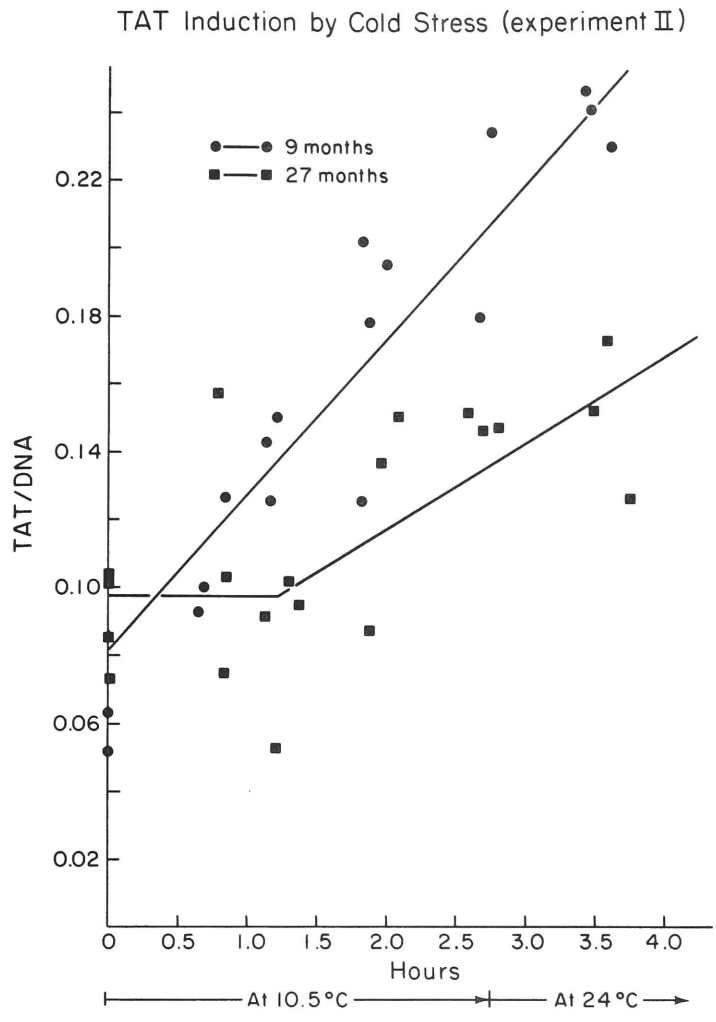
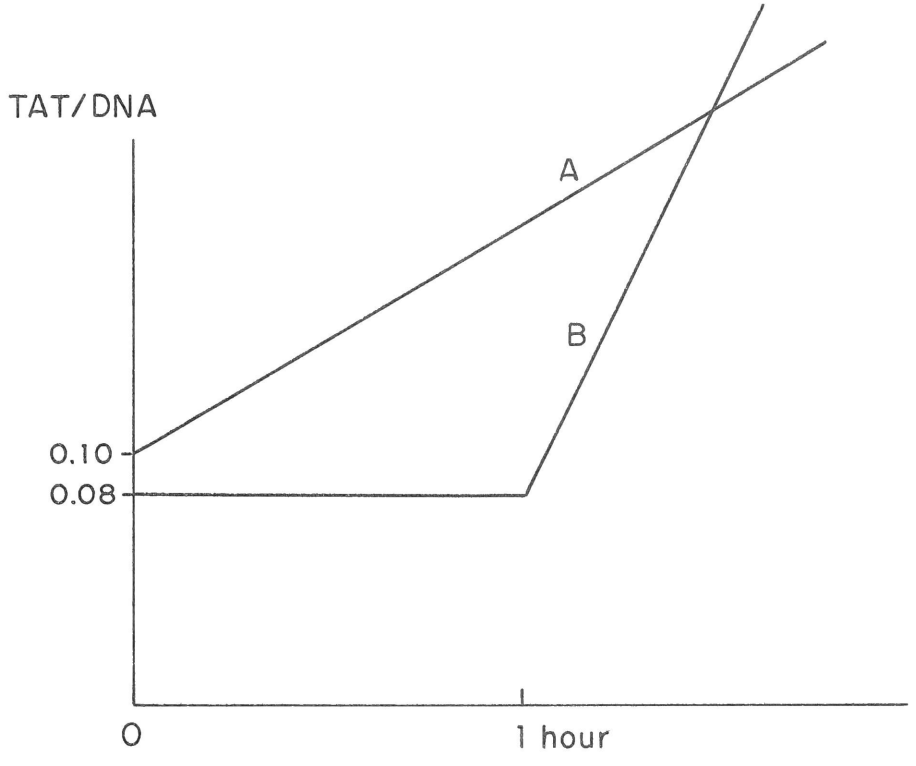


Figure 21: The effect of ageing on the induction of TAT by cold. Experiment II. Experiment conducted as described in legend of figure 20.

Figure 22

Determination of the best fitting function (hypothetical example)



function	insert lag	start TAT	slope	RMS error
A	0	.10	.10	.5
B	1	.08	.20	.1

chose function B - has lowest RMS error

computer program kindly provided by Mr. Bruce W. Knight, jr. (see Appendix I)

value, the slope of the TAT increase at the end of the lag time, and the root mean square deviation. A series of "lag times" from 0 to the end of the experiment were empirically evaluated and the minimum root mean square deviation was thereby found. The resulting functions were drawn on the graph (e.g., figures 20 and 21) and confirm the impression that the increase in TAT during cold exposure is delayed in senescent mice.

(iii) Statistical analysis

The statistical confidence in the increase of TAT levels during the experiments were determined by the standard Fisher t-test for comparing the significance of the difference in mean value of two samples. This method of analysis was used because it enabled the comparison of changes in TAT (expressed as the probability, P, that the mice sampled during the cold stress are drawn from the same population as the "control" mice of that age group) in all age groups from a common starting value (P=1.0), of all control levels of TAT. In general, TAT levels increased immediately in young and middle aged mice (6-18 months of age), whereas there was a pronounced lag before an increase of TAT occurred in senescent mice (26-28 months of age). The lag varied from experiment to experiment. On the average, the senescent mice required 70 ± 20 minutes (± S.E.M., 5 experiments) longer than younger mice to increase TAT levels significantly ($P < .05$) above the starting values. (The value P = .05 appears on the graphs (figures 23 and 24) as P = .1, because the standard tables of t values are given for a "two-tailed" distribution. In my experiments, only changes greater than average control levels occur. This corresponds to a "one-tailed" distribution in which the actual P values are 1/2 of the graphed P values for $P < 1.0$ (Bailey, 1959).) The time required to reach $P < .05$ is well correlated with the lag as determined by computer analysis in any experiment.

(iv) Uniformity of response

It is essential for the interpretation of the results of this statistical treatment that the mice of a given age group represent an internally homogeneous population with the same variance about the mean. For instance, if the variance about the mean of the control levels was greater in senescent mice, then a greater average increase in TAT levels would be required to reach the same P value as in younger mice. However, this is not the case. The value of $\sigma/\bar{X}$ does not change with age as shown in table XX.

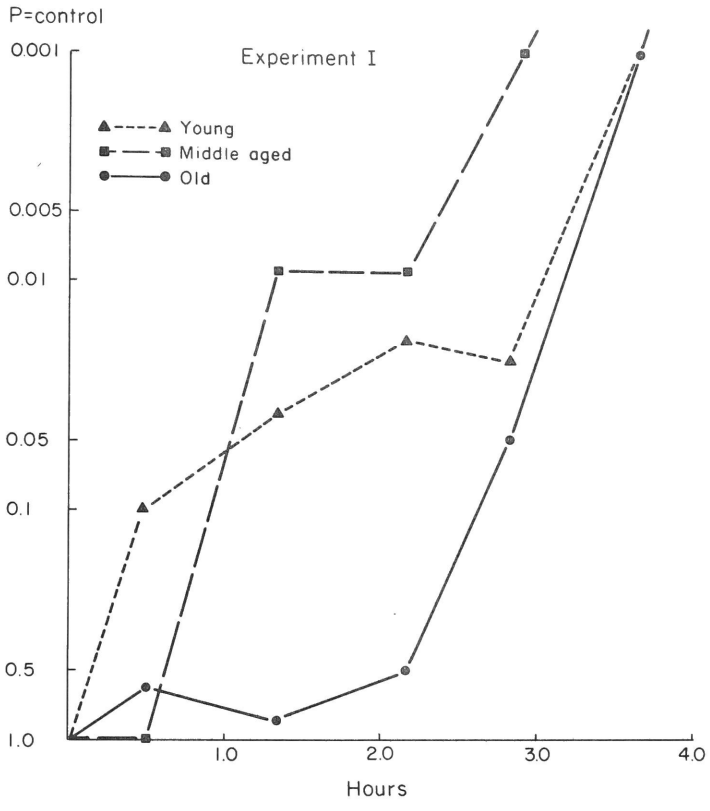


Figure 23: The time course of the change in the probability, P, that the values of TAT during cold stress represent a sampling from the same population as the controls of that age group. Experiment I.

P values calculated from the data of figure 20.

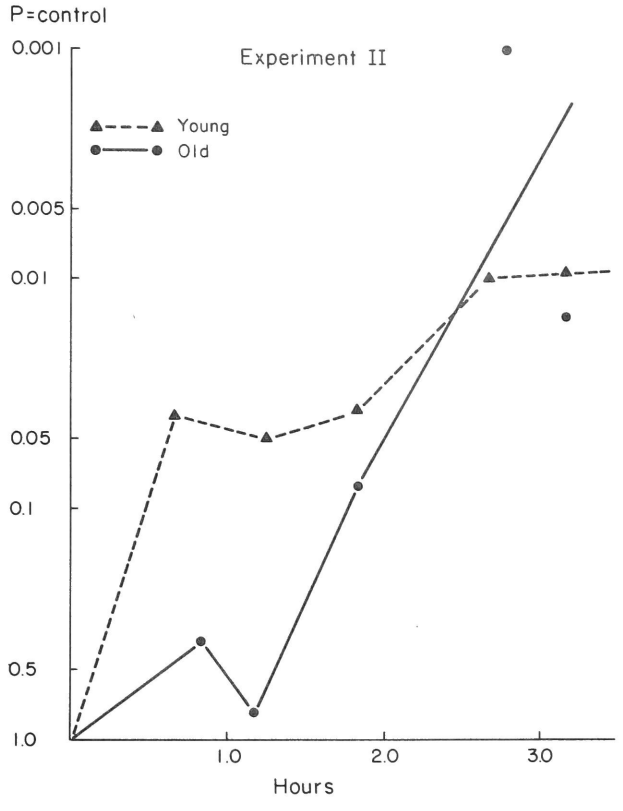


Figure 24: The time course of the change in the probability, P, that the values of TAT during cold stress represent a sampling from the same population as the controls of that age group. Experiment II.

P values calculated from the data of figure 21.

Table XX

TAT levels (20 hour fast)

age months	TAT/DNA ± S.E.M.	$\sigma/\bar{X}$	Number of mice	P = young
5	.045 ± .0046	0.25	5	1.0
16	.026 ± .0029	0.26	5	.025
26	.026 ± .0022	0.22	5	.025

Similarly, the value $\sigma/\bar{X}$ for mice exposed to cold does not change appreciably in any age group except in senescent mice at the time when the increase in TAT begins to occur. Thereafter, $\sigma/\bar{X}$ returns to the range of previous values, as illustrated in figure 25. It can be concluded that senescent mice respond to cold with the same uniformity in any experiment as do younger mice. In all experiments, mice were randomly sampled within their age group.

(v) Rate of TAT increase

The rate of increase of TAT in cold stressed senescent mice after the lag, as determined by computer analysis, varied somewhat, and was not characteristic of any age group (see table XXI).

Table XXI

Rate of Increase of TAT (units/hr.) during Cold Stress after the End of the Lag

experiment	young	senescent
I	0.040	0.065
II	0.045	0.025
III	0.050	0.065
IV	0.036	0.034

(determined by computer analysis)

(vi) Age differences in basal TAT levels

The levels of TAT in control mice were, as a rule, lower in middle aged and senescent mice as seen in table XX. The difference is statistically significant ($P < .025$). In one experiment (experiment II, figure 21), the control levels were higher in the senescent mice. On the average, fasted middle aged and senescent mice have TAT levels about 30% less than young mice. All measurements were made between 9 a.m. and 12 a.m.

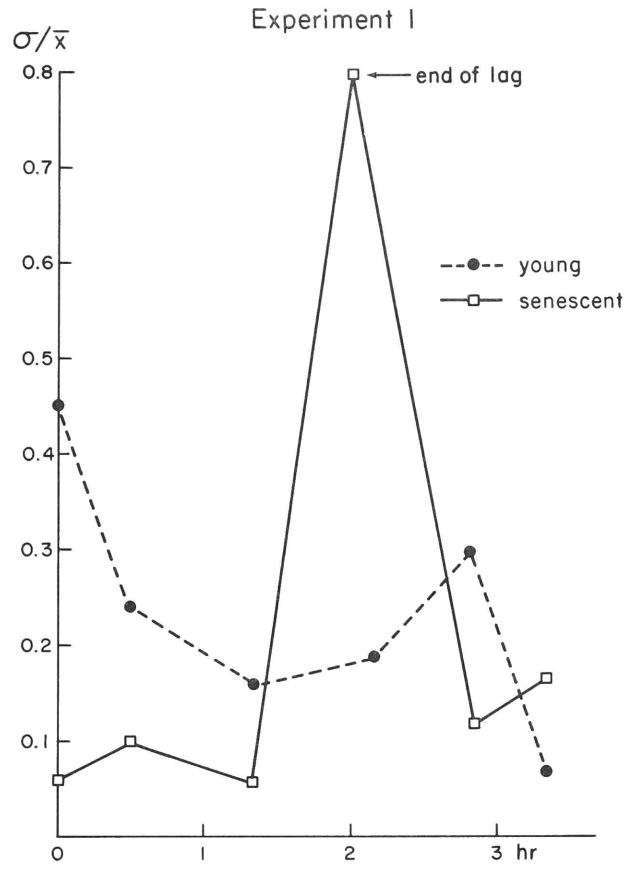


Figure 25: Changes in the coefficient of variation of TAT, $\sigma/\bar{X}$, during cold stress. Calculated from the data of figure 21, Experiment I.

(vii) Experimental variability

During the course of numerous experiments, it became apparent that there was some variability from experiment to experiment in the response of mice of various ages to the stress of cold. This is revealed, for example, in the variations in the rate of TAT increase given in table XXI. It is also manifested in the variability of the lag of TAT increase in senescent mice which ranged from 0.3 to over 2.0 hours under presumably identical conditions. Despite the variability from experiment to experiment, it is clear that in any experiment, mice of any age group responded uniformly. Similar variations between experiments have also been observed with regards to TAT induction (Kenney and Flora, 1961; Grossman and Mavrides, 1967) and repression (Kenney, 1967).

(b) Hormonal induction of TAT

The capacity of the liver to respond to the action of insulin and corticosterone was compared in young adult and senescent mice. In these experiments, the mice were fasted for 20 hours before the start of the experiment. The hormones were injected intraperitoneally with a 23Gl gauge needle. Corticosterone (Mann Research Laboratories) was dissolved initially in ethanol (2mg/ml), a 0.005ml aliquot was rapidly diluted into 0.1ml of warm 0.9% NaCl and injected (30μg/100gm body weight) with a Hamilton microsyringe graduated in units of .005ml. Insulin (Iletin, Eli Lily and Co.) was diluted from the stock 40 i.u./ml with 0.9% NaCl and injected at a dose of 0.75 i.u./100gm body weight in 0.3ml volume. Two experiments were performed with each hormone. No age differences in the temporal pattern of response were detected (see figure 26). Throughout the course of the induction, the livers of young mice had a significantly greater content of TAT/DNA than did livers of senescent mice ($P < .05$). In 3 of 4 such experiments, the net increase above control values was greater (40-90%) in young mice; in 1 experiment (corticosterone induced), the net increase of TAT per mg DNA was the same.

The lower values of TAT (see table XX) in fasted control or hormonally challenged mice 16 months or older are at variance with the observations of Gregerman (1959), which indicated the similarity of control levels of TAT and levels of TAT 4 hours after the injection of hydrocortisone in 13 and 26 month old Wistar rats. In these experiments, enzyme activity was expressed per unit of dried liver tissue. It is possible that the expression of TAT

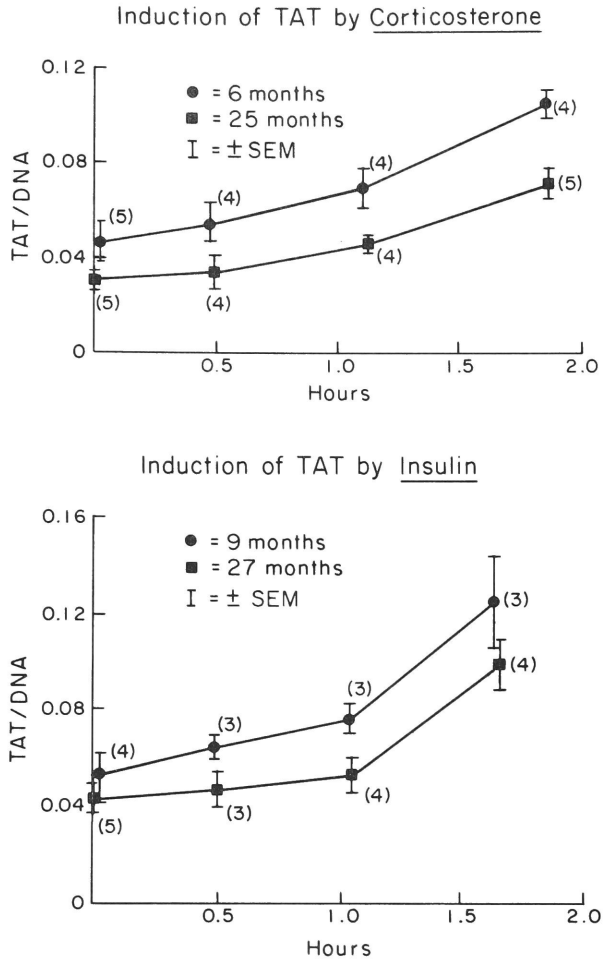


Figure 26: The effect of ageing on the induction of TAT by insulin and corticosterone.

Mice, fasted for 20 hours, were injected i.p. with corticosterone and insulin as described on page 153. The number of mice is given in parenthesis.

activity in terms of tissue DNA content in my experiments is faulted by an increase of connective tissue during ageing (Hinton and Williams, 1968) or by a loss of functioning parenchymal tissue (Falzone et al, 1967). Livers from 5 randomly selected non-fasted mice of each of 3 age groups (5, 16, and 26 months old) were sacrificed at 9 a.m. and were examined for evidence of such changes. Sections were prepared by Miss Lilian R. Gregg of the Tissue Cutting Service of the Rockefeller University. Dr. Arthur Hurvitz kindly assisted in the examination of these sections. The typical post-prandial appearance of liver parenchymal cells, uniformly laden with glycogen, was observed in each case. There were no areas of necrosis such as found by Falzone et al (1967). This is evidence against general parenchymal cell dysfunction. There were occasional regions of low grade hyperplasia of bile duct endothelial cells. Instances of this occurred in all age groups and could not, in a hypothetical case, distort the fraction of parenchymal cells in the liver (presumably the sites of inducible TAT) sufficiently to reduce the fasted TAT/DNA levels by 25% or more in the middle aged and senescent mice. Therefore, the reduced TAT/DNA levels in middle aged and senescent mice appear to represent a resetting of the TAT content per hepatic parenchymal cell. Other examples of changed enzyme content in liver during ageing have been considered in Table I.

It can be concluded from these results that age does not impair the biosynthetic response of the liver cell to insulin and corticosterone, as measured by the time course of TAT induction. Thus, the delayed induction of TAT in senescent mice during cold stress may be considered as the result of an extra-hepatic age change: possibly the release of the hormonal factors (unknown at present) which act on the liver to induce TAT is delayed or their action is temporarily inhibited.

e) Studies on the secretory activities of the adrenal cortex

Adrenal corticosteroids are known to play a vital role in the maintenance of body temperature during cold stress (Roos, 1943). The secretion of corticosterone, an adrenal corticosteroid released during cold stress (Kolthoff et al, 1963), was investigated in young and senescent mice during cold exposure. Blood, collected from the jugular veins of mice immediately after killing by cervical dislocation at various times during cold stress, was allowed to clot for 30' at room temperature, and was then clarified by centrifugation. Duplicate samples of the resulting serum were analyzed for corticosterone content by fluorescence spectrophotometry according to Peterson (1957).

The combined data of 2 experiments are given in figure 27. There is no evidence for a lag of corticosterone secretion in cold stressed senescent mice during that same period of time when a lag occurred before the induction of TAT. This result supports the previous conclusion (Section IIIF9) that corticosterone is not a sufficient cause for the induction of TAT during cold stress. The serum levels of corticosterone were observed to be consistently lower in senescent mice during cold stress. However, the age difference was small and was not statistically significant except at the earliest time point, 15' ($P < .01$). There was no age difference in basal levels.

The effect of ageing on the adrenal response to stress has not been well studied. In the one investigation known to me, age changes in corticosterone levels had only marginal statistical significance: they were slightly lower at 24 than at 13 months in rats stressed with ether, whereas no age differences were found in rats stressed with Nembutal (Rapaport et al, 1964). Basal levels of corticosterone were closely similar in mice of various ages examined in my experiments, as well as in rats (Rapaport et al, 1964) and humans (Grad et al, 1967).

4) Discussion of results

Previous investigations have demonstrated the decreased ability of ageing rats (Weiss, 1959) and mice (Grad and Kral, 1957) to survive in the cold. It is of some interest that senescent mice, like young mice, can successfully adapt to life at 2.5°C if the temperature is gradually lowered and if adequate food and water are provided, even in the absence of nesting material or a companion mouse to huddle with. There was, however, a much greater mortality among senescent mice during these temperature changes (Grad and Kral, 1957). In general, young mice had a greater per cent increase in food intake, oxygen consumption, and blood sugar during this prolonged stress (Grad and Kral , 1957). These later results constitute evidence for age changes in regulation of homeostatic mechanisms. The two studies known to me on the response of ageing humans to brief cold exposure give conflicting results: an earlier onset of oxygen consumption was found in the older human subjects during cold exposure by Krag and Kountz (1950), whereas the older group was notably unreactive with respect to changes in oxygen consumption in the study of Horvath et al (1955). It is of interest that the older subjects were observed to shiver less in both studies, an

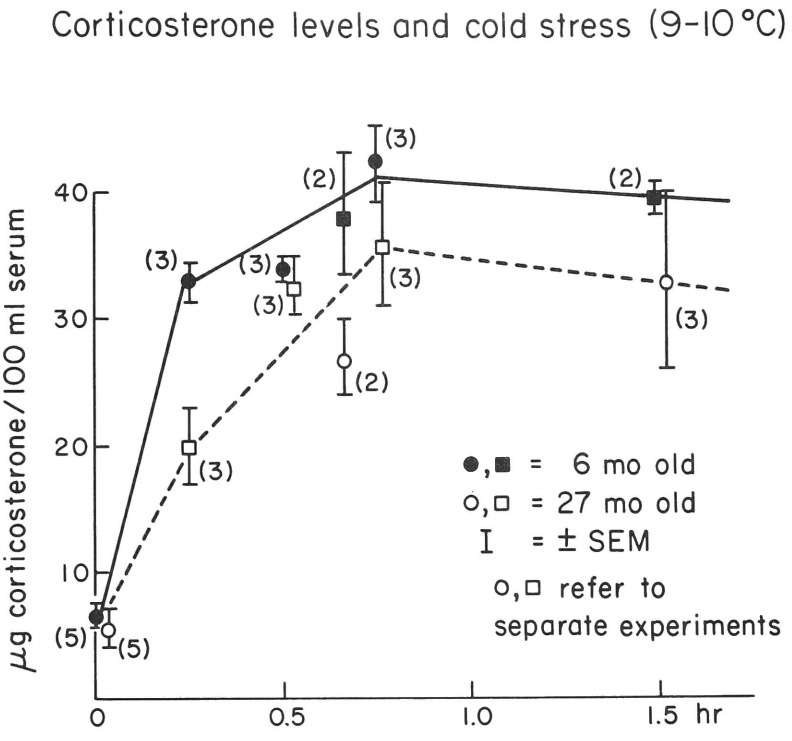


Figure 27: The effect of ageing on the secretion of corticosterone during cold stress.

Mice, fasted for 20 hours, were stressed by cold as described in figure 5. The procedure for corticosterone assay is described on page 156. The number of mice is given in parenthesis.

index of changed reactivity to cold during ageing.

The effect of ageing on the response of mice to cold, revealed in initial experiments (figures 5 and 6) as a differential mortality, and as an inability to maintain body temperature (figures 9 and 10), was investigated in the activities of two cellular systems, the liver and the adrenal cortex, which participate in the metabolic changes associated with cold exposure. These cells were studied because of their sensitivity to changes in the state of the body. The relationship of age changes in cell activities of the liver and adrenal cortex to the decreased ability to maintain body temperature remains obscure.

The liver enzyme, tyrosine aminotransferase (TAT) is rapidly induced by the direct action of insulin, glucagon, and glucocorticoids on the liver (Goldstein et al, 1963; Hager and Kenney, 1968). Increases in TAT activity are known to result from corresponding increases in the synthesis of new TAT molecules (Kenney, 1962). The process of induction is inhibited by Actinomycin D (Greengard and Acs, 1963) and is therefore dependent on gene activity. Levels of TAT change in concurrence with the nychthemeral rhythm of spontaneous activity and feeding in mice and rats. The levels of TAT are therefore sensitively regulated by hormonal and nutritional changes in the body. Experiments described above have shown that rapid increases of TAT also occur following cold exposure; these increases are inhibited by Actinomycin D (figure 16).

The induction of the liver enzyme tyrosine aminotransferase (TAT) by cold stress is manifested as a prompt, linear increase of TAT in young and middle aged mice (6-16 months old). In contrast, the increase of TAT to a level of statistical significance ($P < .05$) is delayed by 70 ± 20 minutes ($\pm$ S.E.M. of 5 experiments) in senescent mice (26 months or older), as illustrated in figures 20-24. After the initial delay, the increase of TAT is linear and occurs at rates closely similar to those observed in younger mice. Senescence has therefore resulted in an altered temporal pattern of cell activity in the mouse liver during cold stress. The question is thus posed: does this age change result from changes in the cellular machinery concerned with genomic expression and protein biosynthesis upon which increases in TAT are known to be dependent?

The ability of the liver cells to induce the synthesis of TAT in response

to injections of insulin and corticosterone was compared in young and senescent mice. The increase of TAT occurred on the same schedule in both age groups as illustrated in figure 26. This result demonstrates that the delayed induction of TAT by cold stress in senescent mice is not the result of a loss of the potential for rapid, gene dependent, cell functions in the liver. The ability of the liver cell to respond, if given the proper stimulus, indicates that genomic expression in liver cells is not fundamentally impaired with age. This is a very sensitive test of cellular competence and depends on the normal function of most of the machinery of gene regulation and the synthesis of RNA and protein. If general damage to the cell nucleus was the cause of senescence, it might have been manifested during such a delicate process.

Experiments have shown that cold stress does not elicit the induction of TAT in young mice during a refractory period which persists for a number of hours after feeding (figure 18). Direct challenge of the liver with insulin injections (a hormone which induces TAT in the perfused, isolated rat liver) in the intact mouse does elicit a vigorous induction of TAT during this refractory period. Therefore, the stimulae which cause the induction of TAT during cold stress are either not released during the refractory period or their effect on the liver is inhibited. The demonstrated sensitivity of the regulation of TAT to a wide variety of hormonal and nutritional conditions in the organism implies that the refractory period is the result of a change in extra hepatic factors. It is likewise inferred from this example that the delayed induction of liver TAT by cold stress in senescent mice is a secondary result of an extra-hepatic age change, possibly in the hormonal or nutritional state of the organism. It is possible that the delayed induction of TAT during cold stress in senescent mice is an extension of the post-prandial refractory period which is known to end earlier than 20 hours post-prandium in young mice.

Hormonal aspects of the response to cold stress were studied through the secretion of corticosterone, although it is not thought that adrenal corticosteroids are the only active agents in the induction of liver TAT during stress. Corticosterone levels were found to increase with the same time course in young and senescent mice during that period of cold stress when the delayed induction of liver TAT occurred (figure 27). This result demonstrates that ageing has a selective effect on the temporal pattern of cell activities during cold stress.

In the experiments involving the induction of TAT by injected hormones (Section IIIF3d10b), the levels of TAT/DNA were significantly lower ($P < .05$) at all time points in senescent mice. This is at variance with the results of Gregerman (1959), who did not find any differences in TAT/liver protein between 13 and 26 month old Wistar rats before, and 4 hours after, the injection of hydrocortisone. Although decreased TAT levels in the ageing C57Bl/6J mouse might possibly be interpreted as evidence of liver cell failure, it would seem more consistant to place the observed age reduction of TAT levels in the context of numerous observations (see tables I - VI) which demonstrate the differential effect of age on enzyme levels: some enzymes are increased, others decreased, while many are unchanged. This array of age changes suggests a shift in regulation, possibly mediated by humoral factors, rather than a general senescence of the liver and other cells.

5) Theoretical interpretations

Most of the theoretical treatments of ageing have been mathematical rationalizations, based on various assumptions, of the increasing death rate during ageing (see review of Strehler, 1962). Focus on the death rate in populations, the most aggregate and abstract measure of ageing, has drawn attention from the available details of cellular changes during the ageing process. In fact, it is not generally realized and not acknowledged in any theory, that ageing has a differential effect on cell activities. The additional fact that ageing changes in any cell activity occur with such regularity and predictability as to establish them as developmental events would seem to invalidate those theories in which ageing is assumed to result from damage to cell nuclei, through the random accumulation of mutations (Szilard, 1959; Curtis, 1963; Wulff et al, 1962 and 1964) or through random cross-linkage of chromatin components which result in the "permanent repression of genes" through the formation of "irreversible bonds" (von Hahn, 1966; Harman, 1968). The accumulation of damage in the genetic apparatus from random mutational and other events is doubtless an inevitable result of the passage of time (e.g., bacteriophages have been found to accumulate mutations in proportion to the duration of storage over a 3 year term in sterile conditions at 0°C or 20°C (Drake and McGuire, 1967)). However, on the basis of evidence presented in this essay, it would appear that the regular and specific aspects of ageing change predominate in most, if not all, tissues of the body. However, if such random changes were restricted to certain tissues, resulting in a localized

dysfunction tantamount to ablation, one consequence might be compensatory changes in physiological regulation with widespread (but reversible) effects on cell activities throughout the body.

Other derivatives of the theories of random nuclear changes are theories of mortality resulting from autoimmune disease, the incidence of which is presumed to increase with age because of accumulated mutations (Walford, 1967b; Burch, 1963 and 1968). There is indeed evidence for the increase of low grade histoincompatibility reactions and other manifestations of autoimmune diseases during ageing (reviewed in Walford, 1967 a, b). The increase is manifested statistically in any ageing population as is the increase in many other diseases, but there is no evidence that all ageing mammals are so afflicted. It does not seem likely that autoimmune phenomena, if caused by random genomic change, can result in the regular, differential changes at the cellular and supra-cellular level which occur in all members of a population during ageing. It is reasonable, however, to infer that autoimmune disease contributes to the increasing mortality during ageing.

Another set of ageing theories is based on the assumption that the exercise of cell function reduces or exhausts the potential for future cell activities. This is an old idea which is consistant at first glance with the existence of maximum potential species lifespans (e.g., "...the matter of life is a veritable 'peu de chagrin', and for every vital act, it is somewhat the smaller", Huxley (1896); Rubner's theory that the total caloric expenditure during life is a constant in many mammals (excluding man), the length of the lifespan thus being an inverse function of the rate of caloric expenditure (Rubner, 1908); and, recently, Hayflick's observations on the limit to diploid cell proliferation in vitro (discussed at length in Section IIIC8a), Hayflick (1968)). However, as described in numerous observations of cell function in senescent mammals (Sections IIIC-F), there is no evidence that ageing of the mature mammal results in a diminution of the potential for any cell activity. It is also obvious from examples of circumstances in which the normal species lifespan limit can be exceeded in certain teleosts (discussed in Section IIB), that the senescent death in these species cannot be the result of the depletion of "vital reserves", the quantity of which are a fixed and immutable characteristic of the species. However, it is possible that the exercise of function in certain cell types does result in their exhaustion and death. Compensatory changes might ensue with widespread,

but reversible, effects on cell activities throughout the body.

G) Ageing, disease, natural selection, and mortality

Death of the mammalian organism is not the result of a preceding, more or less concurrent cell death throughout the body. On the basis of transplantation experiments (Section IIIC12), it would appear that the potential longevity of some cells in the body is in marked excess of the maximum lifespan of the species. The major problem is to elucidate the changes in regulation of cell activities at the supra-cellular, physiological level and to show their relationship to the increasing rate of mortality during ageing.

The incidence of disease, which precedes in parallel the increasing incidence of mortality during ageing (Section IIIB2d), would seem to be a factor of paramount importance. Ageing mammals, for example, are well known to have a decreased resistance to microbial disease. A major cause of this particular susceptibility would appear to be the diminishing vigour of immunological reactions (see Section IIIC8f). For most other diseases (e.g., cancer), the specific aspects of physiological change which increase susceptibility during ageing are almost entirely obscure.

An important aspect of the relationship between physiological changes and disease concerns the ability to withstand stress. It is well known that various stresses can result in disease (e.g., Selye, 1946; Wolff, 1968). As the capacity to withstand stress diminishes with age, it might be anticipated that the threshold for stress-induced disease diminishes with age. The decreased capacity to survive various stresses would also seem to be a major factor in the increase of the mortality rate during ageing, as an ageing mammal would be less able to bear the stress which disease itself imposes on the body.

Certain genetically determined diseases (e.g., Huntington's chorea or acquired agammaglobulinemia) do not develop until many years of adult life have passed. This delay implies that the expression of the characters conferred by these genes is dependent on some particular change in the physiological milieu during ageing. It is possible that many of the diseases which are statistically distributed in ageing populations are the result of similar age changes in the characters of various polymorphic genes. In this way, genetic proclivities for certain diseases manifested during ageing (e.g., cancer) might be superimposed on the pattern of physiological changes during ageing.

The origin of genotypes influencing post-maturational age changes is of special interest. Haldane has pointed out that the genes conferring the presence of various chronic diseases and regulating the precise age of their incidence after the period of the bearing and rearing of the young would not appear to be subject to the laws of natural selection (Haldane, 1941). The example of death after spawning in the Pacific salmon (Section IIB2d) therefore constitutes a case, unusual among vertebrates, of a genotype whose lethal effects (resulting from toxic levels of adrenal corticoids) are manifested concurrently in nearly all post-spawning fish in populations of at least 5 species. The evolutionary persistance of the pattern of post-reproductive age changes in mammals implies that genes influencing these age changes have been selected during mammalian evolution for their role at an earlier stage of life.

IV. SUMMARY

In this essay, on the basis of published data from a diverse literature and on the basis of experiments presented here, I have argued for the following conclusions about ageing phenomena in homeothermic mammals.

1) Most post-maturational ageing changes occur in a regular and predictable temporal pattern during the lifespan. Accordingly, ageing changes may be considered as an aspect of the mammalian, gene controlled, developmental program.

2) The schedule of ageing changes is adjusted in proportion to the lifespan limit characteristic of the species in such a fashion that many events of ageing occur in the same fraction of the lifespan (e.g., in men or mice).

3) At all levels of organization (sub-cellular, cellular, organ, etc.), ageing results in differential changes: some activities or parameters remain unaltered throughout adult life. In particular, the pattern of age changes in cell activities is histotypic.

4) Histotypic age changes in cell activities are considered to be the result of differential changes in gene activity.

5) In several cases, it has been demonstrated that age changes in cell activities are not the result of a loss of the potential for "normal" genomic function, e.g., the slowed growth of hair in senescent mouse is

restored upon transplantation to a young host; experiments described in this thesis have also shown that the delayed induction of the liver enzyme tyrosine amino-transferase in cold stressed senescent mice is not the result of an age change in the potential for the rapid induction of TAT, an event dependent on gene activity. These examples argue powerfully against an intrinsic "irreversible" cellular ageing process, such as might result from the accumulation of random mutations or random cross-links in the chromatin. It is also inferred from these experiments that there are important age changes in the regulation of circulating factors, which, in turn, cause age changes in gene activity.

6) The essence of age changes is therefore considered to be a change in regulation at the supra-cellular level of the organism, which, mediated through circulating factors, affects the pattern of gene activity, differentially and reversibly, in cells throughout the body.

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APPENDIX I

Program for best fitting function, written by Mr. Bruce W. Knight, Jr.

(see figure 22 for a hypothetical example of usage)

```
*               immortality for rats
                dimension data(100), array(100), ramp(100)
                do 11 i=1, 100.
                read 20,data(i), array(i)
20              format(2f10.5)
                if(data(i)+array(i))11,12,11
11              continue
12              ndata=i-1
                write type 21
21              format(26henter turn time at request,///)
                write type 22
22              format(10x,8hconstant,3x,5hslope,3x,9hrms error,//)
13              read type 23,tz
23              format(f10.0)
                a1=0.
                a2=0.
                b11=0.
                b12=0.
                b22=0.
                dd=0.
                d0 403 i=1,ndata
                d=data(i)
                t=array(i)
                if(t-tz)1,1,2
1               t=0.
                go to 3
2               t=t-tz
3               ramp(i)=t
                a1=a1+d
                a2=a2+d*t
                b11=b11+1
                b12=b12+t
                b22=b22+t**2
403             dd=dd+d**2
                det=b11*b22-b12*b12
                c1=(b22*a1-b12*a2)/det
                c2=(-b12*a1+b11*a2)/det
                r=0.
                do 404 i=1, ndata
404             r=r+(c1+c2*ramp (i)-data(i))**2
                s=sqrtf(r/ndata )
                write type 24, c1,c2,s
24              format(10x,3f9.3)
                go to 13
                end
```

End