CRC Press, Florida, USA (1994).

substances *in vivo* and *in vitro*.[25] Mitochondrial inclusions and changes in shape may result from altered metabolism, such as uncoupled respiration, and/or artifacts, such as hypoxia during fixation. Because of damage induced by free radicals originating as by-products of oxygen reduction during respiration (Chapter 6), disorganization of mitochondria has been considered the "Achilles' heel" of the aging cell.[26,27] In the brain, the magnitude of the shrinkage during fixation is more pronounced in tissues from young than old brains, probably because of the higher water content of the former.[28] Tissue shrinkage would, undoubtedly, influence cell density as well as distribution and structure of organelles within the cell.

Mitochondrial Mutations and Degenerative Diseases

The genetics of oxidative mitochondrial process show that mitochondrial DNA (mtDNA) is predominantly inherited from the mother (only 0.1% of mtRNA is provided by the sperm). Mutations of mtDNA have been implicated in the etiology of a number of degenerative diseases with apparently late onset and associated with defects of oxidative phosphorylation (e.g., Alzheimer's disease, Parkinson's disease).[29] It is suggested that mutations may occur early in development and induce cumulative cell damage and loss (e.g., by free radical accumulation) which become manifest in old age (Chapter 6). Prevention of mtDNA damage in the mother may represent an equally or more efficient intervention than prevention of mtDNA damage in the elderly for reducing the risk of age-related degenerative diseases.[29]

It is difficult to measure the volume of *Golgi complex* even with new techniques because it is not contained within a distinct boundary. It appears, however, to be increased in volume in some aging cells, such as rat cerebral cortical and vestibular neurons and fibroblasts.

2.3 Aging Changes in the Nucleus

Aging affects proliferative homeostasis[11] and proliferative capacity of the cells[30,31] under normal conditions and after mitogenic stimulation.[32,33] These changes have been correlated with a number of structural alterations in the nucleus with aging as identified by electron microscopy *in vivo* and *in vitro*. They include: irregularities of shape and invagination, interpreted as consequent to reduced blood supply and cell metabolism; chromatin condensation, suggesting lowered functional activity; presence of inclusion bodies, particularly evident in pineal cells and neurons, perhaps related to alterations of intranuclear microtubules and microfilaments; and increased number of nucleoli *in vivo* but decreased *in vitro*.

Changes in DNA, RNA systems, mutations, errors, and repair mechanisms have been discussed in Chapter 4 together with their possible role in the aging process.

3 ENZYMATIC CHANGES DURING AGING*

3.1 Enzyme Levels During Aging

Changes in enzymatic levels and activities were among the first biochemical observations demonstrated to occur during aging and have been extensively reviewed.[34] The collected information is hard to integrate because of the variable and often contradictory nature of the reported data. Nonetheless, studies on enzymes have stimulated interest in aging research and have contributed considerably to the field.

Enzymes and Enzymatic Activity

Enzymes are specific proteins that

- Catalyze chemical reactions in biological systems
- Have enormous catalytic power
- Are highly specific with regard to both the reaction catalyzed and the choice of reactants, called substrates

Concentrations of enzymes and their rate of synthesis are under genetic controls and are influenced by small molecules such as hormones, substrates, and products of the metabolic pathways. Some enzymes are synthesized in an inactive precursor form and are activated at a suitable physiologic milieu. Another controlling mechanism is the covalent insertion of small groups on the enzyme.

The extent to which an enzyme can increase the rate of a reaction depends on the activity of the enzyme, which is a function of (1) the amount of active enzyme available, (2) the concentration of substrates and the presence of cofactors, inhibitors, and activators, and (3) the innate property of the enzyme itself.

Changes in the levels or properties of enzymes may alter the functional activity of an organism. Since enzymes are responsible for specific functions, the various phases of the lifespan (differentiation, development, reproductive maturity, aging) may depend on the activity of specific enzymes.

Depending on the animal species, *the activities of several enzymes decrease, increase, or remain unchanged with aging*. Even within each class, the activities of most enzymes do not follow a specific age-related pattern; indeed, few single enzymes consistently change activity with aging. However, alterations in the activities of various enzymes may affect the functional ability of the aging organism.

The metabolic status of animals is coordinated by a network of enzymes at different phases of the lifespan. Activities of certain lipogenic and gluconeogenic enzymes in the rat liver change at different ages. Lipogenic activity is diminished because of an age-dependent decline in nicotinamide-adenine dinucleotide (NADP)-malic enzyme and ATP-citrate lyase activity.[35] Studies on oxaloacetate metabolism in the liver and brain of male Wistar rats show that activities of cytoplasmic aspartate aminotransferase and malate dehydrogenase (gluconeogenic enzymes) are higher in older rats.[34] This increase may be correlated with the greater involvement of the enzymes in the conversion of the oxaloacetate pool for gluconeogenesis in older rats.

The activity of poly(ADP-ribose) polymerase (an enzyme involved in DNA repair) is higher in long-lived mammals. Such increased activity may contribute to the efficient maintenance of the genome integrity and stability over a longer lifespan.[36] Long-lived flies *(Drosophila melanogaster)* express an unusually active form of the antioxidant enzyme superoxide dismutase; this higher activity helps to neutralize superoxide quite efficiently, thereby supporting the longer life of these flies.[37] Activities of the antioxidant enzymes, superoxide dismutase and catalase, decrease significantly in the liver of old rats, but can be returned to normal by dietary restriction (which also raises the levels of mRNAs coding for these enzymes).[38] Indeed, the observed decrease or increase in enzyme levels may be correlated with the decrease or increase in the template activity of the corresponding genes and/or their internal regulation, depending on the changing metabolic needs with advancing age.

* This section (pp. 50–52) was contributed by Dr. Ramesh Sharma.

3.2 Changes in Isoenzyme Composition

During development, many proteins change from the fetal to the adult form. Differentiation and development depend on the sequential activation and repression of genes.[39] This dependence is best exemplified during gestation in humans by the shift of hemoglobin from the embryonic to the fetal and finally to the adult form.

Isoenzymes are functionally related proteins present in more than one molecular form within the same individual and species. The best example is lactate dehydrogenase (LDH), a tetramer of two different types of subunits designated M (the predominant form in skeletal muscle) and H (the predominant form in heart). These subunits are controlled by two separate genes. Several LDH isoenzymes are formed by combinations of M and H subunits (M4, M3H, M2H2, MH3, and H4). Each isoenzyme is characteristic of a specific tissue or cell population and is subject to different regulatory signals. Aerobic and gluconeogenic tissues contain mostly the H form, which is primarily concerned with the conversion of lactate to pyruvate. M-type LDH predominates in anaerobic and glycolytic tissues, where it converts pyruvate to lactate.[40] LDH isoenzymic composition is not only tissue specific, but it also changes in the same tissue with development.[41] The greater proportion of M4 isoenzyme is present in the developing embryo of mammals, as their metabolism is mostly anaerobic. A shift towards H4 occurs as development proceeds and the developing organism becomes increasingly dependent on aerobic metabolism. In the chick embryo which develops in an aerobic environment, the H4 isoenzyme predominates and shifts to M4-type isoenzyme during the later stages of development.

These changes in isoenzyme composition are not restricted to development, but extend into adulthood and old age.[42] The proportion of M4-LDH is considerably lower in the heart, skeletal muscle and brain of old as compared to young rats, with a concomitant increase of H4-LDH in old age. This shift in LDH isoenzymes has been correlated with changing metabolic functions with advancing age: decreased M4-LDH may result in a decreased ability of tissues from old animals to cope with anaerobic conditions.[43]

The isoenzyme pattern of isocitrate lyase is markedly altered in old as compared to young nematodes *(Turbatrix aceti)*.[44] Studies on cytoplasmic alanine aminotransferase (c-AAT) in rat liver reveal that the phenomena of sequential changes do extend to old age.[45] Liver from young (5 weeks of age) rats has the A-type isoenzyme, the adult (52 weeks) has both the A and B types, while the old (100 weeks) has only the B isotype. Both subunits are under the control of two separate genes which are sequentially activated and repressed at different ages.[46]

The mono-, di-, and tetrameric forms of the enzyme glucose-6-phosphate dehydrogenase differ quantitatively in the liver of young as compared to old rats.[47] Selective enhancement of isozymic variants of β-D-glucosidase and acid phosphatase has also been reported in aging *Caenorhabditis elegans*.[48] Overall, these isoenzymes shifts may be explained by regulatory changes in the activity of their corresponding genes consequent to endogenous programmed signals.

3.3 Enzyme Induction During Aging

Induction of enzymes is caused by "inducers" or "effectors", which may be either the substrate, a metabolite, or an exogenous factor. Induction is an adaptive process. The ability to initiate adaptive changes in the activity of many enzymes is impaired with old age.[49] The induction of enzymes by hormones during aging has been extensively reviewed.[34] The magnitude of induction of many enzymes decreases, increases, or remains unchanged with the increasing age of the animal.

The expression of age-related adaptive changes in enzyme induction has been categorized into four general patterns of response.[49] With aging, and depending on a number of variables (animal species, strain, sex, and physiologic state, environmental conditions), the response (1) may have an altered adaptive latent period (or initiation time) following the stimulus, without affecting the magnitude of the induction, (2) may decrease or increase in the magnitude of induction without changes in latency, (3) may show alterations in both magnitude and latency, and (4) may fail to show any changes in the induction pattern.

Despite the great variability of responses, an overall picture shows a decrease in the magnitude of the adaptive response with an increase in latency. Identical stimuli elicit weaker responses in older animals, although the reverse is seen in a few cases.[50] Among inducers, hormones are important in the maintenance of overall adaptive responses of enzymes during the lifespan. With respect to hormonal actions, the degree of tissue responsiveness would be directly proportional to the amount of hormone bound to its specific receptor.[51] Alterations in responsiveness may depend on changes in the number of receptors and in the physicochemical properties of receptor molecules[52-54] and/or transacting factors involved in hormonal regulation of gene expression.[55-57]

3.4 Changes in the Kinetic Properties of Enzymes

The hypothesis that proteins are altered during aging is discussed in Chapters 4 and 6. The proposed concept assumes that faulty proteins are formed with aging. The hypothesis creditably stimulated a considerable amount of research both theoretical and practical to confirm or negate the production with aging of error-containing proteins and their possible causative role in aging.[58-61] Search for errors was vigorously pursued by measuring in great detail the kinetic properties, molecular weight, electrophoretic mobility, heat stability, and antigenicity of many enzymes at progressive ages and in several animal species.

Despite some differences in the molecular properties of a few enzymes, the overwhelming evidence negates the idea that errors in proteins may cause aging. Significant differences in young as compared to old enzymes have not been convincingly demonstrated. If errors were made in transcription by RNA polymerase or during translation by tRNA synthetase, then all proteins would contain errors, which in fact does not occur in old animals. Some enzymatic alterations reported in old animals may simply be due to a change in the shape of the molecules with no covalent modifications.[61] For example, aldolase A from rabbit muscle differs in conformation between young and old animals.[62] Comparison of the properties of glyceraldehyde-3-phosphate dehydrogenase (GPDH) and phosphoglycerate kinase (PGK) in tissues from young and old animals indicates lower specific activity of the enzymes and greater heat sensitivity, without differences in amino acid composition, –SH groups, UV spectra, and sedimentation coefficient.

Even in the absence of errors in the protein synthetic machinery, it is after completion of synthesis that the altered enzymes may be produced by altered protein folding. Oxidative damage is one of the possible mechanisms capable of altering proteins and enzymes during aging (Chapter 6). The apparent changes in the activity of enzymes and the shift in isoenzyme patterns may be viewed as expression of adaptive responses, coordinating with intrinsic and extrinsic factors responsible for the hormonal and metabolic status of the organism at progressive ages.

4 CELL INJURY AND CELL DEATH

The more our knowledge of cell complexity increases, the more we become aware of the difficulty of distinguishing between a normal and an abnormal cell and, even more so, between a normal adult and an aging cell. Formerly, it was considered an easy task to identify a living amoeba: it withdrew from a noxious stimulus and, if it failed to withdraw, it was either sick or dead. Today, with rapid advances in cell and molecular biology, the line between health and injury, even in a simple organism such as the unicellular amoeba, is being more finely drawn. Slight swelling of the mitochondria or minute increases in intracellular sodium levels have now become criteria for assessing injury or aging of a cell. Changes in cellular morphology represent only one aspect of cellular aging. Biochemical and functional changes consistently accompany, precede, or cause changes in structure.

The view of aging as a progressive alteration of structure and an impairment of function with time makes it extremely difficult to differentiate cellular changes due to age from those due to injury. In both cases, morphologic changes underlie the functional disturbances: if the injury is severe enough and the aging process sufficiently advanced, they will lead to cellular death. This section considers briefly certain types of morphologic alterations and cell death. Major causes of cellular aging and death have already been discussed in Chapter 4 with the theories of aging.

4.1 Cell Degeneration

Cellular degeneration has already been defined as reflecting altered structure and impaired function. It may be (1) reversible or (2) irreversible and lead to cell death (when aging or injury are applied over a longer period of time or to a more intense degree). In this sense, cellular degeneration and cellular death merely reflect two levels of severity of cell damage, the former compatible with recovery and the latter resulting in death. Cell degeneration is often accompanied by infiltration, that is, entrance (and accumulation) into the cell of normal or abnormal substances usually kept outside. Cellular degenerations and infiltrations have classically been subdivided into specific morphologic patterns based on damage location or nature of the metabolite that accumulates within the cell. Some of the principal changes of this type are listed here; for more information on this subject, the reader is referred to specialized textbooks of pathology.

4.2 Cloudy Swelling/Dehydration

Changes in cell membrane transport and in cell metabolism result in abnormal movements of water and solutes in and out of the cell. Water accumulation in the cell is referred to as "edema" or "swelling" and, when associated with high solute content, as "cloudy swelling". The opposite condition, i.e., water loss, is referred to as "dehydration".

Intracellular water accumulates in the ER or the mitochondria, probably due to impairment of respiratory energy-releasing mechanisms. The loss of this energy would inhibit continued excretion of sodium and water. Whether sodium is primarily affected and increased intracellular water is secondary or whether changes in membrane permeability directly promote the passage of water remains to be established. Edema is often preceded by a decrease in protein synthesis, but the relationship between this decrease and edema of mitochondria and ER is not clear. A more severe form of intracellular edema, "hydropic" or "vacuolar" degeneration, is produced in the same way as cloudy swelling but implies a greater accumulation of water, often in vacuoles and throughout the cytoplasm.

A certain degree of dehydration occurs usually with age, as manifested in the shrunken appearance of skin in general and the "dried-up look" associated with senility. Water content decreases during growth, from a total body water value of approximately 80% at birth to about 50% in the adult; however, further decrease of body water with advancing years occurs at a very slow rate. Indeed, some investigators claim that dehydration *per se* is not a characteristic of aging and that changes in body water with age involve a redistribution of water between extracellular and intracellular compartments rather than a net loss. Such a redistribution may be consequent to a number of factors related either to the cell population of the tissue considered (e.g., loss of cells, redistribution of cell population, replacement of specific cells with fibroblasts) or to shifts in water between intracellular and extracellular spaces (e.g., alterations in membrane permeability, ion distribution, energy required for transport). For example, in skeletal muscle of old rats, the extracellular fluid is nearly double that found in young and mature animals, whereas intracellular water and potassium remain relatively constant. In heart, brain, and liver tissue of rats, little change occurs in the amount and composition of either extracellular or intracellular compartments. Conversely, chemical analysis of the kidney in both humans and rats indicates an increase in extracellular water and a considerable decrease in cell mass with age (Chapter 21). The decrease of protoplasmic units (e.g., loss of muscle fibers) would show a positive correlation with an increase in the proportion of extracellular water to total body water and a negative correlation with the quantity of intracellular constituents.

4.3 Fat/Glycogen Changes

With middle and advanced age, whole-body adipose (fatty) tissue has the tendency to accumulate in many mammals including humans.[63] In addition to overall increased body fat, abnormal accumulation of fat occurs within cells (other than those of the adipose tissue), *Fatty change* (also referred to as fatty degeneration, fatty infiltration, fatty metamorphosis) represents a common response to a derangement of cellular metabolism. It is indicative of severe cellular dysfunction and, although in itself it is a reversible alteration, it is often the harbinger of cell death. Examples of fatty change can be found in the lipid (fatty) loading of smooth muscle "foam" cells in the arterial wall and in the lipid accumulation in the atheroma, both characteristic lesions of atherosclerosis (Chapters 16 and 17).