PHYSIOLOGICAL BASIS OF GERIATRICS

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CHAPTER

6 ENZYMATIC CHANGES DURING AGING

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Enzymes are specific proteins that catalyze chemical reactions in biologic systems. Enzymatic studies were among the first biochemical changes demonstrated to occur during aging. Subsequently, a large literature has developed concerning changes in enzyme levels and has been well reviewed (Finch, 1972; Wilson, 1973, 1981; Kanungo, 1980). At present, this collected information is hard to correlate owing to the variability and contradictory nature of the reported results. Nevertheless, these studies have stimulated interest in aging research and for this reason they have benefited the field. One of the problems in obtaining consistent results is that the enzymes have been studied individually without relationship to others in a particular metabolic pathway. To study all the enzymes of one particular metabolic pathway would provide a complete profile of their biologic function during aging. In the present chapter, enzyme levels and changes in isoenzyme composition with aging are presented first and information thereon is summarized in corresponding tables. Following this, enzyme induction and kinetic changes during aging are described.

ENZYME LEVELS DURING AGING

It is difficult to draw from the current data (see Table 6–1) a unified pattern in enzymatic changes during aging. There is little consistency in the data from different workers. This is mainly owing to the different experimental procedures in measurement of enzyme activity and criteria for unit expression, such as per mg protein, per g wet weight of tissue, or per mg DNA. Moreover, animal strain and maintenance conditions also vary widely. Most enzyme assays have been performed with crude preparations, and results are related to wet weight and protein content, which themselves are subject to vary with age, sex, and strain. Most of the tissues analyzed consist of a heterogeneous cell population in which the changes in enzyme activity may not be similar or of the same magnitude in all cells.

Characteristics and Function of Enzymes. The most striking characteristics of all enzymes are their enormous catalytic power and specificity. They accelerate reactions by factors of 10^6 to 10^{12} as compared to uncatalyzed reactions. Enzymes are highly specific both in the reaction catalyzed and in their choice of reactants, called substrates. An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions. The catalytic ability and specificity are determined by certain amino acid side chains of an enzyme molecule. These amino acids need not be adjacent to one another in the linear sequence of polypeptide and are brought into proximity, i.e., active site, by the folding of the primary sequence of amino acid residues. The binding of substrate to an enzyme usually involves formation of several types of noncovalent bonds: ionic, hydrogen, hydrophobic, and Van der Waals interactions. The active site of enzymes has an array of chemical groups that are precisely arranged so that the specific substrate can be more tightly bound than any other molecule.

Enzymes catalyze a wide variety of chemical reactions. Some, which catalyze oxidation/reduction, are grouped as *oxidoreductases*. Others, which catalyze transfer of different groups such as -CH3, PO4, NH3, etc., are termed *transferases*. *Hydrolases* catalyze hydrolytic reactions whereas *lyases* catalyze the addition of groups to double bonds, or vice versa. Isomerization reactions are catalyzed by *isomerases*, whereas *ligases* (synthetases) catalyze the condensation of two molecules coupled with the cleavage of a pyrophosphate bond of ATP or other triphosphates.

The activity of many enzymes is regulated. Their rate of synthesis, as well as their final concentration, is under genetic control and is 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 a small group on the en-

Table 6–1. CHANGES IN ENZYME LEVELS DURING AGING

ENZYME	ANIMAL	TISSUE	AGES STUDIED (MONTHS)	UNITS/MG	EFFECT OF AGE	REFERENCE
Oxidoreductases						
Cytochrome oxidase (T)	Mouse (M)	Liver	4,18,30	DNA; Protein	Decrease	Wilson, 1972
Glucose-6-phosphate dehydrogenase	Rat (M) (Sprague-Dawley)	Liver	3,24	Protein	Decrease	Wang and Mays, 1977
	Rat (M) (Fischer)	Liver	3,24	Protein	Increase	Wang and Mays, 1977
	Mouse (M)	Liver	4,18,30	DNA; Protein; Wet weight	Increase	Wilson, 1972
α-Glycerophosphate dehydrogenase (c)	Rat (F)	Liver	6,24	Protein	Decrease	Bulos *et al.*, 1971
Isocitrate dehydrogenase (c)	Rat (F)	Liver	6,10,13	Wet weight	Decrease	Webb and Bailey, 1975
Lactate dehydrogenase (c)	Rat (M)	Liver	6,21	DNA	Increase	Ross and Ely, 1954
	Rat (F)	Liver	12,24	Protein	No change	Schmuckler and Barrows, 1966
	Rat (F)	Liver	3,24	Wet weight	Decrease	Singh and Kanungo, 1968
	Mouse (M)	Liver	4,18,30	DNA; Protein; Wet weight	Increase	Wilson, 1972
	Mouse (F)	Liver	4,18,30	DNA; Protein; Wet weight	No change	Wilson, 1972
Malate dehydrogenase (c)	Rat (M)	Liver	2,18	DNA	Increase	Ross and Ely, 1954
	Rat (F)	Liver	6,21	Protein	Increase	Kanungo and Gandhi, 1972
	Rat (M)	Liver	1.5,8,24	Protein	Increase	Sharma and Patnaik, 1982b
Succinate dehydrogenase (T)	Rat (M)	Liver	6,21	Wet weight	No change	Ross and Ely, 1954
Glucose-6-phosphate dehydrogenase (c)	Mouse (F)	Brain	5,25	Wet weight	Increase	Wulf and Cutler, 1975
Lactate dehydrogenase (c)	Rat (F)	Brain	12,24	Protein	Decrease	Schmuckler and Barrows, 1967
	Rat (F)	Brain (CH)	3,10,13,24	Wet weight	Decrease	Singh and Kanungo, 1968
Malate dehydrogenase (c)	Rat (M)	Brain	1.5,8,24	Protein	Increase	Sharma and Patnaik, 1982b
Cytochrome oxidase (T)	Rat (F)	Kidney	13,27	Wet weight	Decrease	Barrows *et al.*, 1958
	Mouse (F)	Kidney	18,30	DNA; Protein; Wet weight	No change	Wilson and Franks, 1971
Glucose-6-phosphate dehydrogenase (C)	Mouse (M)	Kidney	6,18,30	DNA	Increase	Wilson and Franks, 1975
	Mouse (F)	Kidney	6,18,30	DNA	Decrease	Wilson and Franks, 1975
Lactate dehydrogenase (c)	Rat (F)	Kidney	12,24	Protein	No change	Schmuckler and Barrows, 1967
	Mouse (F)	Kidney	4,18,30	DNA; Protein; Wet weight	No change	Wilson and Franks, 1971
	Mouse (M)	Kidney	4,18,30	DNA; Protein; Wet weight	Increase	Wilson and Franks, 1971
Succinate dehydrogenase (c)	Rat (F)	Kidney	13,27	Wet weight	Decrease	Barrows *et al.*, 1958
	Rat (M)	Kidney	13,27	Wet weight	Decrease	Barrows *et al.*, 1958
Lactate dehydrogenase (c)	Rat (F)	Heart	12,24	Protein	No change	Schmuckler and Barrows, 1967
	Rat (F)	Heart	3,24	Wet weight	Decrease	Singh and Kanungo, 1968
Malate dehydrogenase (c)	Rat (F)	Heart	5,24	Wet weight	Decrease	Singh, 1973
Lactate dehydrogenase (c)	Rat (F)	Skeletal Muscle	3,30	Protein; Wet weight	Decrease	Schmuckler and Barrows, 1966
	Rat (F)	Skeletal Muscle	3,24	Wet weight	Decrease	Singh and Kanungo, 1968
Transferases						
Alanine aminotransferase (c,m)	Rat (F)	Liver	1.5,21	DNA; Protein	Decrease	Patnaik and Kanungo, 1976

Table 6–1. CHANGES IN ENZYME LEVELS DURING AGING (Continued)

ENZYME	ANIMAL	TISSUE	AGES STUDIED (MONTHS)	UNITS/MG	EFFECT OF AGE	REFERENCE
Aspartate aminotransferase (c)	Rat (M)	Liver	1.5,8,24	Protein	Increase up to 8 mo. then constant	Sharma and Patnaik, 1987
Aspartate aminotransferase (m)	Rat (M)	Liver	1.5,8,24	Protein	No change	Sharma and Patnaik, 1987
Collagen glucosyl transferase (T)	Rat (F)	Liver	3,8,14	Protein	Increase	Risteli and Kivirikko, 1976
Hexokinase (c)	Rat (M)	Liver	3,18	Protein	Decrease	Bartoc *et al.*, 1975
Phosphoenolpyruvate carboxykinase (c)	Rat (M)	Liver	1.5,8,24	Protein	Increase up to 8 mo. then decrease	Sharma and Patnaik, 1983
Phosphofructokinase (T)	Rat (F)	Liver	6,10,13	Wet weight	Increase	Webb and Bailey, 1975
Pyruvate kinase (c)	Rat (F)	Liver	6,10,13	Wet weight	Increase	Webb and Bailey, 1975
t-RNA methylase (c)	Mouse (M)	Liver	12,18,30	Protein	Decrease	Mays *et al.*, 1973
Tyrosine aminotransferase (c)	Rat (M)	Liver	1.5,19	Protein	No change	Ratha and Kanungo, 1977a
Tyrosine aminotransferase (m)	Rat (M)	Liver	9,19	Protein	Decrease	Ratha and Kanungo, 1977a
Aspartate aminotransferase (T)	Rat (−)	Brain	3,11,24	Protein	Increase	Oeriu, 1963
Aspartate aminotransferase (c)	Rat (M)	Brain (CH)	1.5,8,24	Protein	Increase	Sharma and Patnaik, 1985
Aspartate aminotransferase (m)	Rat (M)	Brain (CH)	1.5,8,24	Protein	Decrease	Sharma and Patnaik, 1985
Cholineacetyltransferase	Mouse (−)	Brain (Hipp)	8,24	Protein	Decrease	Vijayan, 1977
	Rat (M,F)	Brain (CH)	10,20	Protein	Decrease	James and Kanungo, 1978
Glutamic-pyruvic transaminase (T)	Rat (−)	Brain	3,11,24	Wet weight	Increase	Oeriu, 1963
Phosphoenolpyruvate Carboxykinase (c)	Rat (M)	Brain (CH)	1.5,8,24	Protein	Increase up to 8 mo. then decrease	Sharma and Patnaik, 1983
Pyruvate kinase (c)	Rat (M,F)	Brain (CH)	9,19	Protein	Decrease	Chainy and Kanungo, 1976
Pyruvate kinase (c)	Mouse (M)	Kidney	6,23	Protein	No change	Zorzoli and Li, 1967
	Rat (M)	Heart	9,19	Protein	No change	Chainy and Kanungo, 1978a
	Rat (F)	Heart	9,19	Protein	Decrease	Chainy and Kanungo, 1978a
	Rat (M)	Skeletal Muscle	9,19	Protein	Decrease	Chainy and Kanungo, 1978a
	Rat (F)	Skeletal Muscle	9,19	Protein	No change	Chainy and Kanungo, 1978a
Hydrolases						
Acid Phosphatase (T)	Rat (M)	Liver	6,21	Wet weight	No change	Ross and Ely, 1954
	Mouse (M,F)	Liver	4,18,30	DNA; Protein; Wet weight	Increase	Wilson, 1972
ATPase	Rat (M)	Liver	6,21	Wet weight	Increase	Ross and Ely, 1954
Fructose-1,6-diphosphatase (c)	Rat (M)	Liver	3,18	Protein	Decrease	Ross and Ely, 1954
Glucose-6-phosphatase	Rat (M)	Liver	6,10,13	Wet weight	Decrease	Singhal, 1967
	Mouse (M,F)	Liver	4,18,30	DNA; Protein; Wet weight	Decrease	Wilson, 1972
Lysyl hydroxylase (c)	Rat (F)	Liver	3,8,14	Protein	Decrease	Risteli and Kivirikko, 1976
Acetylcholinesterase	Rat (F)	Brain (CH)	2,6,16	Protein	Decrease	Moudgil and Kanungo, 1973
	Rat (M,F)	CH	10,20	DNA; Protein	Decrease	James and Kanungo, 1978
Cholinesterase	Rat (F)	CH	1.5,24	DNA; Protein	Decrease	Kaur and Kanungo, 1970
ATPase	Mouse (M,F)	Brain	4,28	DNA	Decrease	Hollander and Barrows, 1968
Glucose-6-phosphatase (T)	Rat (M)	Kidney	6,24	Protein	Increase	Grinna and Barber, 1972
	Mouse (F)	Kidney	4,18,30	DNA; Protein; Wet weight	Decrease	Wilson and Franks, 1971

Table 6–1. CHANGES IN ENZYME LEVELS DURING AGING (Continued)

ENZYME	ANIMAL	TISSUE	AGES STUDIED (MONTHS)	UNITS/MG	EFFECT OF AGE	REFERENCE
Acid phosphatase (T)	Rat (–)	Kidney	6,13,24	Protein	Decrease	Franklin, 1961
	Mouse (M)	Kidney	3,19	Wet weight	Decrease	Kurnick and Kernen, 1962
Acid DNase (T)	Rat (F)	Kidney	5,25	Protein nitrogen	Increase	Franklin, 1962
Alkaline DNase (T)	Rat (F)	Kidney	5,25	Protein nitrogen	Increase	Franklin, 1962
Alkaline phosphatase (T)	Mouse (M)	Kidney	4,18,30	DNA; Protein; Wet weight	Increase	Wilson and Franks, 1971
ATPase (T)	Rat (–)	Heart	5,20	Protein	No change	Honorati and Ermine, 1974
Cathepsin (T)	Rat (F)	Heart	3,12,24	Wet weight	Increase	Barrows and Roeder, 1961
ATPase (c)	Rat (M)	Skeletal Muscle	9,16,26	Wet weight	Decrease	Rockstein and Brandt, 1961
Lyases						
Aldolase (c)	Mouse (F)	Liver	3,31	Protein	Decrease	Gershon and Gershon, 1973
	Rat (M)	Liver	3,18	Protein	Decrease	Bartoc et al., 1975
	Rat (M)	Liver	2,24	Protein	No change	Anderson, 1976
ATP-citrate lyase	Rat (F)	Liver	6,10,13	Wet weight	Increase	Webb and Bailey, 1975
Ornithine decarboxylase	Rat (M)	Liver	2,12,30	Wet weight	Decrease	Ferioli et al., 1976
Citrate synthetase (m)	Rat (M)	Liver	1.5,8,24	Protein	Increase	Sharma and Patnaik, 1984
	Rat (M)	Brain	1.5,8,24	Protein	Increase	Sharma and Patnaik, 1984
Lygases						
Glutamine synthetase	Rat (F)	Liver	4.5,17	Protein	No change	Rao and Kanungo, 1972

c, cytoplasmic; m, mitochondrial; T, total; M, male; F, female; CH, cerebral hemispheres; Hipp, hippocampus.

zyme. A different kind of regulatory mechanism affects many reaction sequences where an enzyme that catalyzes the first step is inhibited by the end product. 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 the amount of active enzyme available and of the presence of substrates, cofactors, inhibitors, and activators. Changes in the levels or properties of these enzymes may alter the functional ability of an organism. Since enzymes are responsible for specific functions, the various phases of the lifespan of an organism, such as differentiation, development, and reproductive maturity, may depend on the activity of specific enzymes or their isoenzymes.

Some of the changes in enzyme levels due to differences in sex and strain are as follows: The activity of glucose-6-phosphate dehydrogenase increases in the kidney of aging male mouse whereas it decreases in the females (Wilson and Franks, 1971); that of lactate dehydrogenase increases in liver of aging male mice but does not change in females (Wilson, 1972); that of glycine-*n*-methyltransferase decreases in liver of aging mice but increases in the liver of aging rats (Mays *et al.*, 1973). In aging Sprague-Dawley rats, liver glucose-6-phosphate dehydrogenase activity is increased by half, but it is doubled in Fischer 344 rats (Wang and Mays, 1977). A 10-fold greater activity of L-glutamine D-fructose-6-phosphate aminotransferase was reported in the submandibular gland of female compared to male mice (Hosoi *et al.*, 1978). The activity of pyruvate kinase does not change in the heart of old male rats but it decreases in the same tissue of old females (Chainy and Kanungo, 1978a). Conversely, the same enzyme decreases in the skeletal muscle of old male rats whereas it remains unchanged in females.

Such variability in the data on enzyme level may also be due to the animal care and their physical activities. If older animals consumed more or less food than young ones or became more sedentary in old age, they would undergo differences in intermediary metabolism and hence variable enzyme levels (Rothstein, 1982). However, when animals are maintained under the same conditions, the variability in enzyme activity persists. This shows that enzymes are always subjected to varying degree of regulatory mechanisms depending on the food, environmental fluctuations, and hormonal and metabolic status of the animals.

Depending on the animal species, the activities of several enzymes decrease or increase or remain unchanged (Table 6–1). Even within each class, the activities of certain enzymes do not show a specific age-related pattern. There is not a single enzyme that shows consistent age-related changes in its activity in all species. However, alterations in the activities of various enzymes may affect the functional ability of organisms with age. The metabolic status of animals is coordinately regulated by a network of enzymes at different phases of the lifespan. Changes in the activities of certain lipogenic and gluconeogenic enzymes in rat liver have been reported at various ages, and lipogenic activity is diminished because of an age-dependent decline in nicotinamide-adenine dinuleotide (NADP)-malic enzyme and ATP-citrate lyase activity (Vitorca *et al.*, 1981). Enzymes of the oxaloacetate metabolism in the liver and brain of male rats of Wistar strain were studied as a function of age (Sharma and Patnaik, 1982a,b, 1985, 1986). The activities of cytoplasmic aspartate aminotransferase and malate dehydrogenase (gluconeogenic enzymes) are higher in these tissues in old rats and may be correlated with the higher involvement of the enzymes in the conversion of the oxaloacetate pool for gluconeogenesis in older rats.

The observed decrease in the levels of certain enzymes and the increase in others may be correlated with the decrease or increase in the template activity of the corresponding genes (Kanungo, 1980) and/or their internal regulation, depending on the need of metabolites as a function of age.

CHANGES IN ISOENZYME COMPOSITION

It is well known that during development, many proteins change from fetal to adult form. Differentiation and development occur because of sequential activation and repression of genes (Davidson and Britten, 1979). This is best exemplified by the shift of various chains of hemoglobin during the gestation period in humans (see Chapter 4).

Isoenzymes are enzymes performing the same function, but present in more than one molecular form within the same tissue or species. The best example is lactate dehydrogenase (LDH), a tetramer made of two different types of subunits called *M* (predominant form in skeletal muscle) and *H* (predominant in the heart). These two subunits are controlled by two separate genes. Various isoenzymes of lactate dehydrogenase are formed by combination of M and H subunits (M4, M3H, M2H2, MH3, and H4). Each isoenzyme is characteristic of individual tissue or cell population and is subject to different regulatory changes. Aerobic and gluconeogenic tissues contain mostly the *H* form, which is primarily concerned with the conversion of lactate to pyruvate. M-type LDH is predominantly found in anaerobic and glycolytic tissues, where it converts pyruvate to lactate (Markert and Ursprung, 1962). LDH isoenzymic composition is not only tissue specific, but also changes in the same tissue during development (Markert and Möller, 1959). The greater proportion of M4 isoenzyme is present in developing embryo of mammals as their metabolism is mostly anaerobic in nature. A shift toward H4 occurs as development proceeds. Contrary to this, a greater proportion of H4 isoenzyme is found in developing chick embryo, which grows in an aerobic milieu, and shows a shift toward M4 type during later stages of development.

These changes in the composition of isoenzymes are not restricted to development, but extend into adulthood and aging (Kanungo, 1980). The proportion of M4-LDH is considerably lower in the heart, skeletal muscle, and brain of older rats, with concomitant increase of H4-LDH in old age. The shift in isoenzymes of LDH has been correlated with the differing metabolic functions of the organisms as a function of age. The decrease in proportion of M4-LDH may cause a decrease in the ability of the tissue to cope with anaerobic conditions (Singh and Kanungo, 1968). Later work on pyruvate kinase, having four isoenzymes (PK1, 2, 3, and 4), shows that PK-4, the predominant form at birth, is replaced by PK3 after 14 days of postnatal life in the skeletal muscle of rats (Osterman *et al.*,

1973). Reiss and Rothstein (1975) reported a considerable alteration in the pattern of isoenzymes of isocitrate lyase in aged versus young nematodes, *Turbatrix aceti*. Further studies on cytoplasmic alanine aminotransferase (c-AAT) of the rat liver reveal that the phenomena of sequential changes do extend to old age (Patnaik and Kanungo, 1976). Young (5-week) rat liver has A type and adult (52-week) has both the isotypes (A and B) while old (100-week) has only the B isotype. Both subunits are under the control of two separate genes (Chen and Giblett, 1971). Genes for both the isotypes of hepatic c-AAT are sequentially activated and repressed at different phases of the lifespan in rats (Patnaik and Kanungo, 1976). Wang and Mays (1977) reported a quantitative difference in the mono-, di-, and tetrameric forms of glucose-6-phosphate dehydrogenase in the liver of young and old rats. The possible reason for such a shift in isoenzymes may be the regulatory changes in the activities of their corresponding genes, which may be brought about by certain endogenous factors according to a specific program (Kanungo, 1980).

ENZYME INDUCTION DURING AGING

Induction of enzymes is caused by several inducers or effectors, which may be either the substrate or a hormone or a metabolite or even an exogenous factor. This phenomenon is an adaptive process. The ability to initiate adaptive changes in the activity of many enzymes is impaired in old age (Adelman, 1975, 1981). The induction of enzymes by hormones during aging has been extensively reviewed (Pitot and Yatvin, 1973; Adelman, 1975; Dickerson and Basu, 1975; Kanungo, 1980). The magnitude of induction of many enzymes decreases with the increasing age of the animal. These enzymes are: tryptophan pyrrolase by tryptophan and estradiol (Haining and Correl, 1969; Patnaik and Sarangi, 1980), glucose-6-phosphatase, fructose-1, 6-diphosphatase, phosphoenolpyruvate carboxykinase by glucocorticoids (Singhal, 1967; Sharma and Patnaik, 1983), glucokinase by glucose (Adelman, 1970), malate dehydrogenase (MDH) (Kanungo and Gandhi, 1972; Sharma and Patnaik, 1982b), arginase (Rao and Kanungo, 1974), alanine aminotransferase (Patnaik and Kanungo, 1976), tyrosine aminotransferase (Ratha and Kanungo, 1977a), aspartate aminotransferase (AsAT) (Sharma and Patnaik, 1985, 1987), ornithine aminotransferase (Rahman and Peraino, 1973) of the rat liver by glucocorticoids; and phosphofructokinase and phosphohexose isomerase of the uterus (Singhal *et al.*, 1969), acetylcholinesterase (Moudgil and Kanungo, 1973), and pyruvate kinase (Chainy and Kanungo, 1978a) of the brain by sex steroids.

The degree of induction of glutamate synthetase (Rao and Kanungo, 1972) and creatine phosphokinase (Srivastava and Kanungo, 1980) increases with the increasing age of the rat. The lag period for the induction of certain enzymes, such as tyrosine aminotransferase by cold exposure (Finch *et al.*, 1969) and glucokinase by insulin (Adelman, 1970), increases in old age. However, the lag period for the induction of mitochondrial glycerophosphate dehydrogenase by thyroxine (Bulos *et al.*, 1972) and tyrosine aminotransferase by corticosteroids or insulin (Finch *et al.*, 1969) in the rat liver does not change with age. Age-related variations in the response of isoenzymes to hormones have been well studied. Cytoplasmic-MDH but not mitochondrial-MDH is inducible by cortisone and hydrocortisone in aged rats (Kanungo and Gandhi, 1972; Sharma and Patnaik, 1982b). Furthermore, cytoplasmic-AsAT is inducible in the liver and brain of rats of all the ages but mitochondrial AsAT remains unresponsive (Sharma and Patnaik, 1985, 1986).

The expression of age-related adaptive changes in enzyme induction has been categorized into four general patterns of response, as shown in Figure 6–1 (Adelman, 1975): (1) The response has an altered adaptive latent period or initiation time following stimulus without affecting the magnitude of induction; (2) the response shows a decrease or increase in the magnitude of induction with no change in the latent periods; (3) the response alters both the latent period and the magnitude of induction; and (4) age-related changes do not occur in the induction pattern.

The patterns of enzyme adaptation, as is

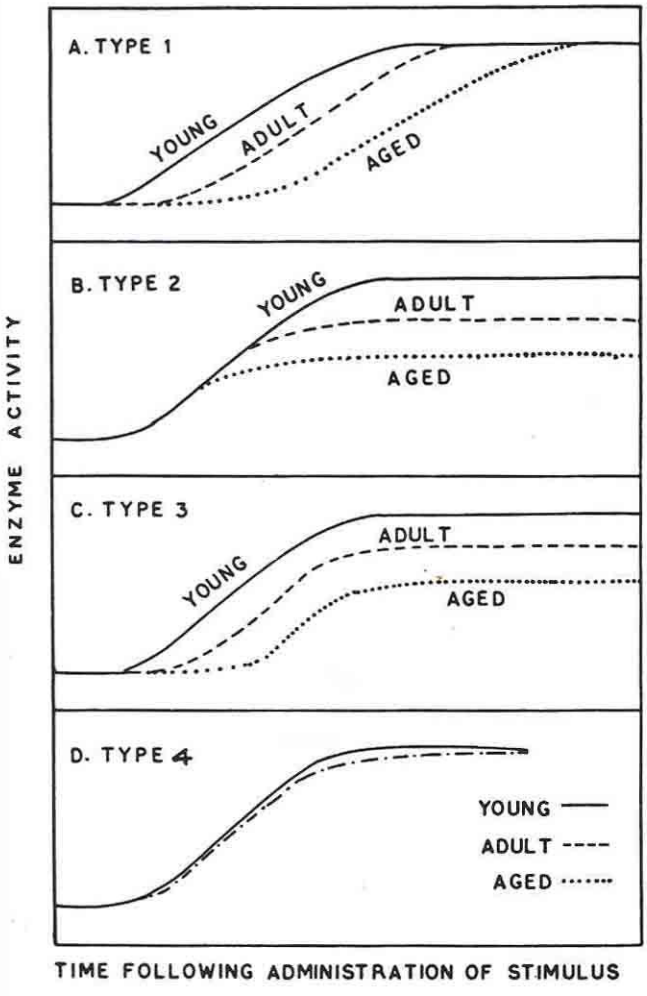

Figure 6–1. *Patterns of age-dependent enzyme adaptation.*

The expression of age-related adaptive changes in enzyme induction has been categorized into four general types (*A, B, C,* and *D*). Each type has been discussed in the text. (From Adelman, R.C.: In Parke, D.V. (ed.): *Enzyme Induction,* Plenum Press, New York, 1975.)

the case for normal enzyme levels, are also susceptible to considerable variation related to difference in species, strain, sex, and conditions of environmental maintenance and the physiologic state of the animals. In general, the magnitude of the response is decreased and the lag period is increased in old age (Table 6–2). Identical dosages of stimuli per unit of body weight elicit weaker biochemical responses in older animals, although the reverse is seen in a few cases (Gusseck, 1972). The degree of tissue responsiveness would be directly proportional to the amount of steroid hormones bound to specific receptors (Baxter and Forsham, 1972). Age-dependent impairment in responsiveness to the hormonal and other biochemical stimuli are dependent not only on receptor level, but also on the physicochemical properties of receptor molecules (Kalimi, 1984; Sharma and Timiras, 1987).

Hormonal Actions. The first step in the action of a hormone is the binding to a specific receptor, which is located on the cell surface or in the cytosol and/or nucleus of the target cells. Hormones act as the signaling molecules and are composed of several distinct classes of biologic compounds, such as glycoproteins, steroids, polypeptides, and modified amino acids. All hormones act via receptor proteins. The function of these specialized receptors is at two levels: (1) specific hormone recognition and (2) initiation of a series of cellular events leading to characteristic responses to the hormone. Hormone receptors reside at different cellular locations, depending on the hormone ligand. For example, receptors are on cell surface for polypeptide hormones, cytoplasm and/or nucleus for steroids, and nucleus for thy-

Table 6–2. ENZYME INDUCTION DURING AGING

ENZYME	ANIMAL (SEX)	TISSUE	AGES STUDIED (MONTHS)	EFFECTORS	MAGNITUDE OF RESPONSE*	REFERENCE
Acetylcholinesterase	Rat (M)	Brain (CH)	10,20	Estradiol & Testosterone	D	James and Kanungo, 1978
Adenylate cyclase	Rat (M,F)	Liver	3,12,24	Epinephrine	I	Kalish *et al.*, 1977
	Rat (M)	Fat cells	2,12,24	Epinephrine	D	Cooper and Gregerman, 1977
Alanine aminotransferase (c)	Rat (F)	Liver	7,21	Hydrocortisone	D	Patnaik and Kanungo, 1976
Alanine hydroxylase	Rat (F)	Liver	1.3,9.9,19.8	Phenobarbital	D	Kato and Takanaka, 1968
Alkaline phosphatase	Mouse (F)	Uterus	6,11,21	Estradiol	D	Holinka et al., 1977
Arginase	Rat (F)	Liver	2,17	Cortisone	NC	Rao and Kanungo, 1974
Aspartate aminotransferase (c)	Rat (M)	Liver; Brain (CH)	1.5,8,24	Hydrocortisone	D	Sharma and Patnaik, 1985, 1987
Choline acetyltransferase	Rat (M,F)	Brain (CH)	10,20	Testosterone	I	James and Kanungo, 1978
Fatty acid synthetase	Mouse (M)	Liver	1,8,18	Fatless diet	D	Eisenbach *et al.*, 1976
Fructose-1,6-diphosphatase	Rat (M)	Liver; Kidney	2,12,24	Hydrocortisone	D	Frolkis *et al.*, 1979
Glucokinase	Rat (M,F)	Liver	2,14,24	Glucose	NC	Adelman, 1970
Glucose-6-phosphatase	Rat (M)	Liver	1,6,12,15	Dexamethasone	D	Singhal, 1967
Glutamine synthetase	Rat (F)	Liver	2,18	Cortisone	D	Rao and Kanungo, 1972
α-Glycerophosphate dehydrogenase	Rat (F)	Liver	12,28	3,3,5-Triiodothyronine	NC	Bulos *et al.*, 1971, 1972
Malate dehydrogenase	Rat (F)	Liver	3,18	Cortisone	D	Kanungo and Gandhi, 1972
(c)	Rat (M)	Liver; Brain	1.5,8,24	Hydrocortisone	D	Sharma and Patnaik, 1982b
Ornithine aminotransferase	Rat (M,F)	Liver	1,2	High-casein diet	D	Rahman and Peraino, 1973
Phosphoenolpyruvate Carboxykinase	Rat (M)	Liver	1.5,8,24	Hydrocortisone	D	Sharma and Patnaik, 1983
Phosphofructokinase	Rat (M)	Prostate	1,3,12	Testosterone	D	Singhal, 1967
Pyruvate Kinase	Rat (M,F)	Brain (CH)	9.5,19.5	Estradiol	D	Chainy and Kanungo, 1976
Serine dehydratase	Rat (M)	Liver	1,12	High-protein diet	D	Rahman and Peraino, 1973
Tryptophan pyrrolase	Rabbit (F)	Liver	2,4,10	Fasting	D	Wu and Rosenthal, 1966
Tyrosine aminotransferase (c)	Rat (M)	Liver	9,19	Hydrocortisone	D	Ratha and Kanungo, 1977a
Tyrosine aminotransferase (m)	Rat (M)	Liver	9,19	Hydrocortisone	I	Ratha and Kanungo, 1977a

*Old values compared to young values.
I, increase; D, decrease; NC, no change; M, male; F, female; c, cytoplasmic isoenzyme; m, mitochondrial isoenzyme.

roid hormones. The effects of most peptide hormones that bind to cell surface-associated receptors are mediated by the generation of second messengers, which, in turn, activate various intracellular processes. In contrast, steroid hormones appear to exert their effects by the translocation of cytoplasmic steroid-receptor complexes to specific acceptor sites on the chromatin within the nucleus. The potential nuclear receptor sites for steroid-receptor complexes are specific and nonspecific DNA sequences, nuclear-associated proteins, and chromatin (DNA + proteins). The specific molecular mechanism by which nuclear-associated steroid-receptor complexes alter gene expression is unknown. Possibly, they alter the localized chromatin structure by modifying DNA structure and/or directly affecting RNA polymerase activity. It is possible that more than one mechanism operates in a particular target tissue. They may also elicit primary (short) and/or secondary (long-term) responses.

Hormone-mediated responses are controlled partly by binding to specific intracellular receptors and then by translocation of hormone-receptor complexes to nuclear acceptor sites (Yamamoto and Alberts, 1976). All of the molecular events in hormone actions subsequent to receptor binding are subject to alteration with age (Roth, 1981). The impairment in the magnitude of induction of various enzymes by hormones may be due to either decreased concentration of receptor binding sites or altered properties of receptors molecules as a function of age (Kanungo, 1980; Roth, 1981; Kalimi, 1984; Sharma and Timiras, 1987). It should be stressed here that hormones and their receptors are very important in the

maintenance of overall adaptive response of enzymes at various phases of the life-span. Changes in these molecules may lead to an altered synthesis of enzymes as a function of age, which may cause functional deterioration of organisms.

CHANGES IN THE KINETIC PROPERTIES OF ENZYMES

The hypothesis that proteins are altered during aging was proposed by Orgel (1963) and has been discussed in Chapter 4. The proposed concept was based on the assumption that faulty proteins would be formed as a function of age. The hypothesis creditably stimulated a great deal of work, both theoretical and practical (Gershon and Gershon, 1973; Gallant and Palmer, 1979; Kirkwood, 1980; Kanungo, 1980; Rothstein, 1982), to demonstrate whether or not old organisms would produce error-containing proteins. Search for errors was vigorously pursued by measuring in great detail the kinetic properties (K_m, K_i), molecular weight, electrophoretic mobility, heat stability, and antigenicity of various enzymes among different ages and species of animals. Such studies were useful for detecting changes in structural and functional aspects of enzymes present in old age. The initial evidence for altered enzyme properties emerged from the studies of Gershon and Gershon (1970) on isocitrate lyase from senescent free-living nematodes. Subsequent to this report, several enzymes were reported to be altered in old free-living nematode, *Turbatrix aceti*: isocitrate lyase (Reiss and Rothstein, 1975), phosphoglycerate kinase (Gupta and Rothstein, 1976a), enolase (Sharma *et al.*, 1976), and aldolase (Reznick and Gershon, 1977). Altered properties of superoxide dismutase (Reiss and Gershon, 1976) and phosphoglycerate kinase (Sharma *et al.*, 1980) were reported from liver and muscle of aged rats, respectively.

Subsequently, many enzymes have been purified and their kinetic properties reported to be unaltered with age. These enzymes are: acetylcholinesterase (Moudgil and Kanungo, 1973), pyruvate kinase (Chainy and Kanungo, 1978b) in brain; malate dehydrogenase (Kanungo and Gandhi, 1972), tyrosine aminotransferase (Ratha and Kanungo, 1977b), alanine aminotransferase (Patnaik and Kanungo, 1976), and aspartate aminotransferase (Sharma and Patnaik, 1982a) in liver of old rats. Triosephosphate isomerase from nematodes (Gupta and Rothstein, 1976b) and enolase from rat muscle and liver (Rothstein *et al.*, 1980) remain unaltered in aged animals.

For a few enzymes, there is still disagreement: mouse liver aldolase has been reported to be altered (Gershon and Gershon, 1973) or not altered (Petell and Lebherz, 1979; Burrows and Davison, 1980). Reiss and Gershon (1976) reported an altered superoxide dismutase in old rat liver, while the same enzyme appears unaltered in the liver of old mice and dogs (Burrows and Davison, 1980). Most molecular properties of enzymes remain unaltered with aging. This is true for properties such as molecular weight, K_m, K_i, and N- and C-terminal residues, number of sulfhydryl (-SH) groups, gel electrophoretic pattern, and immunodiffusion pattern (Rothstein, 1979, 1982; Kanungo, 1980). In some cases, however, differences have been reported for heat sensitivity and antigenic response. Heat sensitivity is generally altered in most enzymes studied, the old enzyme being less stable. Again the exceptions: nematode aldolase and phosphoglycerate kinase from rat muscle are more heat stable in old age (Rothstein, 1982), but nematode phosphoglycerate kinase does not differ in heat sensitivity (Gupta and Rothstein, 1976a). Thus, heat sensitivity acts as a tool to measure an altered enzyme but lacks uniformity.

In spite of some differences in the molecular properties of a few enzymes, there is enough evidence that negates the idea of errors in the proteins as a function of age. Most strikingly, there are no changes in young versus old enzymes using highly specific techniques of isoelectric focusing. Sequential changes and the possibilities of phosphorylation, acylation, esterification, and deamidation are hard to occur, otherwise they would have been detected by isoelectric focusing of enzymes. Moreover, if errors were made in transcription by RNA polymerase or during translation by tRNA synthetases, then all proteins would con-

tain errors. Many proteins remain unaltered in old animals. Some enzymatic alterations in old animals may simply be due to a change in the shape of the molecules with no covalent modifications (Rothstein, 1982). Demchenko and Orlovska (1980) reported that aldolase A from young and old rabbit muscle differs in conformation. Gafni (1981) studied the properties of muscle glyceraldehyde-3-phosphate dehydrogenase from young and old rats. The old enzyme had lower specific activity and was more sensitive to heat without any detectable differences in amino acid composition, -SH groups, UV spectra, and sedimentation coefficient. Although there is no error in the protein synthetic machinery of old animals, it is after completion of synthesis that the altered enzymes may be produced by conformational changes. The apparent changes in the levels of enzymes and the shift in isoenzyme patterns express an adaptive response, coordinating with the intrinsic and extrinsic factors, of the hormonal and metabolic status of the animals as a function of age.

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