Developmental and hormonal regulation of malate-aspartate shuttle enzymes in chicken

By

Herbert Goldfield Lyngdoh

Department of Biochemistry

Submitted in partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Biochemistry

Of

North Eastern Hill University
Shillong, India
2001

North Eastern Hill University

December, 2001

I, Herbert Goldfield Lyngdoh, hereby declare that the subject matter of thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the **North Eastern Hill University** for the degree of **Doctor of Philosophy** in **Biochemistry**.

Prof. Ramesh Sharma 19/12/01
Supervisor & Head
Department of Biochemistry

HEAD
Deptt. Biochemistry
NEHU, Shillong-22

Herbert Goldfield Lyngdoh
(candidate)

DEDICATED TO

BELLA

AND

MY PARENTS

List of Contents

ACKNOWLEDGEMENTS

At the outset, I glorify the Almighty God for this privelege in presenting this thesis with happiness and satisfaction.

My heartful gratitude and compliment goes to my wonderful guide, Prof. Ramesh Sharma for his unsparing valuable guidance and inspiring supervision throughout the entire period of work and completion of this thesis.

I convey my regards to Prof. A.N. Rai, Prof. R Lalthantluanga, Prof. R. N. Sharan, Dr. A Alam and Dr. A. K. Singh for the efforts they have done for the best of my career.

I cannot but express my indebtedness to Dr. Don Syiem for his initial contributions and the time spared for the countless discussions that have added some dimensions to my work and thinking without which this work would have been that much less.

My special thanks goes to my labmate Harmit for the help, assistance, discussions and cooperation. Thanks also to Gareth, Onarlin, Imliwati, Debipreeta and Indrani for their helping hands.

I also wish to express my sincere gratitude to Rev. Fr. Stephen Mavely, Principal, St. Anthony's College, Shillong where I teach for allowing me to carry out the research work to its completion.

The help and cooperation of Dr. S. R. Joshi, Dr. M. B. Syiem, Dr. M. A. Laskar and Mr. Phrang Majaw, especially in the elctrophoresis experiment done in the college, is gratefully acknowledged.

I sincerely offer my humble gratitude and deepest love to Della, 'Mei', 'Pa', Sheppard (brother), Cornelia (sister), Larisa (sis-in-law) and little Alvi for the unremitting love, support, faith, patience, confidence, encouragements and at times sacrifices that has made this work worthwhile. Their contributions were unfathomable.

I also express my sincere gratitude to Kong Ester, Kong Diborrah and Kong Betty for all the help and support.

I wish to thank Bijoy Das, Bani Laloo, Mani Babu, Kong Judy and all the non-teaching staff of this department for their assistance.

Last but not the least, I am grateful to the department of Biochemistry, North Eastern Hill University, Shillong for providing research facilities. Initial research fellowship from Meghalaya State Merit Research fellowship and The North Eastern Council, Government of India is also gratefully acknowledged.

Date: 17th December, 2001

(Herbert Goldfield Lyngdoh)

List of Figures

List of Tables

ABBREVIATIONS

BSA	:	Bovine serum albumin
HC	:	Hydrocortisone
T_3	:	Triiodothyronine
c-MDH	:	Cytosolic malate dehydrogenase
c-AsAT	:	Cytosolic aspartate aminotransferase
m-MDH	:	Mitochondrial malate dehydrogenase
m-AsAT	:	Mitochondrial aspartate aminotransferase
PAGE	:	Polyacrylamide gel electrophoreses
EDTA	:	Ethylene diamine tetra acetic acid
CM	:	Carboxy Methyl
Tris	:	Tris-(hydroxymethyl) aminomethane
TEMED	:	N,N,N',N'-tetramethyl-ethylediamine
NADH	:	Nicotinamide adenine dinucleotide
NaCl	:	Sodium Chloride
HCl	:	Hydrochloric acid
$MgCl_2$	:	Magnesium chloride
KCl	:	Potassium chloride
$(NH_4)_2SO_4$	:	Ammonium sulphate
NaOH	:	Sodium hydroxide
AoAA	:	Amino-oxyacetic acid
α-KG	;	α-ketoglutarate
K_2HPO_4	:	Dipotassium hydrogen orthophosphate
KH_2PO_4	:	Potassium hydrogen orthophosphate

INTRODUCTION

Living organisms have evolved a very complicated regulatory mechanisms for controlling the levels of their enzymes, specially those catalyzing critical reactions involved in adaptation to environmental changes. Enzymes are specific proteins that catalyze chemical reactions in biological systems. The feature, which generally characterizes all multicellular organisms, is the progressive development and gradual impairment in the ability to adapt to the environmental changes. At the biochemical level, adaptation can be expressed as the alterations in the rates of synthesis and degradation of enzymes as well as changes in physiological activities. It is facilitated by regulated rate of synthesis and/or degradation and by post-translational modification of enzymes. Higher organisms, including humans, are like cellular cities in which groups of cells perform specialized functions and cooperate by intricate system of communication. Each specialized group of cells, termed as tissues, requires different rates and patterns of metabolic adjustments during the development of animals. Essentially, all the metabolic reactions are catalysed by enzymes. Therefore, enzymes are one of the prime factors that play pivotal role in regulation of metabolic adjustments as an adaptation to the changing demands made upon developing animals.

Life span is a continuum of development and growth at one end and deterioration of functions or senescence or aging at the other end. In between is the reproductive phase or adulthood. Life span is programmed into the developmental patterns of the organism. The programme onsets as soon as the fertilized egg begins to grow (Hanawalt, 1987). This genetic programme determines the maximum life span for each species, for example, mayflies live only 1 day, houseflies 30 days, rats 3 years, horses 25 years and humans 100 years. Further, all individuals of a species have a more or less similar life span, long-lived individuals have generally long lived progeny, identical twins have almost similar life span. Life span can be defined as the duration of life of an individual or organism in a particular environment. The life span of a multicellular organism may be broadly divided into three phases: development (growth), reproductive period (adulthood) and senescence (aging). The time of onset, duration and rate of each phase (Kanungo,1994) is dependent on the vigour and vitality of the other, and are therefore interrelated. Development normally involves both quantitative and qualitative changes leading to suitable

specialization of various cells, tissues and organs of the body, which is usually referred to as differentiation. The development of many animals is divided into prenatal and postnatal periods. For many years, attention was focused on events taking place before birth, when the most striking advances occur in the animals. Prenatal and postnatal stages are characterized by certain distinguishing features, such as morphologic, physiologic, biochemical and psychological features (Timiras, 1994). Accordingly, prenatal developmental studies encompass events that take place right upto birth or hatching, whereas, postnatal developmental studies extend upto at least the attainment of reproductive maturity which include the period of infancy and/or weaning, childhood, adolescence and adulthood. Aging, on the other hand, has been described as a process that causes a gradual decline in the function and adaptability to environmental changes following the onset of reproductive maturity.

The biological nature of aging was postulated to be pleiotropic in nature resulting from the normal by-products of living processes which may be from evolutionary non-selected endogenous properties of an organism. Aging is defined as the time-dependent drifting away of cells, cell organization and homeostatic control from their most optimum state of functions (Cutler, 1984). Two classes of pleiotropic aging processes were postulated to exist. One is associated with energy metabolism and has been called as continuously acting biosenescent processes. The second is associated with developmental processes and has been called developmentally linked biosenescent processes. It is not known which of these two classes might play the most important role in causing aging. Williams (1957), Medawar (1957) and Hamilton (1966) have proposed that a special class of pleiotropic gene whose expression is linked to development causes aging. There are also experimental data indicating that developmentally-linked hormonal processes such as those involved in sexual maturation can act to accelerate the aging process. Thus, aging is the result of pleiotropic effects of development, differentiation and maturation. These processes act to accelerate the destabilization of the differentiated state of a cell, cell organization in tissues and the general homeostatic state of organisms.

One of the basic characteristics of living organisms is their capability to adapt to an ever changing environment. Vertebrates such as fishes and amphibians are capable of an independent existence at relatively immature stages(larvae), with most or much of their development still before them. It is quite otherwise with birds, reptiles and mammals. The

newborns are anatomically quite complete, yet utterly dependent on their elders for food and care. Throughout infancy, childhood and adolescence (for humans) come the completion of some organs and a gradual remolding of body shape (Arrey, 1966). These progressive changes continue and stabilize only on reaching adulthood and/or maturity. In higher animals realization of the full genetic potential involve increase in size, changes in structure and function (Kanungo, 1994).

Developmental processes and their regulation by heredity and environment have engaged biologists for many decades. Heredity and environment have been implicated to determine the physiological competence and length of the life span(Timiras, 1994). Longevity is determined by processes governing the time-dependent stability and differentiation. These processes are encoded into longevity-related genes and their levels of expression are governed by other regulatory genes. Environment supplies the external factors that make development possible and allows inherited potentials to find expression.

The transformation from egg to adult is fundamentally important to the study of avian biology. This has long been recognized by developmental biologists and poultry scientists, but only recently by most avian biologists (Ricklefs,1983). Growth of a chick is to traverse the developmental gulf between neonate and adult with a minimum of risks to itself and with efficient utilization of the care provided by its parents. The exact course of development depends to some extent upon the size and body proportions of the neonate and adult and the environment of the chick during its development. A general trend during postnatal development is from greater to lesser dependence upon parental care, and finally to complete independence. Chick development and parental care are complementary in that the behavior of adults is adjusted to the needs of their offspring. Development is gradual, but the progress of the chick can be measured against certain landmarks like hatching, acquisition of flight and independence.

Development begins with a single fertilized egg which in turn gives rise to cells that have different developmental fates. It includes an increase in the number and size of the cells, their differentiation to perform specialized functions and formation of organs. At the molecular level, several genes that play specific roles at specific times have been visualized and that each cell type may be characterized by its pattern of gene expression (Kanungo,1994). The development of an adult organism from a fertilized egg follows a

predetermined path in which the principal controlling factor is gene expression at transcriptional level. However, there may be regulation at the processing, transport, and stability of RNA transcript. There also exists post-translational control of gene expression. Gene expression can also be controlled by signaling due to cell-to-cell interaction (Lewin, 2000).

One of the classical examples of sequential activation and repression of genes during development is that of hemoglobin during gestational period in human (Zuckerkandle, 1965). Haemoglobin, a tetrameric metalloprotein, consists of $\alpha_2\varepsilon_2$ chains in the fetus at the age of 1 to 2 months of gestation. In the later phase of gestation, the ε chain is replaced by the γ chain and just before birth, the γ chain is in turn replaced by β chains which together with the α chains comprise the adult haemoglobin characterized by differences in the electrophoretic mobility. The synthesis of these chains is governed by different sets of genes which are sequentially activated and repressed during the development of human fetus.

Genetic control of development can be best exemplified by studies on the fruitfly, *Drosophila melanogaster*. These studies demonstrate that development involves an orderly pattern of gene expression in which individual genes have both temporal as well as spatial specificity in their expression, and that some important controlling genes apparently maintain this pattern by regulating the action of other genes (Russel, 1987). Similar studies on the soil nematode (*C. elegans*) showed valuable informations relating to switching on and switching off of genes during development and aging process (Edgar and Wood, 1977: Sulstan and White, 1980). These studies indicate that the sequential activation and repression of genes could be a continuing process and may operate throughout the life span of an organism.

During senescence, on the other hand, the functional abilities of most organs and organism decrease. The decline becomes apparent towards the later part of the reproductive phase. Thus, the reproductive phase smoothly merges into senescence phase, unlike the transition from the developmental to the reproductive phase wherein specific genes are expressed and confer reproductive ability to an organism. An important feature of senescence is that reproduction ceases in this phase and no special structure or function appears, rather than those already present undergo change (Kanungo, 1994).

To explain the phenomena of aging, various theories have been proposed. These theories can be broadly divided into molecular, cellular and system level theories (Sharma, 1988;1994).

Molecular theories begin with the following assumptions-

(i) All individuals within a species have an almost similar length of life span.

(ii) Individuals from different species have different life span.

According to these theories, it is presumed that there is some genetic programme which determines the maximum life span for each species. An equally significant contribution to a genetic basis of aging is deduced from the duration of the three phases of life span. Molecular theories of aging include-

(a)*Codon restriction theory* which is based on the assumption that the fidelity or accuracy of translation in a cell depends on its ability to decode the triplet codons in mRNA (Strehler et al., 1971)

(b)*Somatic mutation theory* which states that mutations that occur randomly and spontaneously destroy genes and chromosomes in post-mitotic cells during the life span of an organism. This process causes a gradual increase in the mutation load which in turn decreases the production of functional proteins (Szilard, 1959)

(c)*Error theory* postulated by Orgel (1963) states that error occurring during information transfer, that is, transcription and translation may cause accumulation of defective proteins that may lead to the aging of an organism.

(d)*Gene regulation theory* proposed by Kanungo (1980,'94) states that senescence may occur due to the changes in the expression of genes after the onset of reproductive maturity. It is based on the presumption that senescence would follow a pattern similar to that of differentiation and growth, that is, a sequential activation and repression of certain genes which are unique to these phases.

Cellular theories relate to changes that occur in structural and functional elements of cell with the passage of time. These theories include Wear and tear, Age pigments, and Cross linking theory (Sharma, 1994).

(a) *Wear and tear-* Living organisms may be compared with the machines and as with repeated use, parts of machine wear out and become defective and the machinery finally fails to function (Sacher, 1977). However, the same cannot be applied to organisms since they have a well defined self controlled repair mechanisms which when fail can destabilize the system lending to aging and death.

(b) *Age pigments-* A marked change that occurs in the cell composition during aging is the increasing accumulation of age pigments (lipofuscin) in both the pre- and post-mitotic tissues of animals (Strehler, 1964: Reichel, 1968: Toth, 1968). It is one of the common morphological features associated with aging and has been correlated with the loss of neurons in old age (Brizee et al., 1969, 1975; Miquel et al., 1978).

(c) *Free-radical and cross-linking theory–* Harman (1986) proposed that the decrease in the adaptability of an organism during aging may be partly due to free radical-mediated damages in the body. Both the "Cross linking" theory of Bjorksten (1964) and the free radical ftheory of Harman (1986) are almost similar as both involve the process of inactivation of biomolecules by cross-linking due to free radical damages.

System level theories include neuroendocrine and immunologic theories. The overall performance of an animal is closely related to the effectiveness of a variety of control mechanisms that regulate the interplay between different organs and tissues (Shock, 1979). With aging, the homeostatic adjustments decline with consequent failure of adaptive mechanisms and that aging and death may be viewed as the result of this failure (Frolkis,1982; Timiras, 1994). The efficiency of immune system also decreases as a funtion of age (Walford,1969). The immune system has also been known to be influenced by hormones. Thus, a decline efficiency of endocrine and neural system with age also influence the immune system in several functions.

During the early period of development, the mother bears much of the responsibility for the maintenance of homeostasis, which serves to maintain a normal state in case of imbalance, and is necessary for growth. Homeostatic regulation together with inherited traits, define the competence with which the individual will continue to respond to environmental changes (Timiras, 1994). When the fetus leaves the uterus or when the egg hatches, there is an abrupt change in the manner and form in which the nutrients enter into the body for postnatal development of organisms. Metabolic routes change as an adaptation to the changing demands made upon them. Adaptation to these changes involve changes in the activities of many important enzymes in the body's tissue which in turn play a key role in bringing about developmental changes ensuring better adaptability of growing animals.

Enzymes during development:

Enzymes are specific proteins, baring some RNA, that catalyse chemical reactions in biological systems. Essentially, all biochemical reactions are enzyme catalysed. The most striking features of enzymes are their enormous catalytic power and specificity. They accelerate reactions by factors of 10^6 to 10^{12} as compared to uncatalysed reactions. They catalyse a wide variety of chemical reactions, such as oxidation-reduction, transfer of chemical moiety such as tranferases, hydrolytic reactions and lyases. Each enzyme catalyses only one type of reaction showing a high selectivity for both reactants and products. Within a single species, there may exists different forms of enzyme catalyzing the same reaction. These could differ from one another in terms of amino acid sequences and termed as isoenzymes. Isoenzymes are the enzymes that arise from genetically determined differences in amino acid sequences. For instance, malate dehydrogenase has an isoenzyme present in cytoplasm as well as in mitochondria of many different tissues (Price, 1989). Enzymatic studies were among the first biochemical changes demonstrated to occur during development and aging. Subsequently, a large literature has developed concerning the age-related changes in enzyme levels and has been well reviewed (Kanungo,1980; Walker,1983; Sharma,1988).The rate of synthesis of some enzymes changes in response to changes in the extracellular environment. In bacterial cells, these changes in enzyme synthesis are quite pronounce with many fold increase in the activity level of specific enzymes being observed. In animal cells, however, the situation is relatively different, although whole organism may be exposed to marked

changes in the environment, mechanism exists which minimizes the number of cells or tissues to exhibit response (Walker, 1983). The vast majority of enzymes in multicellular organisms are expressed constitutively, differing from adaptive enzymes, a term which was introduced by Karstrom (1936) to describe those enzymes which are induced in a microorganism only when they grow in presence of specific substrates in contrast to constitutive enzymes which are present in a given organism irrespective of the composition of the medium in which the organism grows. Later, the term induction was defined as an increase in enzyme activity caused by a change in substrate concentration or by hormonal action (Cohn et al., 1953), whereas enzyme repression was defined as a relative decrease in the rate of synthesis of a particular apoenzyme resulting from exposure of cell to a given substance (Vogel, 1957). Induction of enzyme is attributed to several inducers, or effectors which may either be a substrate or a hormone or a metabolite or even an exogenous factor (Sharma, 1988). A substantial amount of literature and reviews are available on the relationship of mammalian hormone, enzyme levels and induction (Pitot and Yatvin, 1973; Dickerson and Basu, 1975; Kanungo, 1980; Walker, 1983; Sharma, 1988).

Growth and development proceed with characteristic alterations of the enzymes through which the organism acquires the capability of coping with the demands of altered environmental conditions as imposed by birth and postnatal life (Bohme et al., 1986). A number of studies have reported changes in enzyme activities with development and aging (Benzi et al., 1980; Nordenberg, 1981; Sharma, 1988,1994). Expression of enzymes during development varies with age, sex and strain of an organism. Glucose-6-phosphate dehydrogenase increases in the kidney of aging male, whereas it decreases in the females (Wilson & Franks, 1971) and that of lactate dehydrogenase increases in liver of aging male mice but shows no change in females (Wilson, 1972).The activity of pyruvate kinase does not change in the heart of old male rats but it decreases in the same tissues of old female (Chainy and Kanungo, 1978). Pyruvate kinase (PK II) isoenzyme is present in fetal rat liver but absent in adult tissue (Walker, 1974).

Most studied example is lactate dehydrogenase (LDH), a tetramer made up of two different subunits called M and H. These two subunits are controlled by two separate genes. Various isoenzymes of lactate dehydrogenase are formed by combination of M and H 'submits (M_4,, M_3H, M_2H_2, MH_3 & H_4). Each isoenzyme is characteristic of individual

tissue or cell population and is subjected to different regulatory mechanisms which are 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 & Ursprung, 1962). LDH isoenzyme composition is not only tissue–specific but also changes in the same tissue during development (Markert & Moller, 1959). The greater proportion of M_4 isoenzyme is present in developing embryos of mammals as their metabolism is mostly anaerobic in nature. A shift towards H_4 occurs as development proceeds. Contrary to this, a greater proportion of H_4 isoenzyme is found in developing chick embryo which grows in aerobic milieu and shows a shift towards M_4–type during later stages of development.

These changes in the composition of isoenzymes are not restricted to development but extent into adulthood and aging (Kanungo, 1980). The proportion of M_4-LDH is considerably lower in the heart, skeletal muscle and brain of older rats, with concomitant increase of H_4-LDH. The shift in isoenzyme of LDH has been correlated with the differing metabolic functions of the organism as a function of age. The lower proportion of M_4-LDH may cause a decrease in the ability of the tissue to cope with anaerobic conditions (Singh and Kanungo, 1968). Studies on cytoplasmic alanine aminotransferase (c-AlAT) of the old rat liver revealed that the phenomena of sequential changes in the expression of proteins do extent to old age (Patnaik and Kanungo, 1976). Young (5 weeks) rat liver has A-type and adult (52 weeks) has both the isotypes (A- & B-) while old (100 weeks) has only the B-isotype. Both subunits are under the control of two separate genes (Chen and Giblett, 1971) and are sequentially activated and repressed at different phases of life span in rats (Patnaik and Kanungo, 1976).

Many studies have been done on changes in the activity of individual enzyme with aging (Benzi et al., 1980; Kanungo, 1980). However, the key enzyme of a particular metabolic pathway might provide a complete profile of their biological functions during development and aging (Sharma,1988). Studies on the developmental expression of arginine synthesizing enzymes during lactation and aging reveal that these enzymes are highly expressed in the small intestine of sucking and weaning rats. The activity of malate-aspartate shuttle enzymes in the liver and kidney of mice was differentially expressed in an age- and tissue-specific manner (Sharma et al.,1992;Santa, 1997). This may reflect differential metabolic transfer of reducing equivalents to commensurate the specific

tissue's requirements at various developmental ages. Studies on the activities of key enzymes of glycolysis, krebs cycle and pentose phosphate pathways of several tissues at different ages have shown that these enzymes show different patterns of expression in different tissues at various ages (Keast, 1989).

Enzyme induction during development:

Similar to the cellular requirements for various proteins vary, the mechanism by which their respective genes are regulated also vary. The degree and the type of regulation reflect the function of the protein product of that gene. Some gene products are required all the time and hence they are expressed at a more or less constant level in virtually all the cells of an organism. Many of the genes for enzymes those catalyse reactions to control metabolic pathways, such as the citric acid cycle fall in this category. These genes are often referred to as housekeeping genes. Unregulated expression of such genes is called constitutive gene expression. The amounts of other gene products rise and fall in response to molecular signals. Gene products that increase in concentration under defined molecular circumstances are referred to as an inducible and the process of increasing the expression of such genes is called induction. Expression of many genes encoding DNA repair enzymes, for example, is induced in response to high level of DNA damage. Conversely, gene products that decrease in concentration in response to a molecular signal are referred to as repressible, and the decrease in gene expression is called repression. For example, high level of tryptophan leads to repression of the genes for the enzymes catalyzing tryptophan biosynthesis in bacteria.

A single gene generally contains in an eukaryotic transcription unit and that most of the cellular enzymes and proteins are encoded by solitary non repeated gene. In general, the rate of synthesis of mRNAs from solitary protein coding genes is sufficient to meet the cells requirement of various individual proteins. The rates of their transcription are controlled by several short DNA sequences, called *cis-acting elements*, spread over a region of >100 basepairs either upstream or downstream of the regulated gene. The transcription factors including steroid and thyroid hormone-receptors, termed as *trans-acting factors*, regulate the expression of specific gene in the presence of an enhancer element located at a variable distance from the promoter in either direction and orientation.

The enhancers are tissue-specific and activate the promoter of the gene only in that tissue and not in the other tissues (Gillies et al., 1983).

The promoter region consists of TATA box (centered at –30 basepair), CAAT box (-75 basepair) and GC box (-90 basepair). They are present in most genes and the proteins that bind to them are distributed widely. Therefore, they are not responsible for cell-specific expression of genes and are mainly responsible for generic transcription. Besides these sequences required for both initiation and efficiency of transcription, there are several tissues-specific cis-acting sequences which are involved in regulation of transcription and have specificity for binding to specific trans-acting factors including receptors for steroid and thyroid hormones, retinoic acid and Vit D. Steroid and thyroid hormones as well as retinoic acid receptors belong to a family of receptors that undergo conformational change after binding to their respective ligands. The receptor-ligand complexes then interact with definite cis-acting sequences of genes which are expressed in a tissue-specific manner and are responsive to these hormones (Allan et al., 1991; Wahli and Martinez, 1991; Wiseman et al., 1991).

Impacts of enzyme catalysed reactions may either be (a) by changing the absolute quantity of enzyme present or (b) by altering the pool size of reactants rather than the enzyme, or (c) by altering the catalytic efficiency of the enzyme (Rodwell, 1996). All the three options can be exploited in most organisms with the help of several types of inducers or effectors which may either be the substrate or a hormone or a metabolite or even an exogenous factor.

The expression of age-related adaptive changes in enzyme induction has been categorized into four general patterns of response: (i) the response has an altered adaptive latent period or initiation time following stimulus without affecting the magnitude of induction, (ii) the response shows decrease or increase in the magnitude of induction with no change in the latent periods, (iii) the response alters both the latent period and magnitude of induction and (iv) age-related changes do not occur in the induction pattern (Adelman, 1975; 1981). The patterns of enzyme induction, as in the case for enzyme levels, are also susceptible to considerable variations related to differences in species, strain, sex and conditions of environmental maintenance and the physiologic state of animals (Sharma, 1988).

Age-related changes in the enzyme induction by hormones have been extensively studied. 17β-estradiol was reported to induce acetylcholinesterase and choline acetyltransferase in the cerebral hemisphere of immature and adult ovarectomized rats, but not in old rats. If the level of 17β-estradiol is prevented from alteration, theoretically, it would be possible to prolong the reproductive period and some of the adulthood activities (Kanungo, 1994). The molecular basis for the impairment of induction of acetylcholinesterase by estradiol in old rats appears to be because of a decrease in the estradiol-binding protein (Kanungo et al., 1975). Patnaik and Kanungo (1976) have shown that cytosolic-AlAT is inducible in the liver of immature, adult and old rats by hydrocortisone, whereas mitochondrial-AlAT is inducible only in the older rats. Furthermore, Kanungo and Gandhi (1972) showed that the level of mitochondrial-MDH decreases in the liver of young rats after adrenalectomy, but not in old adrenalectomised rats. Administration of cortisone to these rats causes induction of the enzyme in young rats but not in old rats. In contrast, administration of cortisone in hepatectomized old rats could induce the enzyme.

Homones during development:

Hormones are signal molecules synthesized and secreted in small quantity by specialized group of cells. They act on target cells by interacting with cognate receptors located either on the cell surface (protein and peptide hormones) or within the cytosol/nucleus (steroid and thyroid hormones). Hormones of endocrine, paracrine and autocrine systems control wide variety of cellular and metabolic processes. Adaptation to external and/or internal stresses during development depends on control mechanisms of the combined interplay of nervous and endocrine systems (Timiras, 1994). Neurons in brain of higher animals act as pacemaker, regulating the "biological clock" which govern development and aging (Sharma, 1994). This pacemaker role of neurons coordinates with the hormones resulting dramatic changes in metabolic patterns including altered patterns of gene expression. Changes in the level of hormones and their properties of binding to receptors during development have been extensively studied. Hence, the level of hormones, their receptors and post-receptor events might influence the process of development, growth and reproduction in animals.

A considerable importance to the understanding of ways in which gene expression in animal cells operate *per se*, is to establish whether the activation of genes is under the control of specific developmental factor(s), or whether same hormones that control the enzyme syntheses in the adult are also responsible for switching these genes on, during development. Since hormones are known to play definite roles in the regulation of the appearance of enzymes, therefore, changes in hormone levels together with the hormone-receptor concentration are critically important. Changes in secretion of hormones during development play a major role in the regulation of enzyme synthesis both during development and in adulthood. Some of the studies on the major metabolic hormones in mammals during early period of postnatal development are reported. However, given the distinct differences that exist in hormonal regulation of metabolism between species, references, wherever available have been cited with emphasis on chickens.

In aves, as in mammals, growth and development appear to be under an intricate but judicious control mechanism, which involves endocrine, paracrine and autocrine factors. A number of hormones have long been recognized as playing major roles in the control of growth. These include growth hormone, thyroxine (T_4) and the active triiodothyronine (T_3) and the sex steroids (Scanes et al., 1990). In addition, other growth factors such as epidermal growth factor (EGF), insulin like growth factor (IGF) and nerve growth factor (NGF) have also been found to influence growth (O'Keef et al., 1988).

Knowledge of avian growth hormones have made considerable strides (Harvey and Scanes, 1977; Scanes et al., 1990). In aves, growth hormones (GH) is required for the normal rate of growth as in the case of mammals. It was shown that hypophysectomy reduces growth while replacement therapy with mammalian GH partially restores growth rates (King and Scanes, 1986). However, administration of GH to intact young chickens does not stimulate growth (Libby et al., 1955; Scanes et al., 1984). The absence of major effects of chicken GH in intact young chicks was speculated to be due to the maximal effect exerted by endogenous GH at these ages studied (Scanes et al., 1990). However, repeated administration of biosynthetic chicken GH stimulated growth in the older (12-weeks) chicks but not in the younger (8-weeks) chicks (Scanes et al., 1990). In chickens, plasma GH rises gradually, with larger increase occurring during the week following hatching (Harvey et al., 1979). Plasma GH also decreases considerably before puberty/mid-postnatal period (Scanes and Johnson, 1984). Growth hormone shows similar

developmental patterns in chicken as that of T_3 and a casual relationship has been described to exists between T_3 and GH during the late embryonic and early post hatch development in chickens (Darras et al., 1992).

Adrenal hormones also play an important role in the development, growth and homeostasis. It was shown in the rat that glucocorticoids are present four days before birth, increase and decline after birth and the neonatal period is characterized by relatively low glucocorticoid concentration until 12-14 days of age, thereafter a second surge of glucocorticoid is released by the adrenals between day 12 and 16 (Cohen, 1973; Martin et al., 1977).

In the avian system, the synthesis and secretion of adrenal hormones are also initiated during embryogenesis and has been suspected to be involved in development (Freeman and Vince, 1974). The principal glucocorticoid, corticosterone (Lauber et al., 1987; Bisbis et al., 1994a), is known to increase during the early stages of development with a marked rise at about the 15th day (Wise and Frye, 1973). It was also reported that exogenous hydrocortisone induces several enzymes (Cohen et al., 1972). These were further reviewed by Freeman and Vince (1974). Subsequent studies have shown that corticosterone treatment in chickens impairs growth and induces fattening and insulin resistance (Simon, 1984; Taouis et al., 1993).

The carbohydrate metabolism of birds reportedly differs from that of mammals in many aspects (Pearce, 1977; Hazelwood, 1986). In rats, the late-fetal period is characterized by high concentration of insulin which drops precipitously at birth (Blazguez et al., 1970). This low level persists throughout the neonatal period. After weaning, when the intake of carbohydrates increases, the steady-state level of the hormone increases again. A pronounced hypoglycaemia accompanies birth and the concentration of glucagon increases markedly during the first hour after birth in contrast to that of insulin, producing, reportedly a 20-30 fold decrease in the insulin:glucagon ratio (Girard et al., 1973; DiMarco and Oliver, 1978), which has been correlated to the appearance of gluconeogenic enzymes such as PEPCK (Yeung et al., 1968) and TAT (Ghisalberti et al., 1980) in the new born.

In aves , the role of insulin in development is well acknowledged (Freeman and Vince, 1974). These early work have established the fact that insulin is present in chicks as early as the 4th day of incubation and known to increase glycogen, affect maturation of hepatocytes (Benzo and De La Haba, 1972), enhance lipogenesis (Foa et al., 1965), and uptake of neutral amino acids (Levi and Lattes, 1969). The role of insulin during embryonic development of chicken has been also established (Pablo et al., 1985; Wada et al.,1990).

Aves differ from rodents in certain metabolic and endocrinological aspects (Pearce, 1977). The relative importance of insulin and glucagon on the regulation of hepatic metabolism are dramatically different between the two groups of animals. In chicken development, carbohydrate accounts for less than 1% of available nutrients and this is primarily utilized within the first 7 days of the 21-day incubation period (Yarnell et al., 1966). Throughout the remainder of the incubation, proteins and lipids serve as the major fuel source, for both energy provisions and for production of intermediates for biosynthetic reactions (Freeman and Vince, 1974).

Thyroid hormones also affect various processes by interacting with their intracellular receptors located primarily in the nucleus of target cells. Thyroid hormone receptors are a member of a class of steroid receptors superfamily. These receptors upon interaction with hormone bind to specific DNA sequences, termed as thyroid response elements (TREs), mostly located upstream of the regulated gene. This leads to a modulation of gene expression and consequently alterations in the metabolic activity of cells.

Thyroid hormones have been implicated in controlling development and differentiation of many animals. They are present in low levels in foetal liver of rat and implicated in the appearance of glucokinase and malic enzyme during the late suckling period (Heide and Ende-Visser, 1980). In addition, thyroid releasing hormone (TRH) and thyroid stimulating hormone (TSH) which regulate the activity of the thyroid gland are reported to increase in concentration in the blood preceding the increase in the concentration of thyroid hormone in the neonate (Pease et al., 1980). It has been pointed out that the increase also coincides with the maturation of the hypothalamic-pituitary axis as a functional regulatory element in the brain.

It has been well established that growth in poultry as well as in other species is regulated by thyroidal and pituitary hormones (Freeman and Vince, 1974; Scanes et al., 1984; Harvey, 1990). Evidence for the role of thyroid hormones comes from the reduction in growth of chicks following chemical ablation of the thyroid and the return to normal growth rate following thyroxine replacement therapy (King and King, 1973). Paradoxically, attempts to stimulate growth in intact chicks with exogenous T_3 or T_4 resulted in reduced growth (May, 1980), whereas growth of sex-linked dwarf chicks was stimulated upon such treatments (Marsh et al., 1984a &b; Scanes et al., 1983). In chickens, the biologically active form is triiodothyronine (T_3) which is however predominantly produced by monodeiodination of T_4 (McNabb, 1988). The plasma level of T_3 and T_4 was reported to have an abrupt increase in T_3 between 19 day of embryonic period to day 1 post-hatch, whereas, T_4 shows a gradual increase during the embryogenesis (Darras et al., 1992).

Hormones, even if present, can only function and interact with tissues that possess appropriate receptors with fully functional signal transduction and second messenger systems. As with enzymes and hormones, changes in the development of receptors and post-receptor events have also been advanced. Insulin being a water soluble, protein/peptide hormone binds to specific receptors located on the membrane of target cells and influences a wide variety of cellular and metabolic processes. Its receptor consists of two α-subunits located on the outer face of the plasma membrane and two β-subunits that traverse the membrane and protrude on the cytosolic face. Binding of insulin to the α-subunit triggers autophosphorylation of a tyrosine residue residing in the carboxyl terminal domain of the β-subunit, which allows the tyrosine kinase domain to catalyse phosphorylation of specific target proteins giving rise ultimately to biological response. Insulin receptor concentration also increases markedly in rat paralleling the increase of insulin in blood just before birth (Caliendo, 1981). At birth, the liver cells possess adult levels of insulin receptors which are maintained throughout the neonatal period (Walker, 1983). In avian species, insulin receptors have been characterised from liver, brain (Simon and Leroith, 1986), skeletal muscle (Adamo, 1987) and kidney (Bisbis et al., 1994b). Insulin receptors in chicken have been detected in brain, liver, heart, limb buds and muscles of 18 hour embryo (Serano et al., 1990). They are shown to be regulated in a tissue-specific manner. In addition, levels of receptor measured by northern blot exhibited a 30-fold increase in post-hatch chicks compared to the embryonic stage.

Protein and peptide hormones produce varying metabolic responses upon interacting with cell surface receptors. The binding of these hormones activates an amplifier enzyme through G-protein that generates a short-lived in the concentration of an intracellular signaling molecule termed as second messenger. These second messengers may be cAMP, cGMP, 1,2-diacyglycerol (DAG), inositol triphosphate (IP_3) and calcium (Ca^{2+}). The elevated intracellular concentration of one or more such second messengers triggers a rapid change in the activity of specific enzymes or nonenzymatic proteins which then produce the biological response. Changes in the components of the second messenger cascade, such as adenylate cyclase, phosphodiesterase and cyclic-AMP, have been detected 5 days before birth, increase to reach a maximum 5 days after birth before declining to adult levels. In aves, during incubation period, the adenylate cyclase and cyclic-AMP cascade is relatively immature (Freeman and Manning, 1971), maturing only during and after hatching, so that glucagon can stimulate lipolysis and glycogenolysis.

Glucocorticoid receptors are a group of trans-acting factors present predominantly in the cytosolic fraction of many cell types. Upon interaction with glucocorticoids, they translocate into the nucleus and interact with specific DNA sequences located generally 100-200 basepairs upstream of the regulated gene(s). These DNA sequences are termed as glucocorticoid response elements (GRE's). Binding of cognate hormone-receptor complexed to GRE's modulates the expression of gene which in turn influence the metabolic response (Yamamoto, 1985; Beato, 1989). High affinity glucocorticoid binding is detected in fetal rat liver 6 days before birth. With the onset of birth, there is a marked increase in the concentration of glucocorticoid receptors (Feldman, 1974; Giannopoulos, 1975). Hepatocytes isolated from fetus 3-4 days before birth are reported to be stimulated to synthesize tyrosine aminotransferase (TAT) when incubated with glucocorticoids (Cake et al., 1979). TAT is normally synthesized just before birth. These findings alongwith the interpretations have been quite extensively reviewed (Walker, 1983). The changes in the level of glucocorticoid receptors as well as in the physicochemical properties have been well documented (Kalimi, 1984; Sharma, 1988). Glucocorticoid effect on various processes depends on the level as well as on the physicochemical properties of its receptor. Age-dependent changes in the inducibility of enzymes by glucocorticoids have been reported to be influenced by the level of receptors and also by the post-receptor events (Sharma, 1994). It has also been reported that the concentration of glucocorticoid receptor increases during the prenatal as well as postnatal development (Singer and

Litwack, 1971; Feldman, 1974). Receptors also exert a certain degree of control over the responses that are dependent upon the amount of complexes generated between receptors and hormones (Roth, 1988). An age-related reduced entry of activated steroid-receptor complexes in the nuclei has been observed in rat prostate. In rats, pancreatic glucocorticoid receptor and total plasma corticosterone level change during postnatal development and the peak value of binding corresponds to an increase in the plasma cortisone level during weaning. Hence, there is a close relationship between plasma corticosterone level and pancreatic glucocorticoid receptors and both may play a vital role in pancreatic development in rats (Lu et al., 1987). Glucocorticoid receptor concentration as well as its actvation and nuclear binding has been earlier studied in immature and mature Long-Evans rats (Sharma and Timiras, 1987). A higher amount of receptor with greater actvation characteristics was obtained, which was well correlated with the weaning period in rats. An elevated level of glucocorticoid receptor protein has also been reported in the liver and kidney of 30- and 45-day old mice, respectively (Borbhuiya and Sharma, 1995 a,b).

In recent years, it has been realized that steroid hormone and protein/peptide hormone actions are not solely isolated. They do 'cross-talk' each other and show an inter-dependence (Sharma, 1993). Activators and inhibitors of protein kinases have been reported to modulate accordingly the steroid hormone influence on the induction of TAT and TO (Sharma, 1991, '93). Dopamine and Bt_2-cAMP have been shown to modulate the effect of progesterone via progesterone receptors. Biomedical potential in getting desired effect of steroids in presence of protein kinase activators will avoid toxic effect of higher steroid doses used alone. Even, it might provide a prospective field for study of development and aging processes.

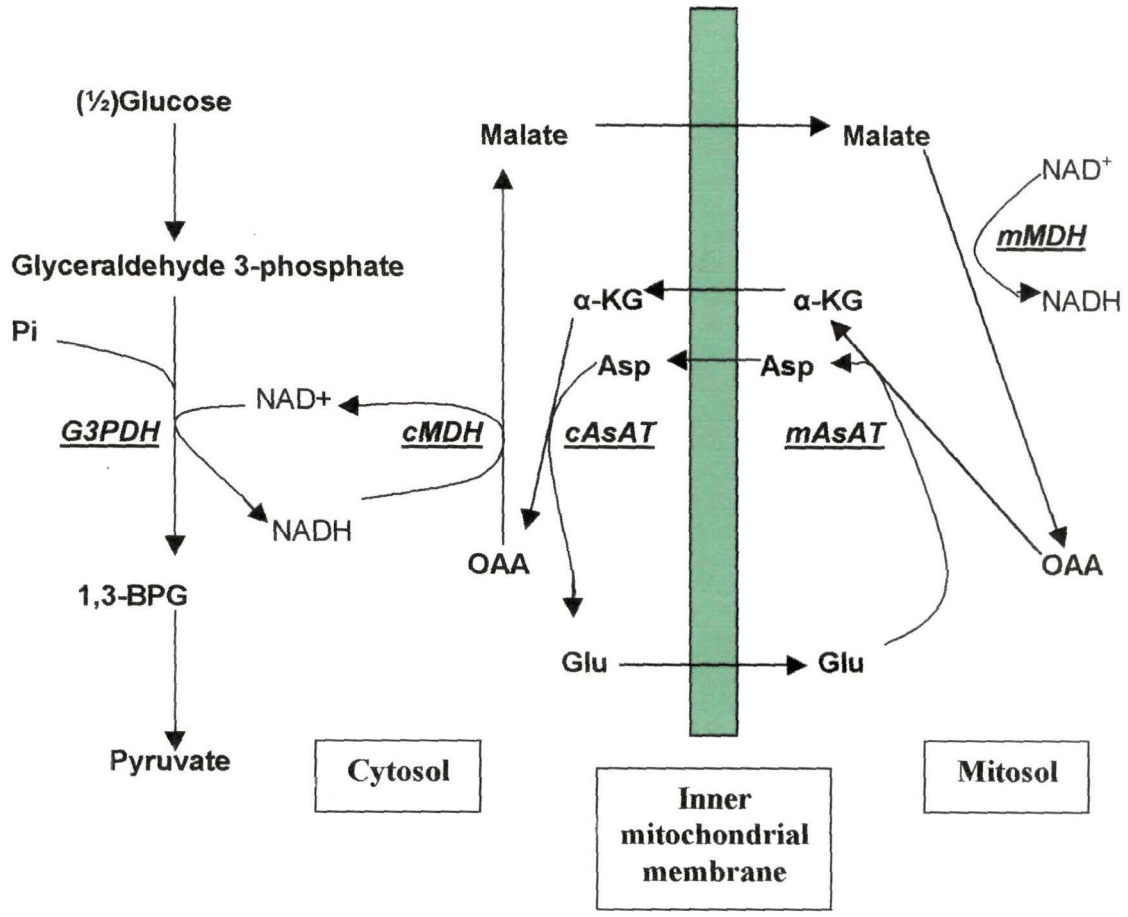
Malate-aspartate shuttle:

Most of the NADH used in electron transport is produced in the mitochondrial matrix space (Garreth and Grisham, 1999) because NADH is oxidized by Complex I on the matrix side of the inner mitochondrial membrane. Lehninger(1951) showed that intact mitochondria are impermeable to externally added NADH. Subsequently it was confirmed that the inner mitochondrial membrane is impermeable to NADH. The mitochondrial inner membrane does not contain a transport system for NAD^+

or NADH. In animal cells, most of the NADH oxidized by the respiratory chain is generated in the mitochondrial matrix by the tricarboxylic acid cycle or by the oxidation of fatty acids. However, a considerable fraction of the total NADH is generated in the cytoplasm during glycolysis, and the transport of these reducing equivalents across mitochondrial membrane is required for respiration in a variety of metabolic processes (Krebs, 1967). The NADH formed in the cytosol during glycolysis must be reoxidized to NAD^+ in order to operate the glycolysis under aerobic condition. It is advantageous to reoxidise the NADH by the respiratory chain rather than by the formation of lactate or ethanol. Approximately, three molecules of ATP are formed for each NADH oxidized in the mitochondria, whereas no ATP is made when NADH is oxidized by the cytoplasmic lactate dehydrogenase or alcohol dehydrogenase. Therefore, eukaryotic cells have a number of *shuttle systems* that harvest the electrons of cytosolic NADH for delivery to mitochondria without actually transporting NADH across the inner membrane. Various mechanisms have been proposed for this transfer, of which the *glycerophosphate shuttle* and the *malate-aspartate shuttle* are the most important (Meijer and VanDam, 1974; Christen, 1985).

The most active NADH shuttle for the movement of reducing equivalents (in the form of NADH) from the cytoplasm to the mitochondria is the malate-aspartate shuttle (Williamson et al., 1971; Cederbaum et al., 1973; Ross et al., 1977; McDonald, 1983). The main enzymes of malate-aspartate shuttle are malate dehydrogenase (MDH; EC 1.1.1.37) (Grimm and Doherty, 1961; Whitt, 1970) and aspartate aminotransferase (AsAT; EC 2.6.1.1) (Whitt, 1970; Braunstein, 1973). The kinetic differences between these two enzymes may be important to the functioning of the shuttle (Majee and Phillips, 1971). Both these enzymes have two homologous and genetically independent isoenzymes; one in the cytosol and the other in the mitosol (Davidson, 1967; Bailey, 1969).

In the malate-aspartate shuttle, the reducing equivalents of cytosolic NADH are first transferred to cytosolic oxalacetate to yield malate by the action of cytosolic malate dehydrogenase (c-MDH). The malate formed, which carries the reducing equivalents donated by cytosolic NADH passes through the inner mitochondrial membrane into the matrix (mitosol) by a dicarboxylate (malate-α-ketoglutarate) transport system. Once inside the mitochondria, the reducing equivalents carried by malate are transferred to matrix NAD^+ by the action of mitochondrial malate dehydrogenase (m-MDH), reducing it to NADH. This NADH then passes its electrons directly to the respiratory chain in the inner



Schematic representation of malate-aspartate shuttle

mitochondrial membrane. Three molecules of ATP are generated as this pair of electron passes to molecular oxygen. The rest of the shuttle is concerned with the regeneration of cytosolic oxaloacetate to start another round of the shuttle and the details of schematic representation of malate-aspartate shuttle is shown above. The malate-aspartate shuttle is also reported to be a primary mode of transfer of NADH reducing equivalents from the cytoplasm in vascular smooth muscle. Glucose oxidation and lactate production are influenced by the activity of the shuttle (Barron et al., 1998). Regeneration of NADH in the cytosol is limited in chicken liver, and that gluconeogenesis is regulated, in part, by alterations in the redox states of mitochondria and cytosol (Sugano et al., 1982). Although

gluconeogenic reactions are similar in all organisms, the metabolic context and the regulations of the pathway differ from organism to organism, and from tissue to tissue (Lehninger et al., 1993). The major gluconeogenic precursor in chicken liver is lactate instead of pyruvate. The crucial gluconeogenic enzyme phosphoenol pyruvate carboxykinase (PEPCK), found predominantly as a cytosolic enzyme in rats and mice, is localized in mitochondria of chicken liver and kidney (Ogata et al., 1982). Oxaloacetate produced in mitochondria is converted directly to phosphoenolpyruvate (PEP) by a mitochondrial PEPCK. The PEP is then transported out of the mitochondria and serves onto the gluconeogenic pathway. It clearly establishes distinct differences in metabolic make up of aves as compared to rodents and other mammalian species.

Malate-aspartate shuttle involves an influx of malate and glutamate and an efflux of aspartate and α-ketoglutarate from mitochondria. The functional significance of this shuttle is to unfold the degree of control points for the glycolysis, gluconeogenesis and krebs cycle (Borst, 1963). The enzymes of malate-aspartate shuttle are freely reversible in their catalysis and generate malate, aspartate, glutamate, α-ketoglutarate which are transported through distinct carrier systems. These carrier systems are located on the mitochondrial membrane (aspartate-glutamate carrier and α-ketoglutarate-malate carrier). The carrier system, first described by Borst (1963), was designated as the malate-aspartate shuttle. A directionality is imposed on the system by two carrier proteins. In chicken, excess cytosolic reducing equivalents generated by the oxidation of lactate to pyruvate are transferred from the cytosol to the mitosol by the malate-aspartate shuttle (Ochs and Harris, 1980).

The malate-aspartate shuttle can be reconstituted, using isolated mitochondria and the extra mitochondrial components of the shuttle (Cederbaum et al., 1973). Dawson (1982) reported that the rate of oxidation of NADH was directly related to the amount of mitochondrial proteins present, while extra mitochondrial reactions become limiting only when the soluble mitochondrial protein ratio fell below 0.8. It has also been observed that a high fat diet increases both the endogenous and total rates of shuttle activity compared to the rates obtained with mitochondria from low fat fed rats (Cederbaum et al., 1973).

Kauppinen (1983) reported aminooxyacetate as a nonspecific inhibitor of pyridoxal phosphate-dependent enzymes. Aminooxyacetate inhibits aspartate aminotransferase, a

pyridoxal phosphate-dependent enzyme, both in the cytosolic and mitochondrial compartments. Aspartate aminotransferase is an essential component of the malate-aspartate shuttle which predominantly transports electrons from cytosolic NADH into the mitochondria. Application of aminooxyacetate demonstrated that the NADH shuttle system was essential for coupling glycolysis with activation of mitochondrial ATP generation to trigger glucose-induced insulin secretion (Eto et al., 1999). In chicken, although the pathway for glucose synthesis from lactate is not thought to involve transamination steps, inhibitors of transamination like aminooxyacetate blocks lactate gluconeogenesis. Thus, aminoxyacetate inhibits the malate-aspartate shuttle and, consequently, glucose synthesis for want of pyruvate (Ochs and Harris, 1980). It has also been reported that β-methyleneaspartate inhibits both the cytosolic and mitochondrial aspartate aminotransferase of synaptosomes and also impairs NADH oxidation in a reconstituted malate-aspartate shuttle (Cheeseman, 1988). Malate dehydrogenase and aspartate aminotransferase are also inhibited at relatively low concentration of bilirubin which has been shown to inhibit the malate-aspartate shuttle (McLoughlin et al., 1987). In the perfused rat liver, the redistribution of cellular Ca^{2+} may activate the efflux of aspartate from mitochondria resulting in an increase in the capacity of the malate-aspartate shuttle (Sugano et al., 1988). Alanine metabolism has been shown to increase the capacity of the malate-aspartate shuttle in perfused liver of cold-exposed and steroid replaced rats (Sugano et al., 1986).

Malate dehydrogenase:

Malate dehydrogenase (MDH ; EC 1.1.1.37) is widely distributed in animal tissues and plays an important role in carbohydrate metabolism. It catalyses the oxidation of malate to oxalacetate with a ΔG^0 of +7 Kcal/mole. MDH has been purified from wide variety of sources ranging from *E. coli* to humans. It catalyses the same reaction, albeit differs in physical and chemical properties. It is one of the several enzymes known to exists in two distinct forms: one in the cytosol (c-MDH) and the other in the mitosol (m-MDH). The two forms have different chemical, physical and kinetic properties and each has a characteristic electrophoretic pattern. The two forms also show a dissimilar developmental pattern and may be regulated differently by endogenous effectors (Malik et al., 1993).

The mechanism of action of MDH is closely related to that of lactate dehydrogenase (Larry and Johannes, 1978). m-MDH plays a crucial role in the reversible conversion of malate to oxalacetate in the Krebs cycle. c-MDH, on the other hand, is critically involved in gluconeogenesis (synthesis of glucose from non carbohydrate sources). The initial step in gluconeogenesis in the carboxylation of pyruvate to oxalacetate which is catalyzed by pyruvate carboxylase, a mitochondrial enzyme. Oxalacetate thus formed in the mitochondria cannot cross the membrane. However, malate diffuses from the mitochondria to the cytoplasm where it is oxidized by c-MDH to oxalacetate. Further, oxalacetate is converted to phosphoenlpyruvate by phosphoenolpyruvate carboxykinase (PEPCK). Thus, c- and m-MDH perform distinct metabolic functions in the different compartments (Lardy et al., 1965). The cytosolic enzyme differs from that of mitochondrial in its catalytic and physicochemical properties. Both the isoenzymes are NAD^+-dependent. However, m-MDH but not c-MDH is inhibited by high concentration of NADH. c- and m-MDH are also under the control of two separate genes (Davidson and Cartner, 1967). It was also reported that nutrition and hormone also regulate the gene for avian malic enzyme (Goodridge et al., 1989). MDH isoenzymes genes were also isolated from mouse and reported to have the DNA sequences required for the promoter activity of these genes. Nuclear binding to the promoter regions was also determined and that a transcription factor, CTF/NFI may contribute to the regulation of these isoenzymes.

Malate dehydrogenase is a dimeric enzyme consisting of identical subunits (M_r= 35,000) (Banaszak and Bradshaw, 1975). Its dissociation and reassociation have been the subject of many studies involving pig heart mitochondrial isoenzyme (m-MDH) (Wood et al., 1978; Muller et al., 1984; Gerl et al., 1985). Malate dehydrogenase from the cytosolic fraction of rat liver has been purified and its molecular weight (71 kDa), isoelectric point (pl 5.1), and amino acid composition were determined. The V_{max} was reported to be 53.3 µmol/min/g wet wt. for oxalacetate and 48 µmol/min/g wet wt for malate oxidation at pH 7.4 (Crow et al., 1982). In chicken, the purified preparations of cytosolic malate dehydrogenase resolved show three electrophoretically distinct species with identical amino-acid compositions and physico-chemical properties very similar to those of the cytosolic isoenzyme of other sources (Domenech et al., 1986). Mitochondrial malate dehydrogenase has been isolated and characterized from a large number of sources (Fahein and Strmecki, 1969; Glatthaar et al., 1974; Hagele et al., 1978; Kuan et al., 1979;

Okayabashi and Nakano, 1984; Cremel et al., 1985). In rat liver, mMDH has a V_{max} of 38 µmol/min/g wet wt. for oxalacetate reduction and 39 µmol/min/g wet wt. for malate oxidation at pH 7.4. Rates of the reaction catalysed by mitochondrial malate dehydrogenase under condition similar to those *in vivo* were calculated and found much lower than the maximum velocity of the enzyme (Wiseman, 1991). Mitochondrial malate dehydrogenase isolated from pig and chicken also known to contain one tyrosine residue which is important for its structure and function, probably involved in a hydrogen bond formation with a carboxylate group (Muller et al., 1983). The polypeptide chain conformation and coenzyme binding site of the crystalline porcine heart mitochondrial malate dehydrogenase by X-ray diffraction studies was determined (Roderick and Banaszak, 1986), indicating that the location of the coenzyme binding site and the catalytically important amino acid residues remain similar in mitochondrial malate dehydrogenase to that of cytosolic MDH and LDH isoenzymes.

The mechanism of substrate inhibition that occurs with pig heart cytosolic and mitochondrial malate dehydrogenase is different from the lactate dehydrogenase from chicken heart (Berstein et al., 1978). The inhibitor constant for oxalacetate is 2.0 mM with mitochondrial isoenzyme and 4.5 mM with the cytoplasmic isoenzyme. *In vivo* concentration of oxalacetate is 10 µM and so the substrate inhibition may not be significant under these conditions. m-MDH exhibits a complex regulatory pattern by citrate, which activates and inhibits mitochondrial malate dehydrogenase in the same direction ($NAD^+ \rightarrow NADH$), and in the same reaction medium, depending on malate concentration (Gelpi et al., 1992). It was also reported that pig heart citrate synthase and mitochondrial malate dehydrogenase do exhibit electrostatic interactions, whereas no interaction is observed between citrate synthase and cytosolic malate dehydrogenase from these sources (Morgunov & Srere, 1998).

Aspartate amonotransferase:

Aspartate aminotransferase (AsAT; EC 2.6.1.1) is the most extensively studied vit B_6- dependent enzyme (Snell and DiMari, 1970; Braunstein, 1973). It is widely distributed and represents the most abundant of the transaminases in mammalian tissues. The enzyme is implicated in maintaining the balance of the four metabolites which are its substrates. It catalyses the transamination reaction representing key steps at the

interconnection between the metabolic pathways of amino acids and dicarboxylic acids. AsAT effects the reversible transfer of an amino group from L-aspartate or L-glutamate to α-ketoglutarate and oxalacetate (Metzler and Snell, 1952). In double displacement reaction, the coenzyme shuttles between the pyridoxal and pyridoxamine p-forms (Gehring and Christen, 1978).

Two homologous genetically independent isoenzymes of AsAT have been found in tissues of higher organisms in two distinct forms, one in the cytosol (c-AsAT) and the other in the mitosol (m-AsAT). The isoenzyme of aspartate aminotransferase differ from one another in chemical, physical, catalytic and immunologic properties (Fleisher et al., 1960; Boyd, 1961; Jaussi et al., 1987; Nagashima et al., 1989). The existence of aspartate aminotransferase isoenzymes has been confirmed in a wide variety of eukaryotic organisms. Several authors have demonstrated (Fleisher et al., 1960; Borst and Poters, 1961; Martinez-Carrion et al., 1967; Banks et al., 1968; Bertland and Kaplan, 1970; Magee and Phillips, 1971; Di Cola et al., 1976) the presence of two main classes of AsAT in animal tissues, located in the mitochondria (m-AsAT) and in the soluble cell fraction (c-AsAT), respectively, with different kinetic and structural properties. Furthermore, several forms have been described for each AsAT isoenzyme and reported that chicken liver contained only the cytoplasmic enzyme (Decker and Rau, 1963;Gil et al., 1987; Morino et al., 1964; Yagi et al., 1979). However, other authors described later the presence of a mitochondrial fraction containing AsAT activity in that tissue (Shrawder and Martinez-Carrion, 1973; Elduque et al., 1982). Some attempts have been made to establish the submitochondrial location of m-AsAT. According to several reports (Landriscina et al., 1970) the enzyme is located exclusively in the mitochondrial matrix (mitosol), while other authors suggest that it is partially bound to the inner side of the inner mitochondrial membrane (Boyd, 1961; Eichel and Bukovsky, 1961; Bhargava and Sreenivasan, 1966; Elduque et al., 1982). This is further supported by the finding that the disruption of mitochondria by freeze-thawing or by treatment with high ionic strength buffers does not result in the complete release of m-AsAT activity, which could be fully achieved only by Triton X-100 treatment (Baumber and Doonan, 1976; Elduque et al., 1982). The mitosolic location of chicken liver AsAT was also ascertained(Gil et al., 1987).

Aspartate aminotransferase isoezymes are well characterized from chicken and pig heart. Borisov et al (1978) have studied the three dimensional structure of c-AsAT from chicken heart at 5 A^0 resolution and have reported that the enzyme molecule appears to consists of two dense and closely packed subunits with a dimension of 40 A^0 x 48 A^0 x 60

A^0 each. Eichele et al (1979) have studied the crystal stucture of m-AsAT from the chicken heart. In the electron density map, the enzyme is clearly seen as anisologous α_2 dimers (105 A^0 x 60 A^0 x 50 A^0). The active sites are located in opposite sides of the dimer, about 30 A^0 apart and close to the inter-subunit boundary so that both the subunits can probably contribute to each active site. X-ray diffraction studies at 2.8 A^0 resolution yielded the 3-D structure of chicken m-AsAT. The subunits are rich in secondary structure and contain two domains, one of which anchors the coenzyme pyridoxal-5'-phosphate (Ford et al., 1980). The cytosolic aspartate aminotransferase from chicken liver also shows molecular heterogeneity in polyacrylamide gel electrophoresis. The enzyme exists as various molecular forms of increasing anodic mobilities with the α-form predominates and its specific activity is the highest (Imperial et al., 1991). These isolated native molecular forms of chicken liver c-AsAT give rise to two kinds of generation processes.

The crystal structure of the stable, closed complexes of chicken mitochondrial aspartate amnotransferase with the natural substrate L-aspartate and L-glutamate have been resolved at 2.4 and 2.3 A^0 resolution, respectively (Malashkevich and Toney, 1993). In both cases, clear electron density at the substrate-coenzyme binding site unequivocally indicates the presence of a covalent intermediate. Crystalline enzyme has a much higher affinity for keto-acid substrates compared to enzyme in solution. The increased affinity is interpreted in terms of perturbation of the open/closed conformational equilibrium by the crystal lattice with the closed form having greater affinity for substrates. Catalytic efficiency of AsAT has been studied by Kohler et al (1994) in different mutants who have shown that improved catalytic efficiency of c-AsAT V_3 92 appears due to closure of the active site upon substrate binding. The primary structure of cytosolic and mitochondrial AsAT of pig heart has also been elucidated. The polypeptide chain of mitochondrial isoenzyme contains 403 amino acid residues, which is 9 residues less than cytosolic form.

The mitochondrial isoenzyme is apparently a matrix bound and is encoded by nuclear DNA (Van Heyningen et al.,1974). Mitochondrial and cytosolic AsAT are two genetically independent isoenzymes that have been found in animal tissues and possibly occur in all eukaryotic cells. Both isoenzymes are composed of two identical polypeptide chains of about 400 amino acid residues and are encoded by nuclear DNA. The mitochondrial isoenzyme is synthesized on free polysomes in the cytosol as a precursor of higher molecular weight (Sonderegger et al., 1980) which is translocated post-

translationally into the mitochondrial matrix. Organ- or tissue-specific forms have not been detected for either isoenzyme (Wada and Morino, 1964; Shrawder and Martinez-Carrion, 1973; Behra et al., 1981).The essential features of catalytic mechanism of both the isoenzymes are identical (Braunstein, 1973). A comparison of the amino acid sequence data of the cytosolic and mitochondrial aspartate aminotransferase from pig heart clearly indicates that these heterotropic isoenzymes are homologous proteins. A comparison of the mitochondrial isoenzyme from two different species, that is, from chicken and pig revealed a close interspecies homology, which appears to exceed the interspecies homology, between the cytosolic and the mitochondrial isoenzymes. Only two amino acid substitutions were found in the sequence of 40-NH_2 terminal amino acid residues.

The genes of mitochondrial and cyosolic aspartate aminotransferase of chicken were cloned and sequenced. In both genes (Juretic et al., 1990), nine exons encode the mature enzyme. The additional exon for the N-terminal presequence that directs mitochondrial aspartate aminotransferase into the mitochondria is separated by the largest intron from the rest of the gene. A comparison of the two genes of chicken with aspartate aminotransferase genes of mouse entails closely similar structures; in the gene of both the mitochondrial and the cytosolic isoenzyme all but one intron positions are conserved in the two species and five introns out of nine are placed at the same positions in all four genes indicating that the introns were in place before the genes of the two isoenzymes diverged. It is proposed that the aspartate aminotransferase genes follow a course of evolution, originating from a common ancestor which probably led to the conservation of certain splicing factors specific for nuclear-encoded mitochondrial proteins.

The degree of structural similarity between the heart mitochondrial isoenzyme of AsAT from pig and chicken was determined by means of their immunological cross-reactivity and compared with the degree of similarity in the cytosolic isoenzyme from the same species. Quantitative micro complement fixation revealed a remarkable similarity of the two mitochondrial isoenzymes corresponding to an immunological distance of 104. The structure of the two cytosolic isoenzymes, on the other hand, diverge with an immunological distance of 203. Thus, a significant structural divergence of the cytosolic isoenzymes appeared to contrast the conspicuous similarity between the mitochondrial isoenzymes (Sonderegger et al., 1977).

Cytosolic and mitochondrial AsAT were purified to homogeneity from human heart and shown to differ distinctly from each other in their structural, immunochemical and kinetic properties. The human isoenzymes were very similar to the corresponding isoenzymes from pig heart in their molecular weight, Michaelis constant (K_m), NH_2- and COOH- terminal amino acid sequences. In electophoretic mobility, the human cytosolic isoenzyme differs from the corresponding isoenzyme from the pig heart while no significant difference was observed between the mitochondrial isoenzymes from the two species (Boyd, 1961; Kagamiyama and Wada, 1975). Cytosolic and mitochondrial isoenzymes of aspartate aminotransferase were also purified to homogeneity from rabbit liver. The rabbit liver isoenzymes were closely similar to the corresponding isoenzymes from other sources, including human heart, pig heart, chicken heart, and rat liver, in their molecular weights, absorption spectra, amino acid compositions, isoelectric points, and Michaelis constants for the substrates. The NH_2-terminal amino acid sequences of rabbit liver isoenzymes were identified up to 30 residues, and showed some differences from those of the corresponding isoenzymes obtained from other animals studied (Kuramitsu et al., 1985). Interspecies comparisons of aspartate aminotransferase was also determined based on the amino acid composition. Subunit molecular weights of various aspartate aminotransferase examined were found to be 46,000 with the exception to that of the *E. coli* enzyme which is 42,700 (Doonan et al., 1981). Compositional difference indices were calculated for pairs of AsAT where indices provide reliable estimates of amino acid sequence differences for those pairs of isoenzymes whose primary structures are known. Comparisons of compositional difference indices also suggested a similar rate of evolution of cytosolic and mitochondrial AsAT throughout the history of the vertebrates examined.

Several workers (Fleisher et al., 1960; Boyd, 1961; Wada and Morino, 1964; Barra et al., 1976) have purified both the cytosolic and mitochondrial isoenzymes of AsAT from various tissues of mammals and have shown that, at a fixed concentration of L-aspartate, the K_m for α-ketoglutarate is much higher for mitochondrial than for the cytosolic enzyme. The reverse applies to the K_m for L-aspartate at a fixed concentration of α-ketoglutarate. The mitochondrial isoenzyme is more susceptible to the inhibition of DL-glyceraldehyde-3-phosphate than that of the cytosolic enzyme (Kopelovich et al., 1970). The inhibition of the mitochondrial isoenzyme is competitive with respect to α-ketoglutarate and noncompetitive with respect to L-aspartate. Aminooxyacetic acid and other aminooxyacetates are

effective inhibitors of both c- and m-AsAT (Braunstein, 1973; Rej, 1977). The inhibitory effect of aminooxyacetic acid is shown to be more pronounced for c-AsAT.

Liver AsAT is responsible for the synthesis of glucose from non-carbohydrate precursors (Lardy et al., 1965). Both the isoenzymes of AsAT are involved in the process of gluconeogenesis. The specific activity of c-AsAT was also reported to be higher in the adult as compared to that of fetal liver (Nakata et al., 1964). Ferre and Williamson (1978) observed that the inhibition of the activity of AsAT in the suckling new born rat causes a decrease in the level of all the gluconeogenic precursors and accumulation of lactate but not pyruvate. On the basis of these observations, it has been suggested that the malate-aspartate shuttle is fully operative in suckling rats during development. Although both the isoenzymes of AsAT are involved in the process of gluconeogenesis, it is the cytosolic isoenzyme which is regulated by dietary and hormonal treatments (Sheid and Roth, 1965).

Herzfeld and Greengard (1971) reported that the expression of AsAT during normal development does not follow the same pattern in all the tissues of rat. In liver, the beginning of rapid enzyme accumulation occurs shortly before birth and just after birth there is a transient rise in the level of AsAT. The developmental formation of AsAT in heart and kidney is gradual over the first few weeks of postnatal age. The beginning of rapid AsAT accumulation in fetal liver coincides with the onset of thyroid and adrenocortical secretions. They demonstrated that the hormones which modify the developmental formation of an enzyme in one organ may have no effect or the opposite effect, on the same enzyme in other tissues of the same animal. Even in the same tissue, the response of the enzyme depends on the age, sex and physiological state of the animal.

Several physiological and biochemical changes occur during development, growth, adulthood and senescence of an organism. Different metabolic events that occur during early life of an organism might influence the later part of life span. To study the activities of all the key enzymes of a particular metabolic pathway would provide a complete profile of their biological function during development and aging. Earlier work on the malate-aspartate shuttle has been done mostly on the mammalian system. However, informations on the developmental and hormonal regulation in enzymes of avian malate-aspartate shuttle are scanty. Keeping in view the importance of studying all the enzymes

of a particular metabolic pathway and with emphasis on avian system, the work embodies in this thesis is directed towards the following:-

1. Assessment of endogenous activity levels of malate-aspartate shuttle enzymes in different tissues of chicken at various postnatal ages (day 0, 30 and 60) in order to find out the changes, if any, in the activity which may predict tissue- and age-specific involvement of malate-aspartate shuttle enzymes in the early postnatal development of chicken.

2. Effects of various hormones such as glucocorticoid and thyroid hormones on the activity of shuttle enzymes in liver and kidney tissues of chicken at various postnatal ages (day 0, 30 and 60) to find out the role of these hormones, if any, in regulating the shuttle activity in a tissue- and age-specific manner.

3. Purification and characterization of one of the shuttle enzymes, that is, cytosolic aspartate aminotransferase from two different ages (day 0 and 60) to determine alterations if any in the physico-chemical and/or kinetic properties accompany this enzyme during the course of development.

MATERIALS

Animals:

Newly hatched male chickens, Rhode Island Red breed (RIR), identified by vent sexing were purchased from nearby Kyrdemkulai farm, which is a Government of Meghalaya undertaking. They were housed in the laboratory animal room with a 12 hr light and maintained at 25 $\pm$ 2^{0}C temperature. Chickens were fed with a chick mash diet purchased locally from Premier Hatchery Ltd., Shillong. It contained the usual supplement of protein, mineral mixture, vitamin and antibiotics. They were fed with the above diet and water *ad libitum.*

Biochemicals and reagents:

Triton X-100, Oxalacetate, NADH, α-Ketoglutarate, Malate dehydrogenase, Aspartate, Coomassie brilliant blue, Bovine serum albumin, Tris (Hydroxy amino methane), EDTA, Mercaptoethanol, ADP, Malate, Glutamate, Hydrocortisone, Triiodothyronine were purchased from Sigma Chemical Company, USA.

Chromatography media and reagent for gel electrophoresis:

CM-Cellulose ion exchanger, Acrylamide, N'N' Methylene bis acrylamide, NNN'N' Tetramethylene diamine, Ammonium persulphate, Bromophenol blue, Coomassie brilliant blue R-250, Glycine, O-Dianisidine tetrazotized salt were also purchased from Sigma Chemical Company, USA.

Other reagents and chemicals:

NaCl, Sucrose, K_2HPO_4, KH_2PO_4, Phosphoric acid, HCl, $MgCl_2$, KCl, Diethyl ether, Ethanol, Urea, $(NH_4)_2SO_4$, NaOH, Sodium acetate, Acetic acid, Glycerol, Methanol and all other chemicals were of analytical grade purchased from Sisco Res. Lab., Hi-Media and Qualigens, India.

Instrumentations:

pH measurements-

A control dynamics digital pH meter model APX 175 E/C was routinely used for all pH measurements at room temperature after calibrating with standard buffer tablets of pH 4, 7 and 9.2.

Absorbance measurements-

A Hitachi U-2000 double beam spectrophotometer was used for all absorbance measurements in the visible and ultraviolet regoin using glass and quartz cuvettes of 1 cm path length, respectively.

Centrifugation-

All centrifugations were carried out in a Hitachi model Himac CR 20B2, high speed refrigerated centrifuge at 4^0C.

Homogenization-

Remi motor type RQ-127 A, HP 8, rpm 8000 (1.1 Amps, 220/230 V) homogenizer fitted with a teflon pestle was routinely used. Glass homogenizing tubes (5-50 ml) were used for homogenizing tissues.

Electrophoresis-

A Bio-Rad slab gel electrophoresis apparatus (Model 122/2.0) and mini protean II chamber was used for polyacrylamide gel electrophoresis.

Weighing balance-

Sartorius balance model 2434 (0.01 mg -160 g), model 2405 (0.001 mg -30 g) and an electronic top pan balance (0.01-600 g) were used for weighing chemicals.

METHODS

Tissue preparation and extraction :

Chicken of various postnatal ages were sacrificed at 9:00 hr by decapitation. Tissues (liver, kidney, heart and brain) were removed immediately and washed in ice cold 0.9 % NaCl. The adhering blood vessels were blotted dry on a filter paper and the tissues

were stored at -80^0C till further used. A 25% (w/v) homogenate of the tissues was prepared in ice cold 0.25 M sucrose using a homogenizer fitted with a teflon pestle.

The homogenates were centrifuged for 10 min at 800 x g at 2^0C to sediment nuclei and cell debris. The resulting supernatant was further centrifuged for 40 min at 14,000 x g at 2^0C to sediment mitochondria. The supernatant thus obtained was used for the assay of cytosolic aspartate aminotransferase (c-AsAT) and malate dehydrogenase (c-MDH). The mitochondrial pellet was washed once and suspended in a solubilizing medium (10 mM potassium phosphate buffer, pH 7.5 / 0.25 M sucrose / 0.5 % Triton X-100) to make a 10% suspension. Various concentrations of phosphate buffer and Triton X-100 were used to solubilize the mitochondrial AsAT before using the most effective one mentioned above. For the assay of m-MDH, the mitochondrial pellets were suspended in 50 mM potassium phosphate buffer, pH 7.5 containing 0.25 M sucrose that gave better assay for mMDH. Assays for m-AsAT and m-MDH were performed within 3 hr of mitochondrial suspension.

Assay of malate dehydrogenase (MDH) isoenzymes:

Both the isoenzymes of MDH (c- & m-MDH) were assayed spectrophotometrically according to the method of Kitto (1969). The method is based on the measurement of the rate of oxidation of NADH (i.e., decrease in absorbance at 340 nm) in the presence of this enzyme and its substrate (oxalacetate). The reaction occurs as follows:

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{MDH}} \text{Malate} + \text{NAD}^+$$

The rate of disappearance of NADH was measured at 340 nm in a spectrophotometer using a cuvette of 1.0 cm light path. The final volume of the reaction mixture was 3.0 ml which contained:

Potassium phosphate buffer, pH 7.5	-	100	mM
Oxalacetate	-	0.5	mM
NADH	-	0.14	mM
Enzyme (suitably diluted)	-	20	µl

The reaction was initiated by the addition of oxalacetate. The decrease in absorbance was recorded at 30 sec. intervals for 3 min and the enzyme activity was

calculated from the initial rate of oxidation of NADH. The amount of enzyme used was adjusted such that the decrease in the absorbance was met 0.10 per min. One unit of MDH activity for both isoenzymes was defined as the amount which is required to oxidize 1 μ mole of NADH per min at 25°C. The specific activity was expressed as units per mg protein.

Assay of aspartate aminotrasferase (AsAT)isoenzymes :

The acivity of both the isoenzymes of AsAT (c- & m-AsAT) was measured according to the method of Karmen (1955) with certain modifications in the concentrations of the substrates and co-factor (Herzfeld, 1971; Sharma & Patnaik, 1982). According to the method, the rate of oxalacetate formation is measured spectrophometrically in a coupled reaction, catalyzed by malate dehydrogenase in the presence of NADH. The reaction occurs as follows;

$$\text{Asp} + \alpha\text{-KG} \xrightleftharpoons{\text{AsAT}} \text{Oxalacetate} + \text{Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^{+} \xrightleftharpoons{\text{MDH}} \text{Malate} + \text{NAD}^{+}$$

For each mole of oxalacetate formed from aspartic acid , 1.0 mole of NADH is oxidised in the coupled reaction. NADH has a characteristic absorption maxima at 340 nm with an extinction coefficient of 6.22 X 10^{6} / mole. The rate of disappearance of NADH was measured at 340 nm using a cuvette of 1.0 cm light path. The final volume of the reaction mixture was 3.0 ml which contained :

Potassium phosphate buffer, pH 7.5	-	100	mM
α-ketoglutarate	-	6.00	mM
Malate dehydrogenase	-	10	units
NADH	-	0.14	mM
Enzyme (suitably diluted)	-	20	μl
Aspartate	-	75	mM

The reaction was initiated by the addition of aspartic acid to the experimental cuvette and decrease in absorbance was recorded at 30 sec. intervals for 3 min. The

linear decrease in absorbance per min was used for the calculation of the enzyme activity. The enzyme was so diluted that the decrease in absorbance did not exeed 0.1 per min.

One unit of enzyme activity was taken as that amount which catalyzed the conversion of one μmole of the substrate per min at 25 ^{0} C. The specific activity was expressed as units per mg protein.

Protein estimation :

Protein concentration of enzyme preparation was determined by the method of Bradford (1976) using BSA as standard. The reagents and procedure were as follows.

Reagent A - *Coomassie brilliant blue G-250 (0.2 g in 100 ml of 95 % ethanol)*

Reagent B - *Phosphoric acid (85%)*

Stock solution was prepared by mixing reagent A (50 ml) with reagent B (100 ml) and then stored in a brown bottle at 4^{0}C for further use.

Bradford working solution was prepared by diluting 15 ml of the stock solution to 100 ml with distilled water and filtered through Whatman No. 1 filter paper just before use. The final concentration of the reagents was 0.01 % (w.v) Coomassie brilliant blue G-250, 4.7 % (v/v) phosphoric acid. 0.1 ml of each appropriately diluted protein sample was added to 5 ml of Bradford working solution, mixed thoroughly with cyclomixer. After incubating at room temperature (10-15 min), the intensity of colour was measured at 595 nm. Protein concentrations were determined using a computed standard curve prepared by different concentrations of BSA (0.01 to 0.1 mg/ml).

Reconstitution of malate-aspartate shuttle :

For the reconstitution of malate-aspartate shuttle, the tissues were homogenized in four volumes of homogenization buffer (50 mM Tris-HCl, pH 7.4/ 0.25 M mannitol / 1 mM EDTA / 2 mM $MgCl_2$ / 30 mM β-mercaptoethanol) and centrifuged at 800 x g for 10 min at 2^{0}C to sediment nuclei. The supernatant was further centrifuged at 14,000 x g for 30 min to sediment mitochondria. The mitochondrial pellet was washed twice, suspended in homogenization buffer, and used for the reconstitution assay. The post-mitochondrial

supernatant was dialyzed for 18 hr at 4^0C against 50 mM Tris-HCl buffer, pH 7.4 containing 1 mM EDTA, 2 mM $MgCl_2$, and 30 mM β-mercaptoethanol. The dialyzed cytosol was centrifuged at 14,000 x g for 30 min to remove traces of particulate materials and the resultant clear supernatant was used for the reconstitution studies.

Reconstitution assay was done according to the method of Cederbaum (1973) and Dawson (1982) with some modifications of our own in the amount of protein taken and in the final assay volume (Sharma et al., 1992). The reaction mixture (final volume, 2.5 ml) contained buffer incubation medium (300 mM mannitol / 10 mM Potassium phosphate buffer, pH 7.4 / 10 mM Tris-HCl pH 7.4 / 10 mM KCl / 5 mM Mg Cl_2/ 2 mM ADP / 2 mM aspartate), 2 mg cytosolic protein and 1mg mitochondrial protein. After setting the baseline to zero, 50 µl of 7 mM NADH was added to the sample cuvette giving the absorbance of 0.70. The slow steady fall in absorbance was monitored for 2 min and then 50 µl of a solution of 0.2 M each of L-malate and glutamate was added to both cuvettes. The decrease in absorbance was followed upto 10 min.

Hormone treatments :

Effects of hydrocortisone and triiodothyronine on the activity of AsAT and MDH were studied in the liver and kidney of 0-, 30- and 60-day old male chicken. Different doses and time durations have been used to check the optimal dose and time response. Animals were grouped in two sets comprising of 4-5 chicks per set. The first set of chicks were administered intraperitoneally (i.p.) with hormone suspending medium and served as the control. The second set of animals were administered with suspended test hormones. To avoid fluctuation which may arise due to circadian variations, hormone administrations were done at a fixed time of day (09:00 hr) in all cases.

Hydrocortisone-

Out of various doses of hydrocortisone administered, a repeated dose of I mg / 100 g body weight was found to be most effective in regulating shuttle enzymes in the liver and kidney of chicken. Finally, hydrocortisone (1 mg / 100 g body weight) was administered in a total volume of 0.3 ml normal saline (0.9% NaCl) containing 10 % ethanol. Hormone was administered three consecutive days at the same time. Chicks were sacrificed 6 hrs after the final injection and tissues (liver and kidney) were removed and washed in normal

saline, blotted dry and stored at –80^{0}C. These tissues were later processed and assayed for MDH and AsAT isoenzymes described under method.

Triiodothyronine-

Again, out of various doses of triiodothyronine administered, a repeated dose of 25 µg / 100 g body weight was found to be most effective. It was finally suspended in saline (0.9 % NaCl) and 10 % ethanol mixture, were administered as a single dose (i.p.) in the same pattern as described for hydrocortisone in a total volume of 0.3 ml. Animals were sacrificed 6 hrs after the final injection and tissues were similarly removed and stored at –80 ^{0} C and later used for assay of isoenzymes.

Purification of cytosolic aspartate aminotransferase (c-AsAT) :

Cytosolic aspartate aminotransferase was purified from immature (0-) and mature (60-day) male chicken liver using Marra et al., (1977) procedure with some modifications as described earlier (Sharma and Patnaik, 1982). All the steps unless mentioned otherwise, were carried out at 4^{0}C.

I- Crude extract

The livers were collected from 10-12 male RIR chicken of each age group. The adhering blood vessels from the tissues were removed. A 25% (w/v) homogenate of liver was made in 0.25 M sucrose using a glass homogenizer fitted with Teflon pestle. Crude homogenate was centrifuged at 20,000 x g for 30 min. The pellet was discarded and the resulting supernatant was used for further purification.

II- Ammonium sulphate fractionation

Supernatant obtained from step I was brought to 40% ammonium sulphate saturation through gradual addition of solid $(NH_4)_2SO_4$. The solution was slowly stirred for 1 hr and was then centrifuged at 10,000 x g for 10 min. The pellet was discarded.

The resulting supernatant of the first $(NH_4)_2SO_4$ precipitation was brought to 80% $(NH_4)_2SO_4$ saturation through a gradual addition of solid $(NH_4)_2SO_4$. After complete solubilization of the added salt into the crude enzyme solution, the pH was maintained at 7.4 by addition of 1 N NaOH. The solution was stirred slowly for 12 hr with the help of a magnetic stirrer. It was centrifuged at 20,000 x g for 30 min and the supernatant was discarded.

III- Dialysis

The final precipitate obtained from step II was dissolved in a minimum volume of 0.02 M sodium acetate buffer, pH 5.4 and was dialyzed for 36 hr against the same acetate buffer. Dialysate was then centrifuged at 10,000 x g for 30 min and pellet was discarded.

IV- CM-Cellulose chromatography

The clear supernatant from step IV was applied onto a column (1.6 x 14 cm) of CM-Cellulose, which was pre-equilibrated with 0.02 M sodium acetate buffer, pH 7.5 at a flow rate of 30 ml/hr. After application of the sample, the column was extensively washed with 0.02 M acetate buffer pH 5.4. Subsequently, a linear gradient of sodium acetate buffer (0.06-0.16 M) was applied with the help of a gradient mixture to elute the bound fractions. The active peak fractions were collected, pooled and were concentrated by ammonium sulphate fraction.

V- Ammonium Sulphate fractionation

The enzyme-rich fractions obtained after CM-Cellulose chromatography were pooled together and were brought to 80% saturation by the addition of solid $(NH_4)_2SO_4$. The solution was stirred slowly for 12 hr and then centrifuged at 20,000 x g for 30 min. The supernatant was discarded and the pellet was dissolved in a minimum volume of 0.02 M sodium acetate buffer pH 5.4. This preparation was further dialyzed for 24 hr to remove the salt present with purified enzyme. The sample was then centrifuged at 10,000 x g for 30 min and the clear supernatant was used as the source of purified enzyme for the kinetic and other analysis.

Polyacrylamide gel electrophoresis of purified c-AsAT :

Native polyacryamide gel electrophoresis (Native-PAGE) of the purified c-AsAT from the liver of 0- and 60-day old chicken was performed according to the method of Davis (1964) with slight modification. A 7.5% slab gel was prepared by taking : 2.5 ml of acrylamide / bisacrylamide solution / 3.73 ml of 1.0 M Tris buffer (pH 8.8) / 3.70 ml of distilled water / 0.033 ml of ammonium per sulphate(APS) (freshly prepared).

Prior to addition of APS, gel solution was degassed for 15 min under vacuum and was immediately casted into slab plates fitted with spacers, sealed at both sides and lower

ends. The gel was allowed to polymerize and then pre-run for 15 min at 12 mA. Purified enzyme preparation (50 µg) from both the ages of chicken were mixed with assay buffer, glycerol (10%) and bromophenol blue (0.1%). 20-40 µl of this preparation was carefully applied with the help of micro syringe onto gel lanes and electrophoresed for 30-40 min at 24 mA in the cold. Subsequently, one set of gel was removed, washed in distilled water and stained with 0.5 % Coomassie brilliant blue R-250 (prepared in 30 % methanol and acetic acid) for 30 min. Background stain was removed by immersing the gel in destaining solution (20% methanol and 7% acetic acid).

The other sets of gel, after electrophoresis, were processed for specific staining of c-AsAT according to the method of Doonan (1981) with certain modifications. The staining mixture contained L-aspartic acid (15 mM), · α-ketoglutarate (6.8 mM), and Tris (100 mM; pH 7.5). Prior to use, O-Dianisidine-tetrazotized (Fast Blue B salt) was added to a final concentration of 10 mM and the staining mixture was stirred vigorously. The mixture was poured onto slab gel and the enzyme activity band appeared as a violet colour within a short span of time. The staining mixture was then decanted and the gels were washed thoroughly to avoid any further development of background colour. These gels were stored overnight in a solution of distilled water, methanol and acetic acid in the ratio of 5:3:1 (v/v) and subsequently photographed.

Kinetic studies :

The kinetic studies for both the immature and mature liver c-AsAT male chicken were carried out using purified enzyme.

Effect of [L-aspartate] and [α-ketoglutarate] on purified c-AsAT-

The activity of purified c-AsAT from the liver of immature and mature chicken was measured at various concentrations of L-aspartate (0-100 mM) and α-ketoglutarate (0.01-10 mM) in separate sets of experiments. The values of K_m for both substrates were determined by Michaelis-Menten and Lineweaver-Burk plots of data using Sigma enzfitter programme (Perella, 1988).

Effect of [Amino-oxyacetic acid] on c-AsAT-

The effect of aminooxyacetic acid (AoAA) on the activity of purified c-AsAT from the liver of immature and mature chicken was studied by using various cocncentrations (0-

0.2 mM) of amino-oxyacetic acid at two different fixed concentrations of L-aspartate (10 and 40 mM) and α-ketoglutarate (1 and 2 mM). The K_i values were determined by Dixon's plot of the data using enzfitter programme.

Effect of urea on c-AsAT-

Using varying concentrations (0.01-8 M) of urea as denaturant in potassium phosphate buffer, pH 7.5, the activities of purified c-AsAT from the two different ages (0-day and 60-day) were studied. The enzyme samples were incubated for 30 min at these concentrations of urea, while the other conditions of assay remained the same. The results similarly expressed as percentage activity retained in presence of specific urea concentration taking no urea as 100%.

RESULTS

The results are represented in the tables which contain the mean values, standard deviation of the mean, level of significance and also the percent increase or decrease between two sets of the data. The results are also represented in figures for clarity. Only the salient features and not the detailed description of the data are mentioned here. The activities of both the isoenzymes of malate dehydrogenase (c- and m-MDH) and aspartate aminotransferase (c- and m-AsAT) have been expressed as Units per mg protein in the tables as well as in the figures. Activities of malate-aspartate shuttle enzymes and the shuttle activity were measured primarily in the early postnatal development of chicken. Hormonal studies were also performed with the same ages. The kinetic characterization of cytosolic AsAT was from the liver of 0- and60-day old chicken in order to find the difference, if any, in the physicochemical and kinetic properties of this isoenzyme at these ages of chicken.

Normal endogenous activity of malate-aspartate shuttle isoenzymes in different tissues of male chicken at various postnatal ages:

Malate dehydrogenase (MDH)

Specific activity (Units per mg protein) of cytosolic malate dehydrogenase (c-MDH) is significantly higher in the liver of chicken at day 30 as compared to day 0 (+53%) and day 60 (+38%) (Fig. 1; Table 1). Mitochondrial (m-) MDH, however, does not exhibit any significant change in the liver upto day 30 (Table1) and declines significantly (-51%) at day 60 (Fig. 1; Table 1). In kidney, both c- and m-MDH show (Fig. 2; Table 1) a significantly lower activity at day 0 and increase sharply at day 30 (8-fold and 4-fold, respectively) and at day 60 (+35% and +14%, respectively). In the heart, c- MDH show a slightly lower activity at day 0 (Table 2) and increases gradually at day 30 (2-fold) and at day 60 (4-fold) (Fig. 3; Table 2). However, m-MDH does not exhibit any significant change with only a slight increase at day 30 but remained unchanged thereafter (Fig. 3; Table 2). Specific activity of MDH isoenzymes, however, does not exhibit any significant change in the chicken brain(Fig. 4; Table 2). From the activity expression pattern, it is

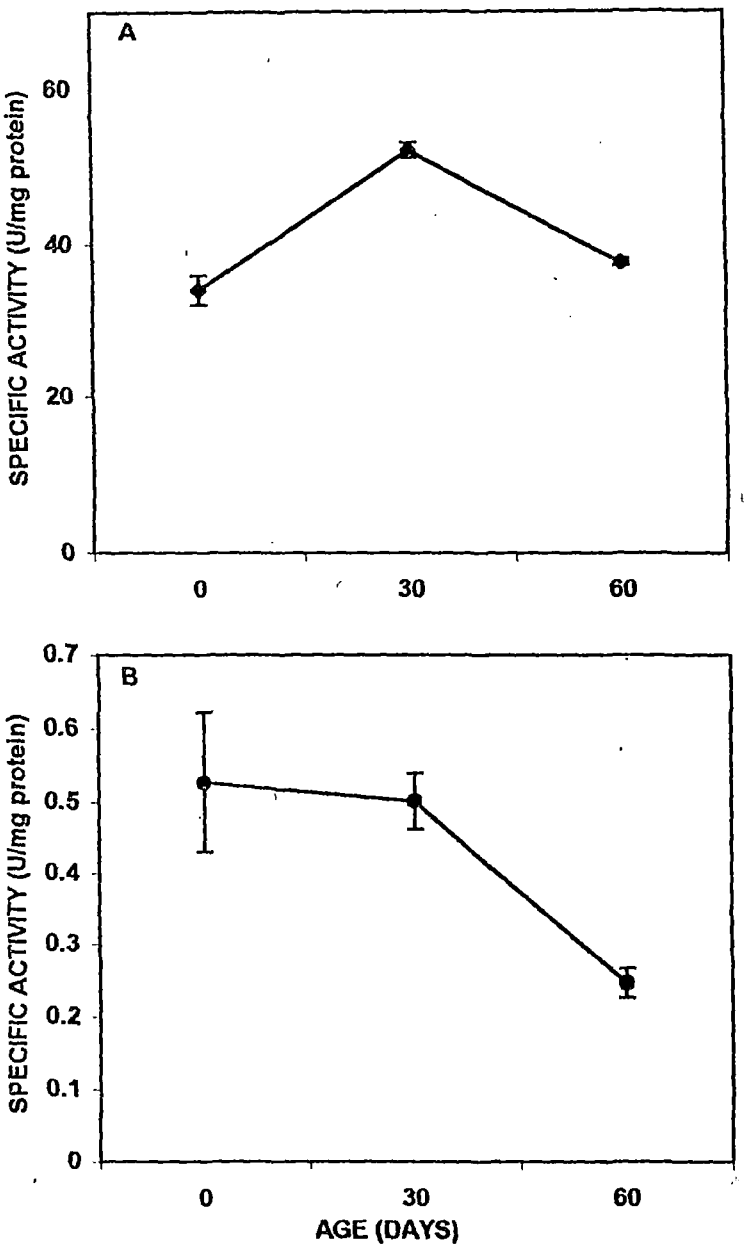


Figure 1. Activity of malate dehydrogenase (MDH) isoenzymes cytosolic(A) and mitochondrial(B) in the liver of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

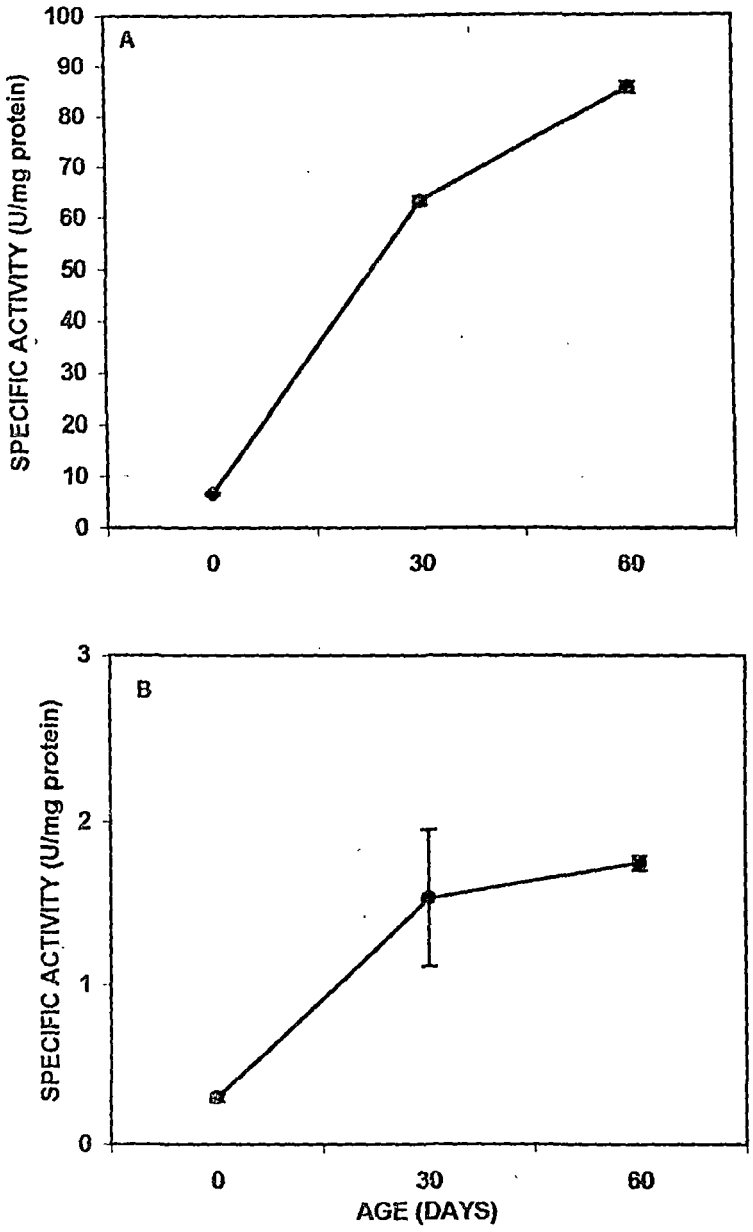


Figure 2. Activity of malate dehydrogenase (MDH) isoenzymes cytosolic(A) and mitochondrial(B) in the kidney of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

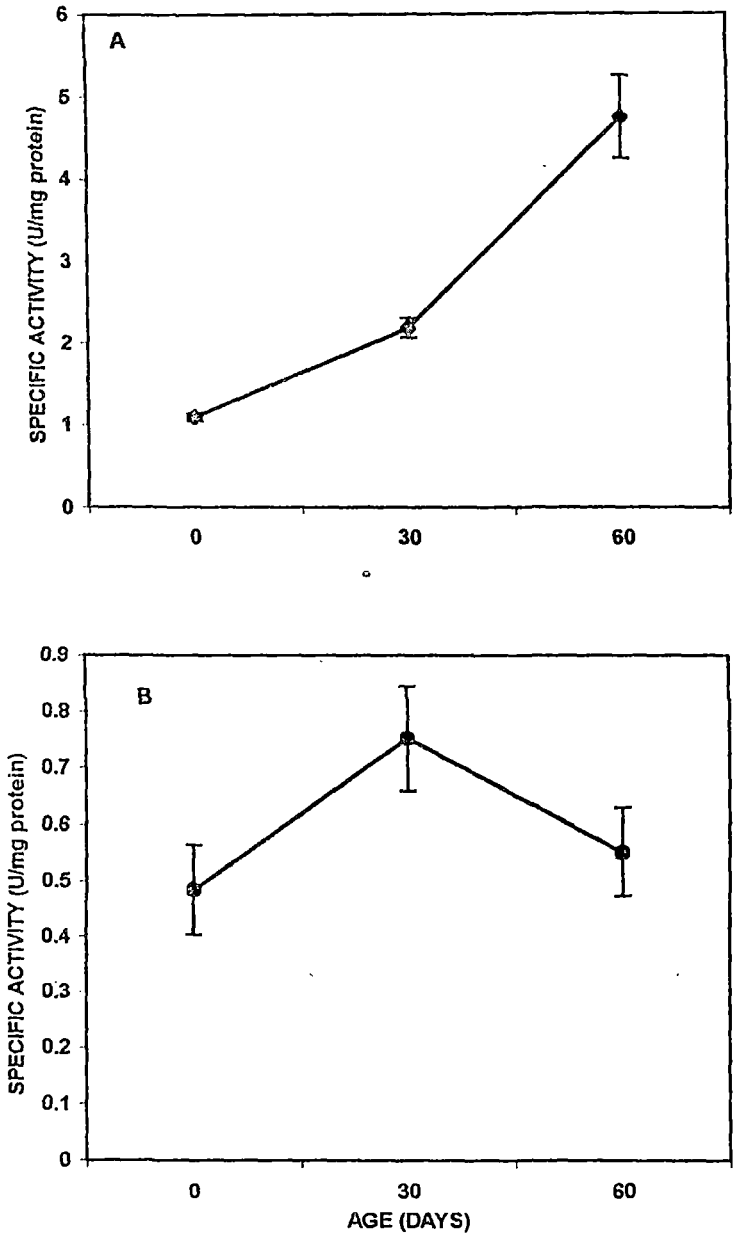


Figure 3. Activity of malate dehydrogenase (MDH) isoenzymes cytosolic(A) and mitochondrial(B) in the heart of normal male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

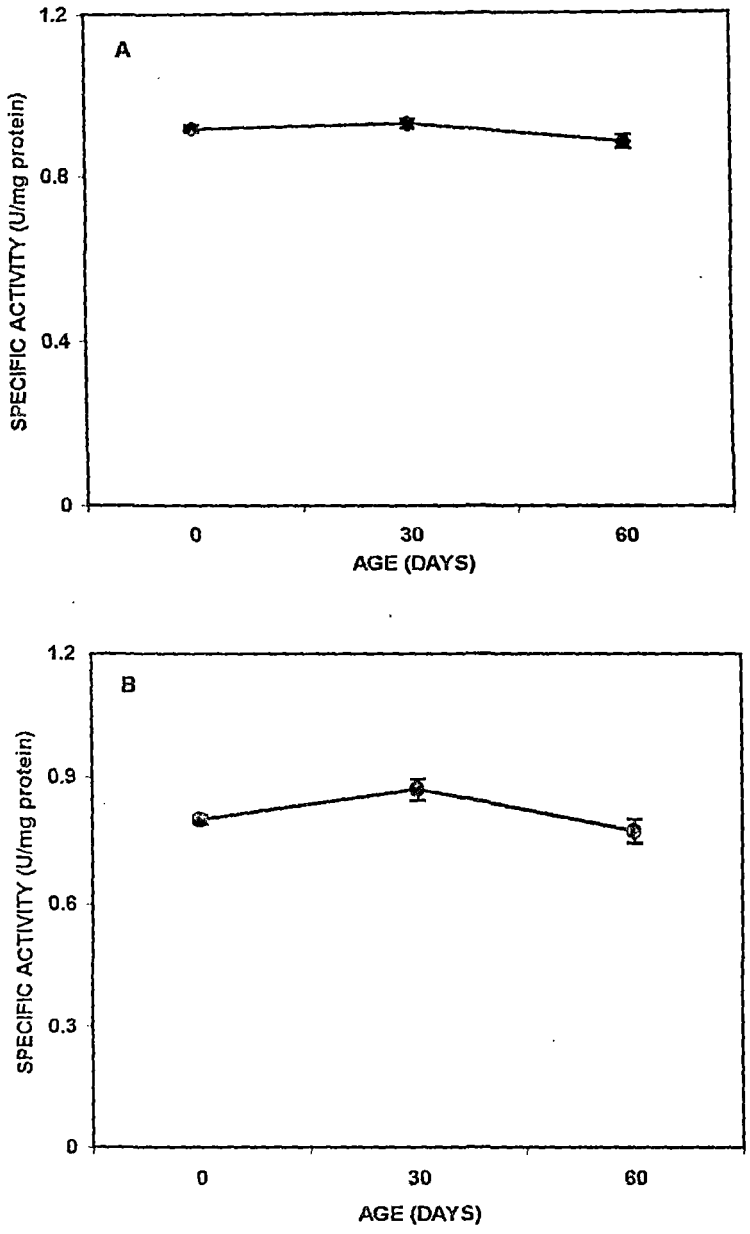


Figure 4. Activity of malate dehydrogenase (MDH) isoenzymes cytosolic(A) and mitochondrial(B) in the brain of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

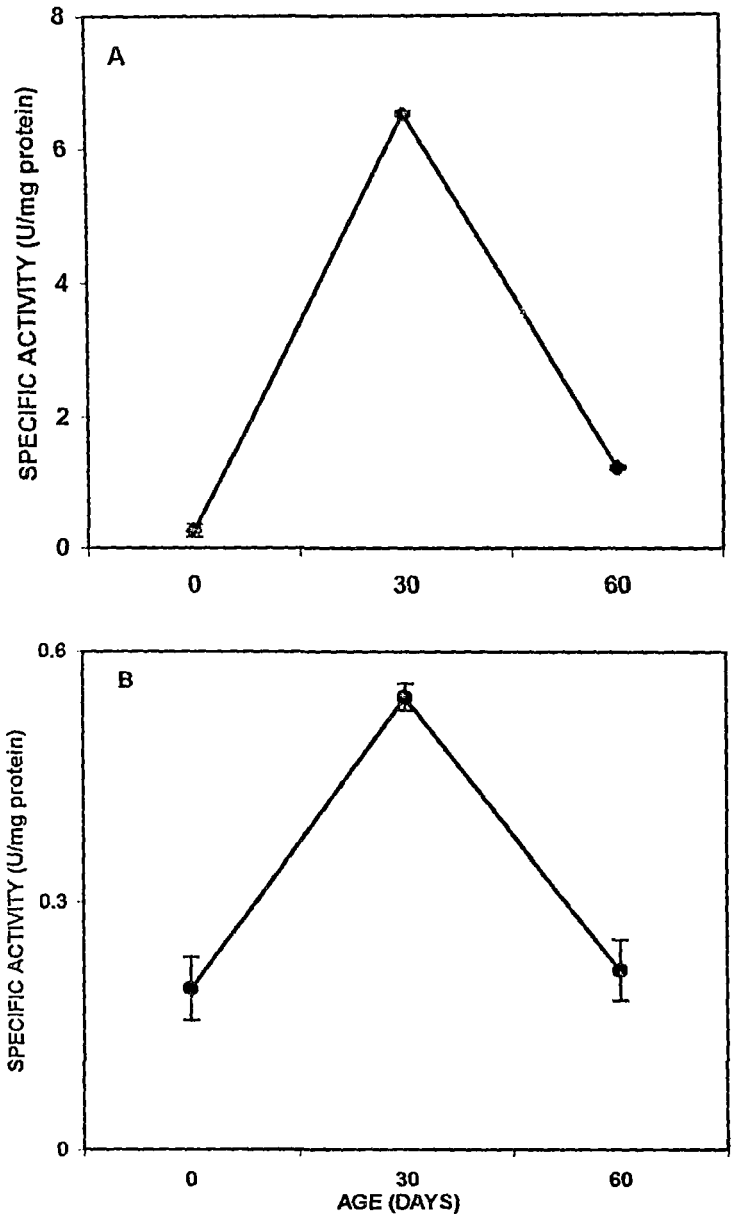


Figure 5. Activity of aspartate aminotransferase (AsAT) isoenzymes cytosolic (A) and mitochondrial (B) in the liver of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

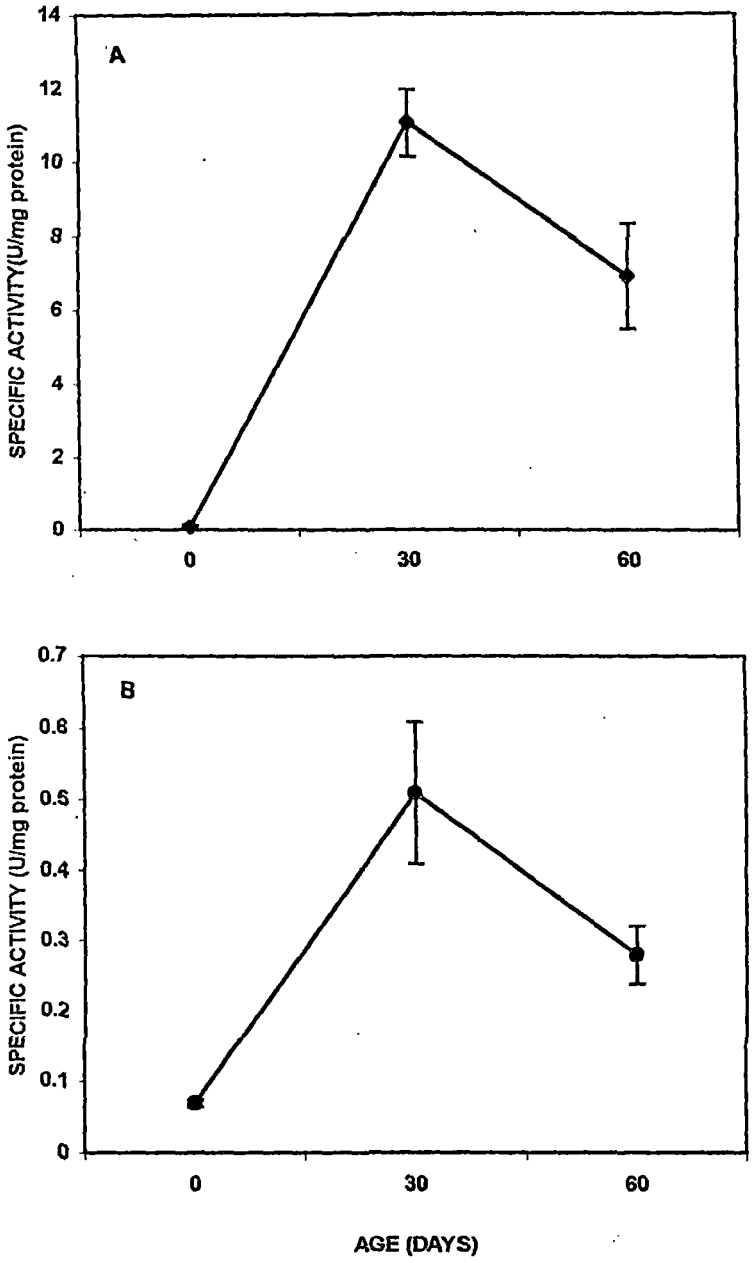


Figure 6. Activity of aspartate aminotransferase (AsAT) isoenzymes cytosolic(A) and mitochondrial(B) in the kidney of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

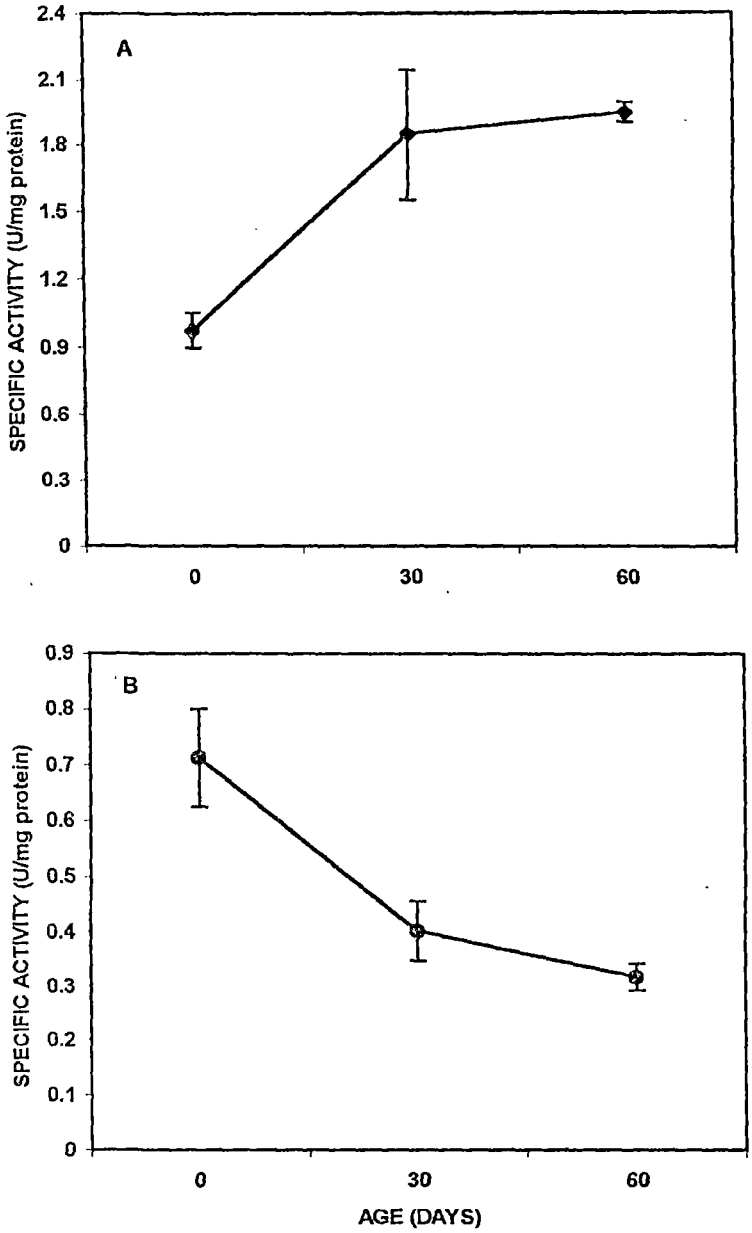


Figure 7. Activity of aspartate aminotransferase (AsAT) isoenzymes cytosolic(A) and mitochondrial(B) in the heart of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

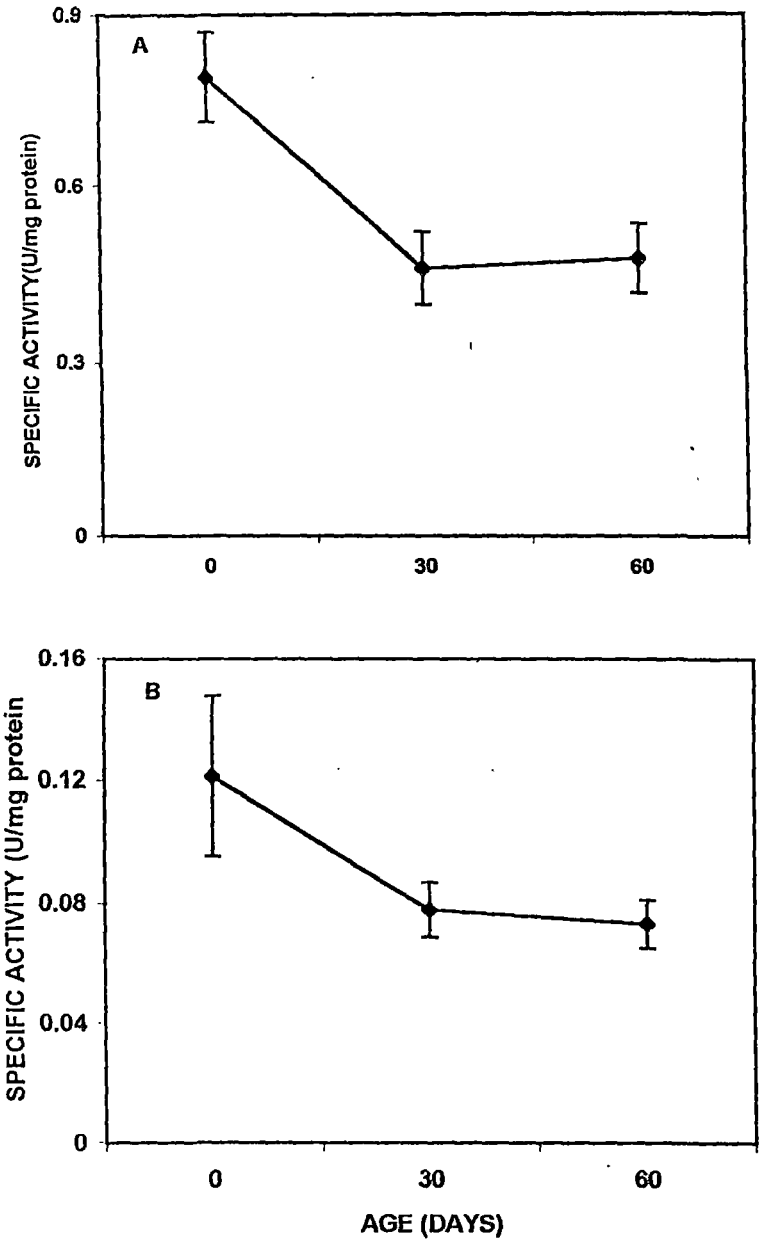


Figure 8. Activity of aspartate aminotransferase (AsAT) isoenzymes cytosolic(A) and mitochondrial(B) in the brain of male chicken of different postnatal ages. Values are means for 4-5 chicks in each age group. Bars represent standard deviation.

Table 1. Activities (U/mg protein) of malate dehydrogenase isoenzymes (cytosolic and mitochondrial) in the liver and kidney of male chicken of various postnatal ages.

Tissue	Age (days)	Cytosolic			Mitochondrial		
		Mean	SD	p	Mean	SD	P
Liver	0	34.013	1.96		0.526	0.095	
	30	52.125	0.927	<0.001	0.501	0.039	NS
	60	37.729	0.45	<0.001	0.247	0.022	<0.001
Kidney	0	6.658	0.197		0.297	0.025	
	30	63.192	0.940	<0.001	1.534	0.417	<0.001
	60	85.645	1.178	<0.001	1.752	0.046	NS

Table 2. Activities (U/mg protein) of malate dehydrogenase isoenzymes(cytosolic and mitochondrial) in the heart and brain of male chicken of various postnatal ages.

Tissue	Age (days)	Cytosolic			Mitochondrial		
		Mean	SD	p	Mean	SD	P
Heart	0	1.084	0.045		0.484	0.081	
	30	2.184	0.117	<0.001	0.752	0.093	<0.05
	60	4.746	0.510	<0.001	0.553	0.078	<0.01
Brain	0	0.916	0.0105		0.799	0.0102	
	30	0.927	0.011	NS	0.869	0.026	NS
	60	0.882	0.016	NS	0.771	0.027	NS

Table 3. Activities (U/mg protein) of aspartate aminotransferase isoenzymes (cytosolic and mitochondrial) in the liver and kidney of male chicken of various postnatal ages.

Tissue	Age (days)	Cytosolic			Mitochondrial		
		Mean	SD	p	Mean	SD	P
Liver	0	0.268	0.029		0.194	0.039	
	30	6.527	0.152	<0.001	0.544	0.016	<0.001
	60	1.249	0.329	<0.001	0.217	0.037	<0.001
Kidney	0	0.079	0.066		0.069	0.005	
	30	11.049	0.919	<0.001	0.509	0.099	<0.001
	60	6.904	1.410	<0.01	0.281	0.041	<0.01

Table 4. Activities (U/mg protein) of aspartate aminotransferase isoenzymes (cytosolic and mitochondrial) in the heart and brain of male chicken of various postnatal ages.

Tissue	Age (days)	Cytosolic			Mitochondrial		
		Mean	SD	p	Mean	SD	P
Heart	0	0.974	0.077		0.713	0.088	
	30	1.847	0.294	<0.01	0.402	0.054	<0.01
	60	1.947	0.047	NS	0.318	0.025	NS
Brain	0	0.791	0.079		0.121	0.026	
	30	0.459	0.062	<0.001	0.078	0.009	NS
	60	0.475	0.059	NS	0.073	0.008	NS

clearly observed that the level of cytosolic MDH is significantly higher as compared to mitochondrial MDH in all tissues at different postnatal ages studied.

Aspartate aminotransferase (AsAT)

Specific activities (Units per mg protein) of cytosolic aspartate aminotransferase (c-AsAT) are significantly higher at day 30 in liver, kidney and heart tissues of male chicken as compared to day 0 and day 60 (Table 3 & 4). In the brain, however, this isoenzyme shows a higher specific activity at day 0 and decrease significantly at day 30 while remains unchanged thereafter. The mitochondrial aspartate aminotransferase (m-AsAT), on the other hand, shows a significant increase in activity at day 30 in the liver (Fig. 5) and kidney tissues (Fig. 6) as compared to day 0 and 60, whereas a slight decrease at day 30 of heart and brain tissues studied (Fig. 7 & 8, respectively). From all the tissues studied, data exhibit a significantly higher activity of cytosolic AsAT as compared to mitochondrial AsAT at all the postnatal ages as observed in case of MDH isoenzymes.

Hormonal regulation of malate-aspartate shuttle enzymes :

Effects of hydrocortisone (HC) and triiodothyronine (T_3)

The effects of hydrocortisone (HC) and triiodothyronine (T_3) were studied on the activity of the shuttle enzymes in the liver and kidney of chicken at three different postnatal ages (0-, 30- and 60-day) in order to understand the involvement of these hormones in regulating the levels of the enzymes of this shuttle. Maximum response of the shuttle isoenzyme was obtained with a repeated dose of 1 mg per 100 g body weight of hydrocortisone and 25 µg per 100 g body weight of triiodothyronine for three days, respectively.

Hydrocortisone administration causes a significant increase in the activity (Units per mg protein) of c-MDH in the liver of chicken at day 30 (+23%) and day 60 (+32%) (Fig. 9; Table 5) while it increases significantly the mitochondrial MDH at day 0 (+141%)in the liver (Fig. 10; Table 5) In kidney it does not influence the c-MDH activity (Fig. 11; Table 6). The activity level of mitochondrial MDH, however, increases significantly at day 30 (+102%) in kidney (Fig. 12; Table 6). On the other hand, administration of hydrocortisone does not show any influence on the cytosolic and mitochondrial aspartate aminotransferase in either

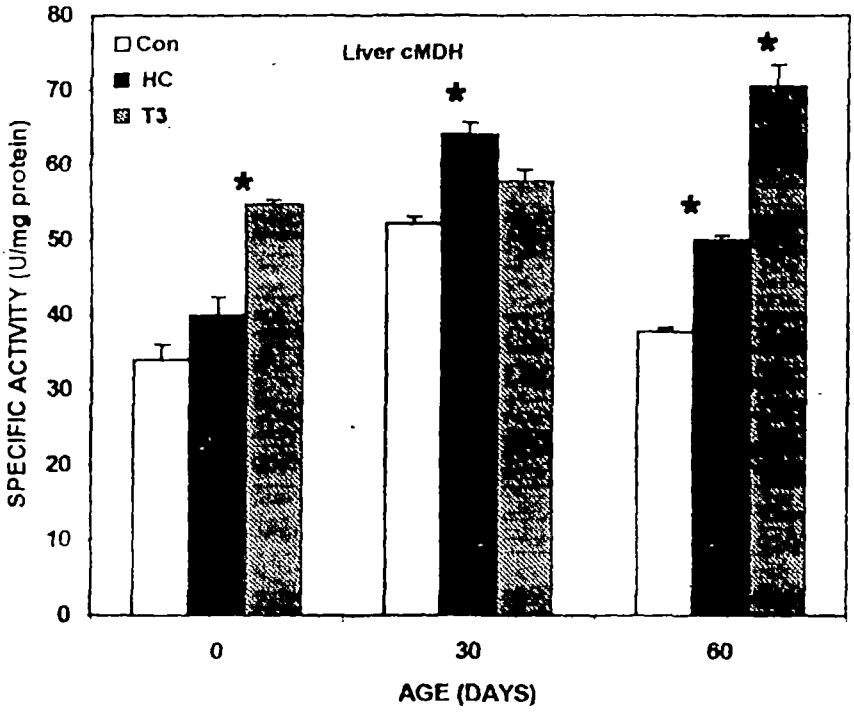


Figure 9. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of cytosolic malate dehydrogenase isoenzyme in the liver of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation. Asterisks (*) exhibit statistically significant values as compared to control

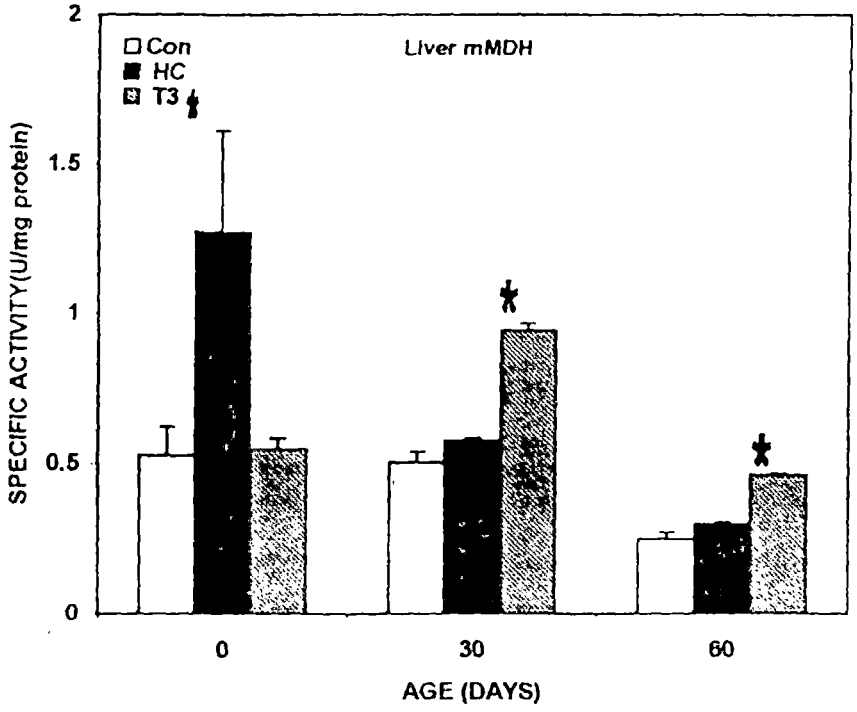


Figure 10. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of mitochondrial malate dehydrogenase isoenzyme in the liver of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation. Asterisks (*) exhibit statistically significant values as compared to control.

Table 5. Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity (U/mg protein) of malate dehydrogenase isoenzymes in the liver of male chicken of different postnatal ages.

MDH	Treated	0- DAY				30- DAY				60- DAY			
		Mean	SD	p	%	Mean	SD	p	%	Mean	SD	p	%
c	Normal	34.01	1.96			52.13	0.93			37.73	0.45		
	HC	39.96	2.34	NS		64.18	1.49	<0.001	+23	49.98	0.58	<0.001	+32
	T_3	54.72	0.68	<0.001	+60	57.81	1.58	NS		70.58	2.76	<0.001	+87
m	Normal	0.53	0.09			0.50	0.04			0.25	0.02		
	HC	1.27	0.34	<0.01	+141	0.58	0.01	NS		0.29	0.008	NS	
	T_3	0.55	0.04	NS		0.94	0.02	<0.001	+88	0.46	0.004	<0.001	+87

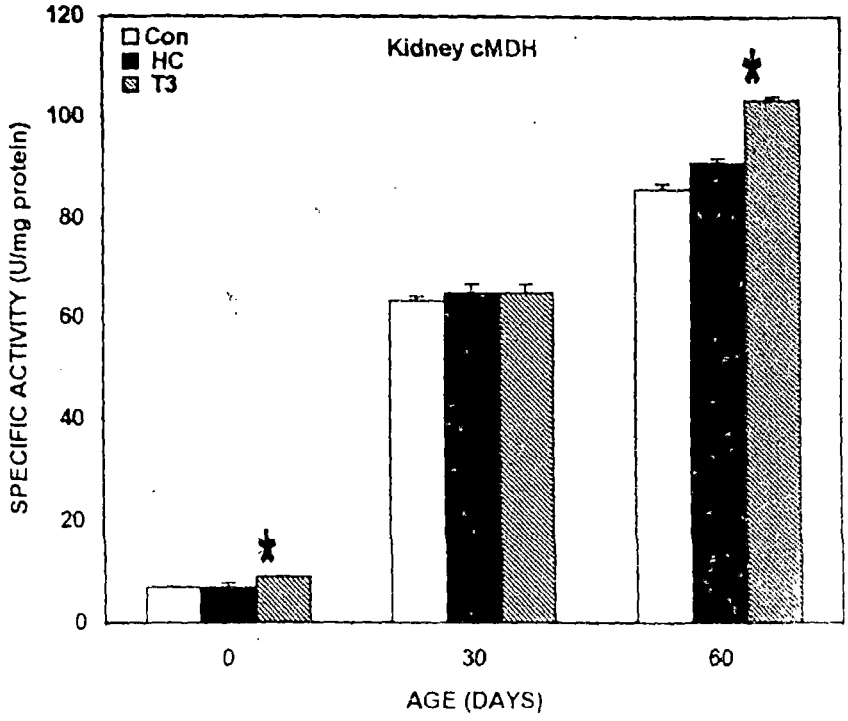


Figure 11. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of cytosolic malate dehydrogenase isoenzyme in the kidney of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation. Asterisks (*) exhibit statistically significant values as compared to control.

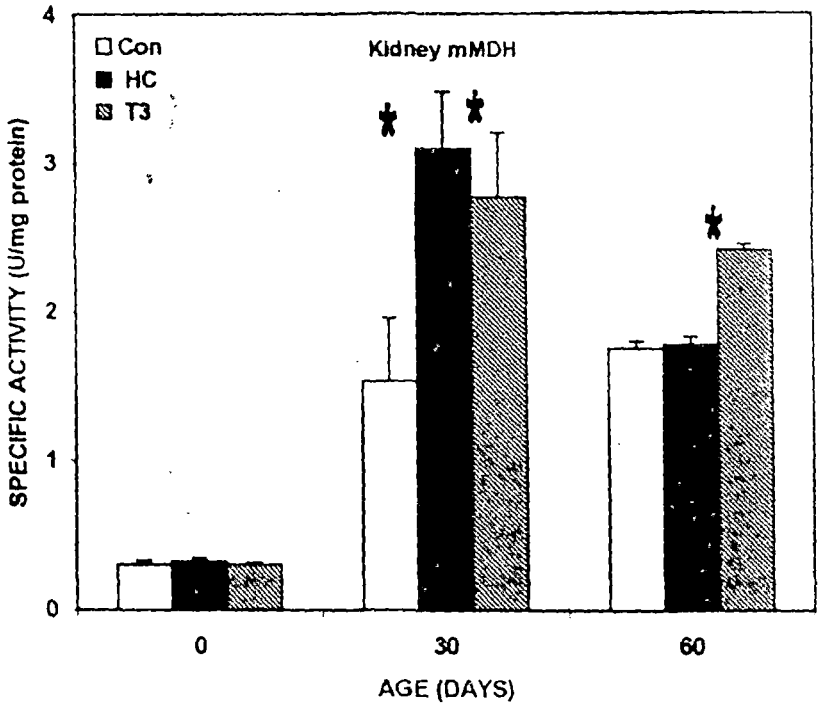


Figure 12. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of mitochondrial malate dehydrogenase isoenzyme in the kidney of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation. Asterisks (*) exhibit statistically significant values as compared to control.

Table 6. Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity (U/mg protein) of malate dehydrogenase isoenzymes in the kidney of male chicken of different postnatal ages.

MDH	Treated	0 -Day				30 -Day				60 -Day			
		Mean	SD	p	%	Mean	SD	p	%	Mean	SD	p	%
c	Normal	6.658	0.19			63.19	0.94			85.65	1.17		
	HC	6.721	0.80	NS		64.89	1.76	NS		90.88	0.9	NS	
	T_3	8.671	0.31	NS		64.93	1.74	NS		103.4	0.73	<0.001	+20
m	Normal	0.297	0.03			1.53	0.42			1.752	0.046		
	HC	0.319	0.02	NS		3.09	0.38	<0.01	+102	1.776	0.049	NS	
	T_3	0.298	0.01	NS		2.77	0.43	<0.02	+80	2.414	0.043	<0.001	+37

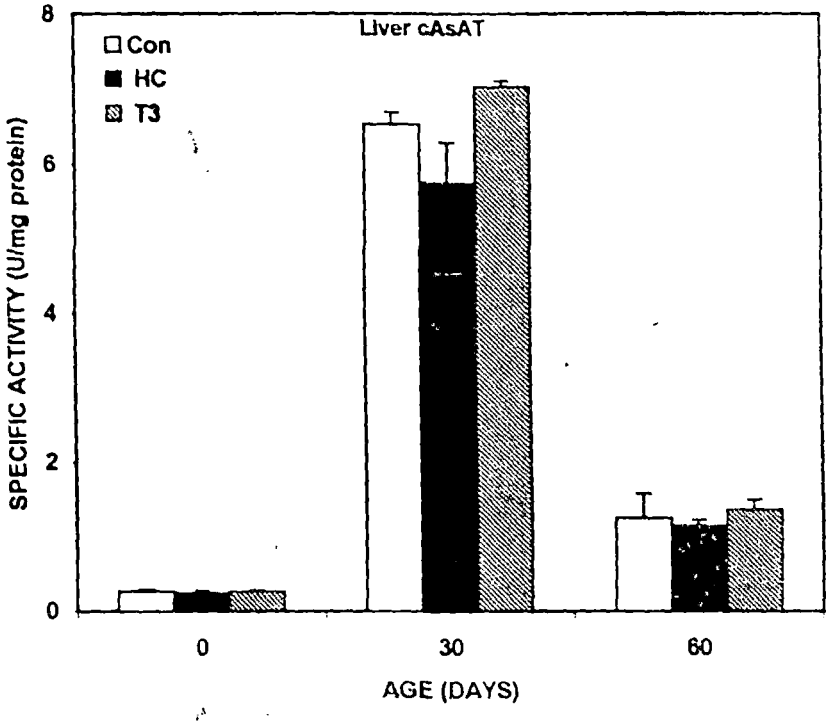


Figure 13. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of cytosolic aspartate aminotransferase isoenzyme in the liver of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation.

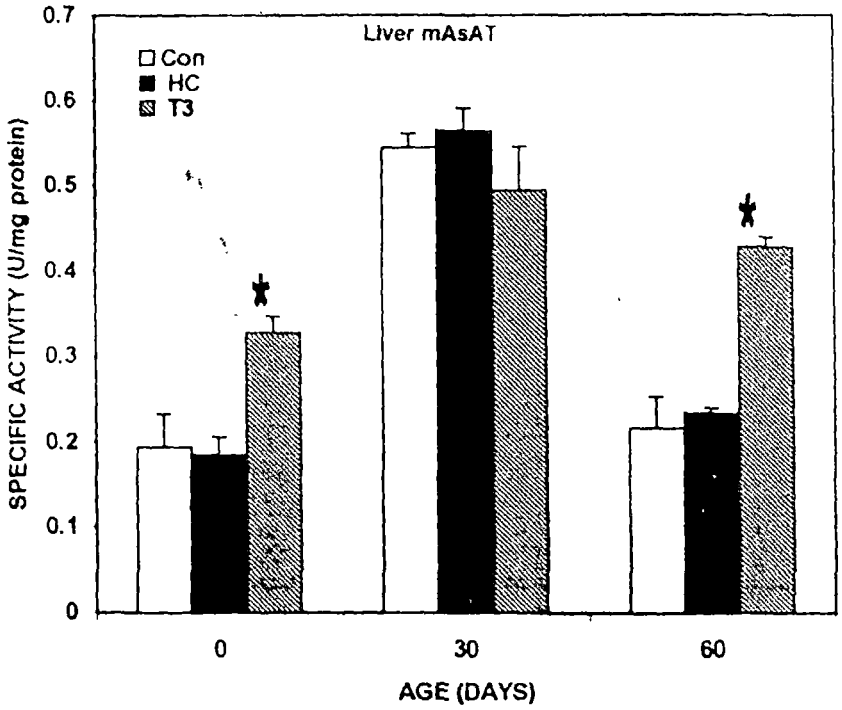


Figure 14. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of mitochondrial aspartate aminotransferase isoenzyme in the liver of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation. Asterisks (*) exhibit statistically significant values as compared to control.

Table 7. Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity (U/mg protein) of aspartate aminotransfearse isoenzymes in the liver of male chicken of different postnatal ages.

As AT	Treated	0 -Day				30 -Day				60 -Day			
		Mean	Sd	p	%	Mean	Sd	p	%	Mean	Sd	p	%
c	Normal	0.268	0.03			6.53	0.15			1.25	0.33		
	HC	0.246	0.04	NS		5.75	0.53	NS		1.15	0.08	NS	
	T_3	0.271	0.01	NS		7.03	0.08	NS		1.36	0.14	NS	
m	Normal	0.194	0.04			0.54	0.02			0.22	0.04		
	HC	0.185	0.02	NS		0.57	0.03	NS		0.24	0.006	NS	
	T_3	0.328	0.02	<0.001	+69	0.52	0.05	NS		0.43	0.001	<0.001	+98

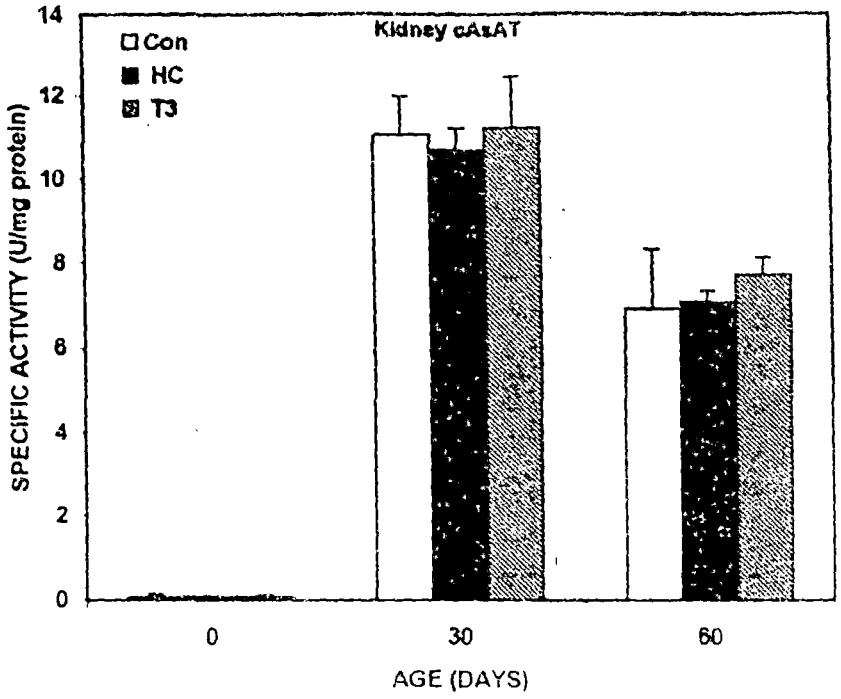


Figure 15. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of cytosolic aspartate aminotransferase isoenzyme in the kidney of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation.

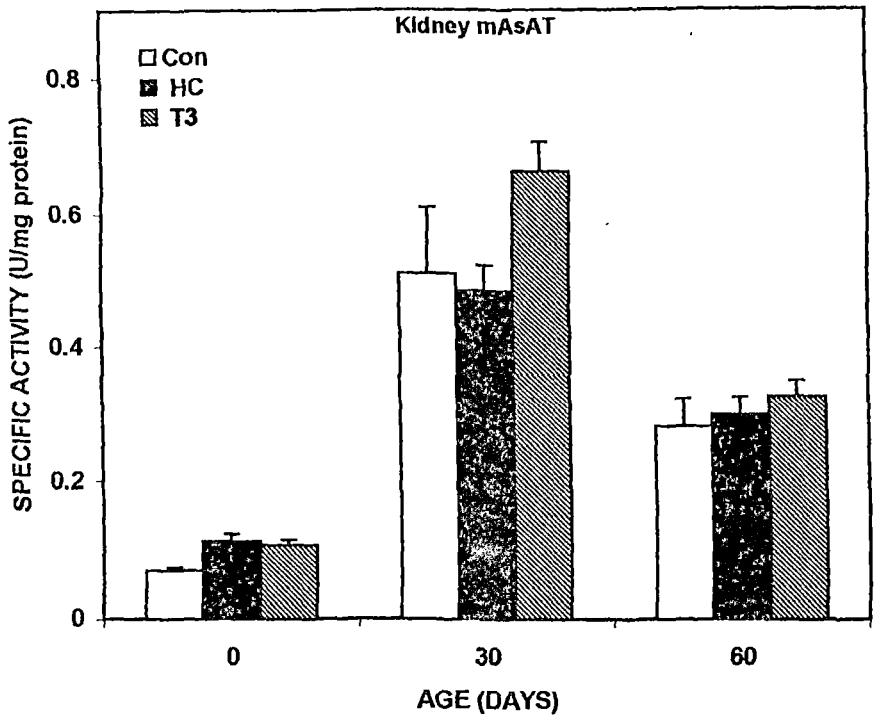


Figure 16. Effects of hydrocortisone (HC) and Triiodothyronine (T_3) on the activity of mitochondrial aspartate aminotransferase isoenzyme in the kidney of male chicken of different postnatal ages. Hormone treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation.

Table 8. Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity (U/mg protein) of asparate aminotransferase isoenzymes in the kidney of male chicken of different postnatal ages.

As AT	Treated	0 -Day				30 -Day				60 -Day			
		Mean	Sd	p	%	Mean	Sd	p	%	Mean	Sd	p	%
c	Normal	0.079	0.07			11.05	0.92			6.9	1.41		
	HC	0.082	0.02	NS		10.68	0.52	NS		7.07	0.25	NS	
	T_3	0.091	0.04	NS		11.21	1.24	NS		7.72	0.419	NS	
m	Normal	0.069	0.005			0.51	0.09			0.28	0.041		
	HC	0.112	0.01	NS		0.49	0.38	NS		0.29	0.026	NS	
	T_3	0.107	0.008	NS		0.66	0.04	NS		0.33	0.025	NS	

the liver (Fig. 13 & 14; Table 7) or kidney (Fig. 15 & 16; Table 8) tissues at any of the postnatal ages studied.

Effects of triiodothyronine were also studied on the activities of MDH and AsAT isoenzymes in the liver and kidney of chicken at three different postnatal ages (0, 30, and 60 day). Administration of triiodothyronine significantly increases the activity of cMDH in the liver at 0 day (+60%) and at 60 day (+87%) whereas, it increases significantly the m-MDH at day 30 (+88%) and day 60 (+87%) of liver (Fig. 9 & 10; Table 5). The activity of c-MDH , however, is moderately influenced by triiodothyronine in the kidney only at day 60, whereas, it increases m-MDH activity significantly at day 30 (+80%) and day 60 (+37%), respectively (Fig. 11 & 12; Table 6). This hormone, however, does not induce c-AsAT in both the liver and kidney tissues at these postnatal ages. However, it increases m-AsAT of liver at day 0 (+69%) and day 60 (+98%) only with no significant change in the kidney isoenzyme at any of the postnatal ages studied (Fig. 13 –16; Table 7 & 8).

Reconstitution of malate-aspartate shuttle:

In order to confirm the differential expression of malate – aspartate shuttle enzymes in the liver and kidney of chicken, the shuttle activity in a reconstituted system was studied. Reconstitution of the malate-aspartate shuttle showed a higher activity (oxidation of NADH as measured by decrease in absorbance at 340 nm) in the liver of 30-day-old chicken compared with that of 0-day-old chicks (Fig. 17). The reconstituted shuttle also showed a notably higher activity in the kidney of 30-day-old chicken as well (Fig. 18).This clearly indicates a greater activity of malate-aspartate shuttle and its enzymes at day 30 in both the liver and kidney tissues of chicken.

Reconstituted shuttle activity was also studied from hormone treated chicken to confirm the induction and regulation of the shuttle enzymes by such treatments. Reconstituted malate-aspartate shuttle showed a higher activity in T_3 treated chicken as compared with that of control untreated male chicken at both the ages studied (Fig. 19 & 20). Similar results were also obtained from the kidney of T_3 treated and untreated control chicken of day 0 and 30 (Fig. 21 & 22).

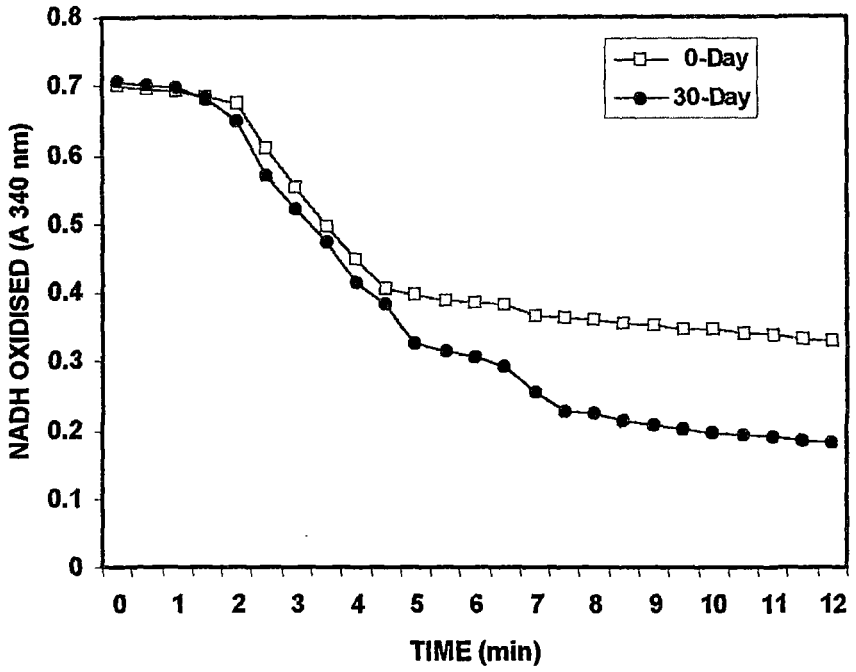


Figure 17. Oxidation of NADH by reconstituted malate-aspartate shuttle from the liver of male chicken. Equal amounts of dialysed clear cytosol (2 mg protein) and mitochondria (1 mg protein) from both the ages (0- and 30-day) were used for the reconstitution assay. Details of the experimental procedures are given in methods section. The traces depict the change in absorbance at 340 nm with the passage of time.

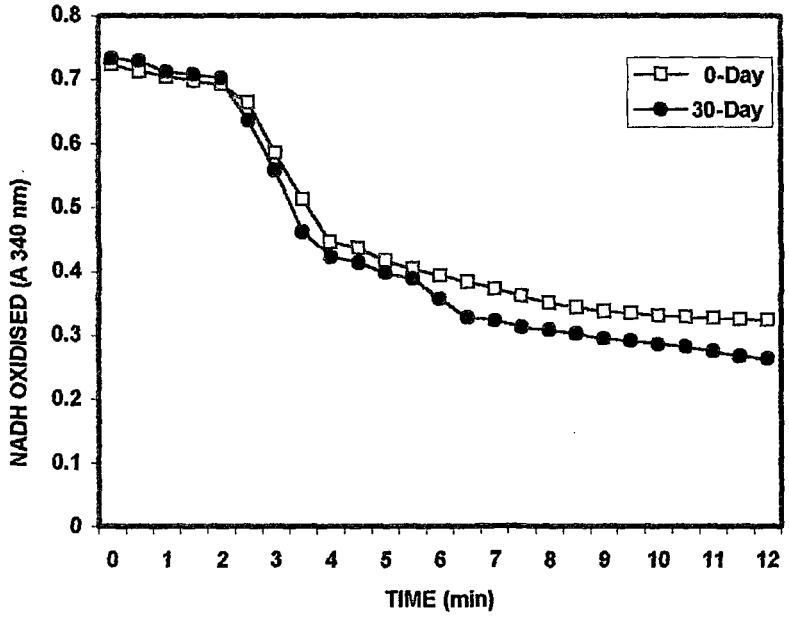


Figure 18. Oxidation of NADH by reconstituted malate-aspartate shuttle from the kidney of male chicken. Equal amounts of dialysed clear cytosol (2 mg protein) and mitochondria (1 mg protein) from both the ages (0- and 30-day) were used for the reconstitution assay. Details of the experimental procedures are given in methods section. The traces depict the change in absorbance at 340 nm with the passage of time.

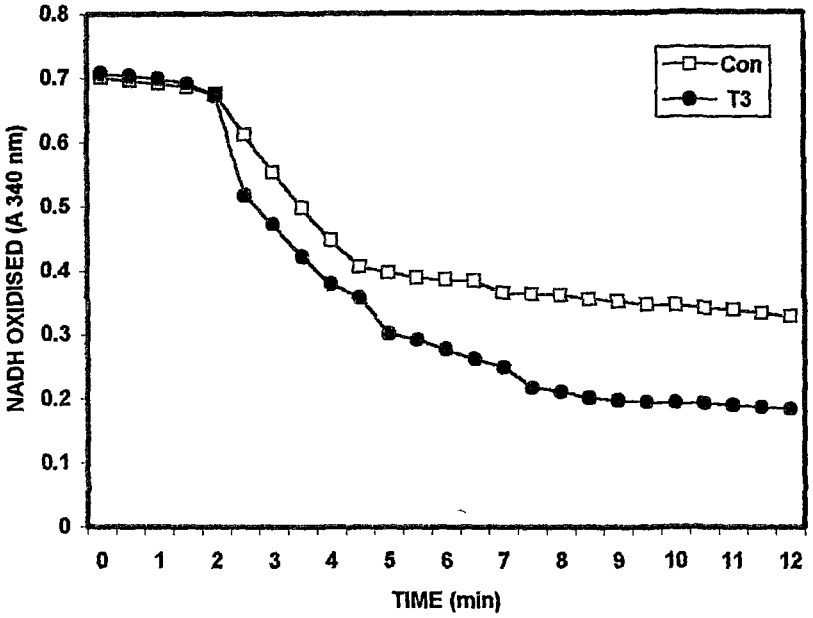


Figure 19. Oxidation of NADH by reconstituted malate-aspartate shuttle from the liver of control and T_3 treated 0-day old male chicken. Equal amounts of dialysed clear cytosol (2 mg protein) and mitochondria (1 mg protein) from both the control and T3 treated 0-day old male chicken were used for both the reconstitution assay. Details of hormone treatment and other experimental procedures are given in methods section. The traces depict the change in absorbance at 340 nm with the passage of time.

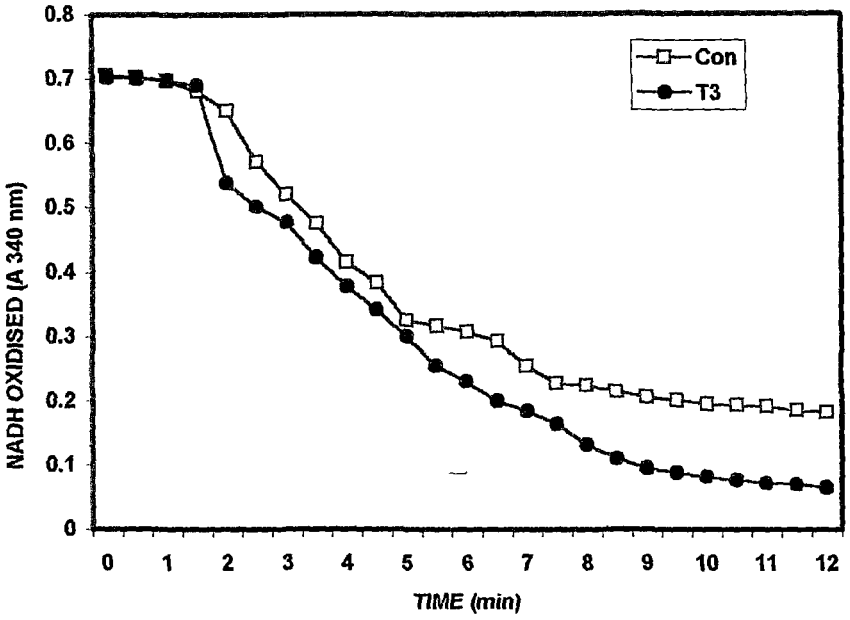


Figure 20. Oxidation of NADH by reconstituted malate-aspartate shuttle from the liver of control and T_3 treated 30-day old male chicken. Equal amounts of dialysed clear cytosol (2 mg protein) and mitochondria (1 mg protein) from both the control and T3 treated 30-day old chicken were used for the reconstitution assay. Details of the experimental conditions are same as given for Figure 19. The traces depict the change in absorbance at 340 nm with the passage of time.

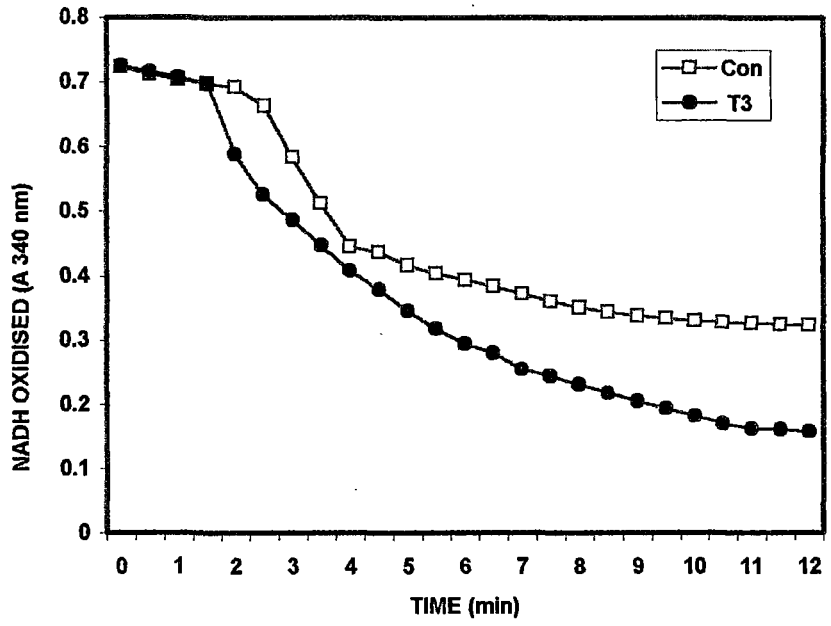


Figure 21. Oxidation of NADH by reconstituted malate-aspartate shuttle from the kidney of control and T_3 treated 0-day old male chicken. Equal amounts of dialysed clear cytosol (2 mg protein) and mitochondria (1 mg protein) from both the control and T3 treated 0-day old male chicken were used for both the reconstitution assay. Details of hormone treatment and other experimental procedures are given in methods section. The traces depict the change in absorbance at 340 nm with the passage of time.

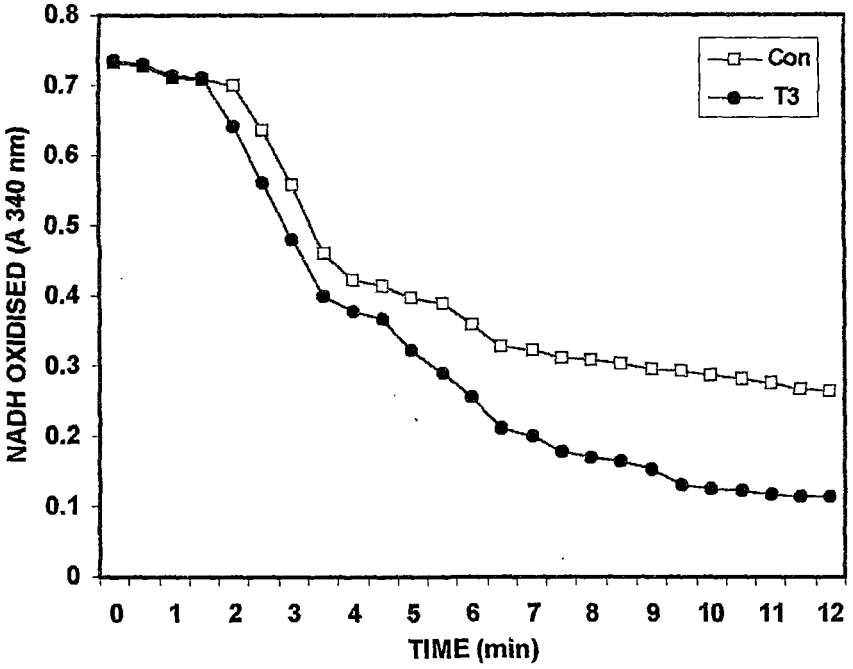


Figure 22. Oxidation of NADH by reconstituted malate-aspartate shuttle from the kidney of control and T_3 treated 30-day old male chicken. Equal amount of dialysed clear cytosol (2 mg protein) and mitochondria (1 mg protein) from both the control and T3 treated 30-day old male chicken were used for the reconstitution assay. Details of the experimental conditions are same as given for Figure 21. The traces depict the change in absorbance at 340 nm with the passage of time.

Isolation and purification of liver c-AsAT:

Using similar experimental conditions, one of the shuttle enzymes, that is, cytosolic aspartate aminotransferase (c-AsAT) was isolated and purified from the liver of chicken of two selected ages, 0 day - and 60 day old chicken. Table 9 and 10 represent the purification protocol of c-AsAT from the liver of 0 day and 60 day old chicken, respectively. The degree of purification achieved was 52- and 41-folds for 0 day and 60 day old, chicken. The yield of purification was 34% and 28%, respectively for 0-and 60-day old chicken. The enzyme was partially purified from the two ages in order to compare the chemical and kinetic properties of this isoenzyme as a function of age. The elution profile of the specific activity of this isoenzyme from the liver of 0 day and 60 day old chicken on CM-Cellulose column is depicted in Fig. 23. c-AsAT isoenzyme from both the ages eluted at a similar ionic strength of 0.11 M sodium acetate buffer.

Polyacrylamide gel electrophoreses (PAGE) of purified c-AsAT:

Using non-denaturing polyacrylamide slab gel of 7.5% crosslinking, the preparation representing the 0-day and 60-day old chicken liver c-AsAT were electrophoresed. One half of the gel was stained for general protein by coomassie brilliant blue and the other half was stained for the enzyme by specific staining of the gel. On the gel stained for general proteins, one major and one or two minor bands could be localized in both the 0-day-old and 60-day-old chicken (Fig. 24 A). Simultaneous staining of the gel after electrophoresis on the other half with specific staining, showed the presence of a single band, exhibiting c-AsAT activity in case in both the ages of chicken (Fig. 24 B). These single bands on specific stain for cAsAT of both the ages correspond with the major band on the gels stained for general proteins. Hence, there is no difference in the rate of migration of this isoenzyme on the acrylamide gel between the two age groups.

Kinetic properties of purified c-AsAT:

The c-AsAT activities of the purified preparation from the liver of two age groups (0-day-old and 60-day-old) were studied at varying concentrations of substrates, that is, L-aspartate and α-ketoglutarate using the normal assay procedure. Data obtained were analysed, computed for K_m, V_{max} and K_{cat} using the enzfitter programmes (Table 11). The figures were

Table 9. Purification protocol of cytosolic aspartate aminotransferase (cAsAT) from the liver of 0-day old chicken.

Fractions	Total Volume (ml)	Total Activity (Units)	Total protein(mg/ml)	Specific Activity (Unit/mg protein)	Purification fold	Yield (%)
Supernatant at 14,000 x g	85	470.28	1383.17	0.34	–	100
First ammonium sulphate fractionation (40%)	80	424.96	566.61	0.75	2.20	90
Second ammonium sulphate fractionation (80%)	13	418.55	240.54	1.74	5.12	89
Dialysis	21	326.42	122.25	2.67	7.85	69
CM Cellulose	27	207.67	14.95	13.89	40.85	44
Third ammonium sulphate fractionation (80%) and Dialysis	8	163.71	9.26	17.68	52.00	34

Table 10. Purification protocol of cytosolic aspartate aminotransferase (cAsAT) from the liver of 60-day old chicken.

Fractions	Total Volume (ml)	Total Activity (Units)	Total protein(mg/ml)	Specific Activity (Unit/mg protein)	Purification fold	Yield (%)
Supernatant at 14,000 x g	85	535.19	1070.38	0.50	–	100
First ammonium sulphate fractionation (40%)	81	449.69	569.23	0.79	1.58	84
Second ammonium sulphate fractionation (80%)	14	398.05	219.92	1.81	3.62	74
Dialysis	23	354.04	123.36	2.87	5.74	66
CM Cellulose	26	178.74	10.58	16.89	33.78	33
Third ammonium sulphate fractionation (80%) and Dialysis	8	151.02	7.45	20.28	40.56	28

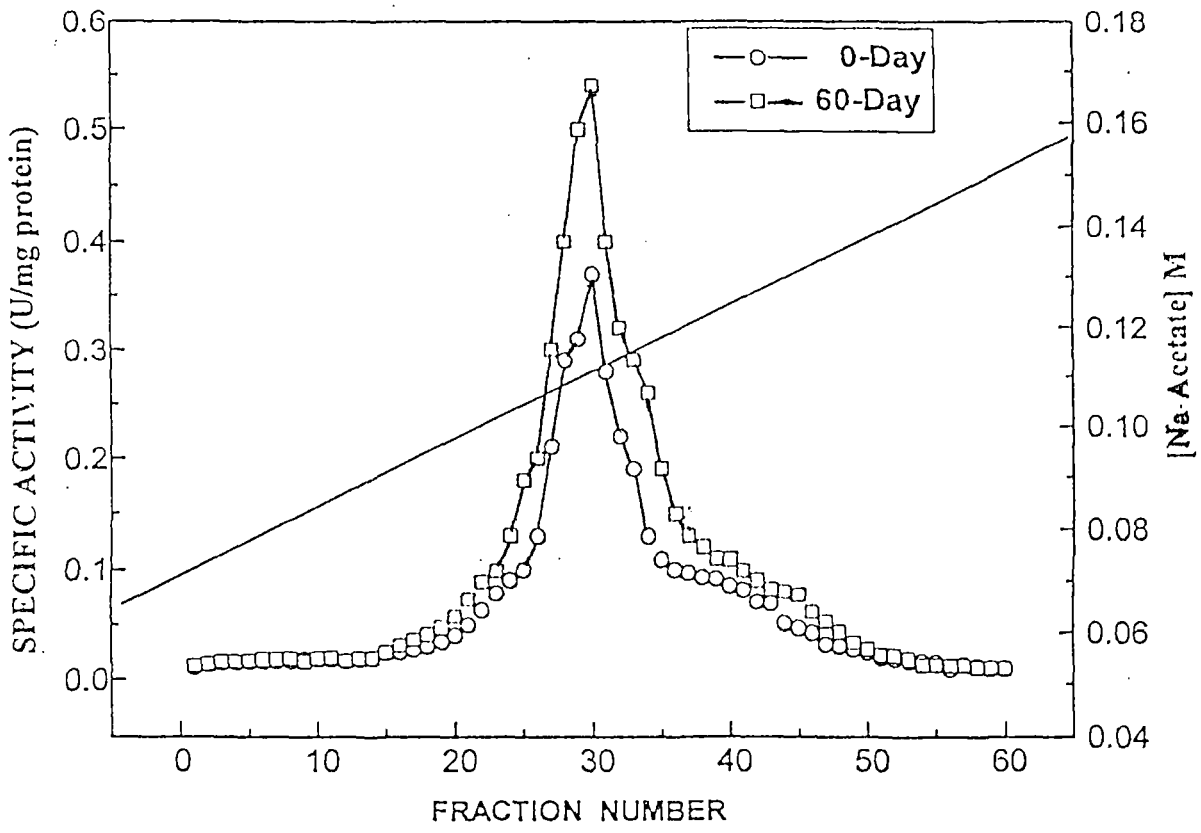


Figure 23. Elution profile of cytosolic aspartate aminotransferase (cAsAT) from the liver of 0-day and 60-day old chicken through CM-Cellulose ion exchange. Details of experimental conditions are described in method section. cAsAT was eluted applying linear gradient of sodium acetate buffer (0.06-0.16 M).

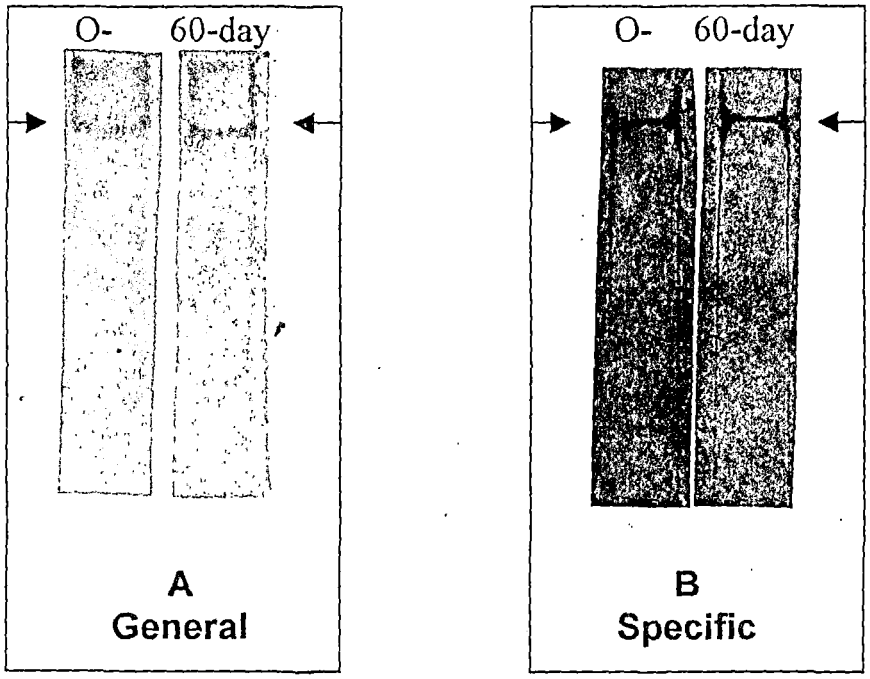


Figure 24. Polyacrylamide gel electrophoreses of purified c-AsAT from the liver of 0-day and 60-day old male chicken.

A. General protein stain using Coomassie brilliant blue (R-250).

B. Specific stain for c-AsAT as detailed in method section.

drawn using Michaelis-Menten equation and the Lineweaver-Burk transformation for the same.

Effect of [L-aspartate] on purified c-AsAT:

Fig. 25 (A&B) and 26 (A&B) show the Michaelis-Menten and Lineweaver-Burk plots of the data for the effect of [L-aspartate] on the activity of purified c-AsAT from the liver of 0-day and 60-day old chicken. The K_m values of liver c-AsAT for L-aspartic acid are 4.59 mM and 4.90 mM for 0-day and 60-day old chicken, respectively (Table 11) indicating no significant difference between the K_m values for [L-aspartate] at these ages of chicken. The V_{max} and K_{cat} values of the isoenzyme are 0.125 µmol/min and 0.129 sec^{-1} for 0-day-old and 0.126 µmol/min and 0.13 sec^{-1} for 60-day-old respectively. These values indicate no significant difference in turnover rates compared between the two ages (Fig. 25 A&B and 26 A&B).

Effect of α-ketoglutarate on purified c-AsAT:

Fig. 27 (A&B) and 28 (A&B) exhibit the Michaelis-Menten and Lineweaver-burk plots of the data for the effect of [α-ketoglutarate] on the activity of c-AsAT from the liver of 0-day and 60-day old chicken. The K_m values of liver c-AsAT for α-ketoglutarate are 0.268 mM and 0.275 mM for 0 day and 60 day old chicken, respectively (Table 11). It indicates no notable difference between the K_m values for [α-ketoglutarate] in both the ages studied. The V_{max} and K_{cat} values of the enzyme are 0.12 µmol/min and 0.127 sec^{-1} for 0-day and 0.124 µmol/min and 0.13 sec^{-1} for 60-day, indicating no marked differences in turnover rates between the two ages (Fig. 25 A&B and 26 A&B).

Effect of [amino-oxyacetic acid] on purified c-AsAT:

Figures 29 and 30 show the Dixon's plots of the data for the effect of aminooxyacetic acid (AOAA) on the activity of purified c-AsAT from the liver of 0-day and 60-day old chicken respectively, at two different concentrations of L-aspartate (10 mM & 40 mM) and α-ketoglutarate (1 mM & 2 mM). Table 12 shows the K_i values of liver c-AsAT for AOAA with respect to L-aspartate and α-ketoglutarate from both the ages of chicken. These values

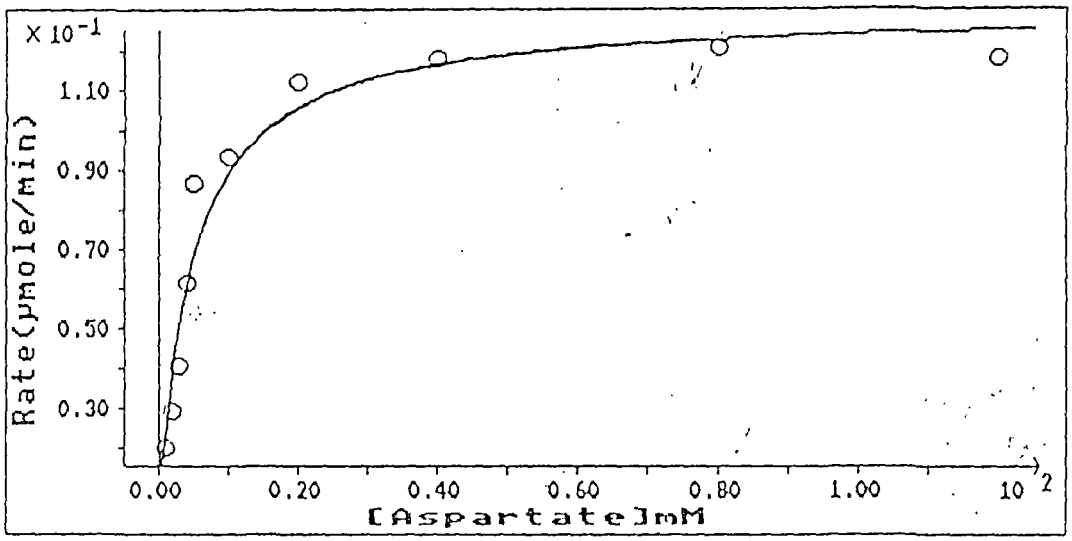


Figure 25 (A). Michaelis-Menten plot for cytosolic aspartate aminotransferase from the liver of 0-day old chicken with respect to aspartate as variable substrate. Data are computed and drawn using enzfitter programme of Sigma.

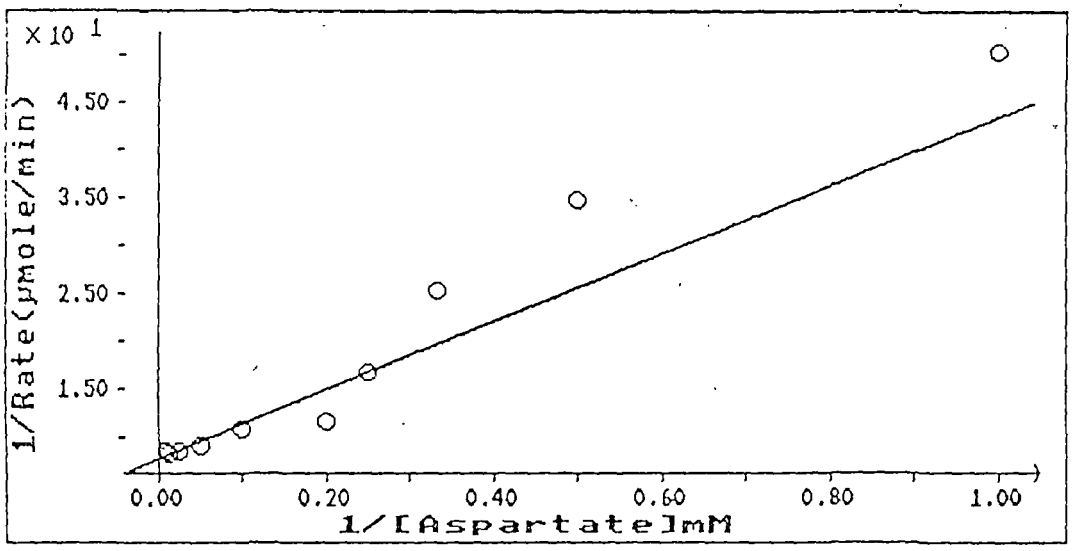


Figure 25 (B). Lineweaver-Burk plot of the same.

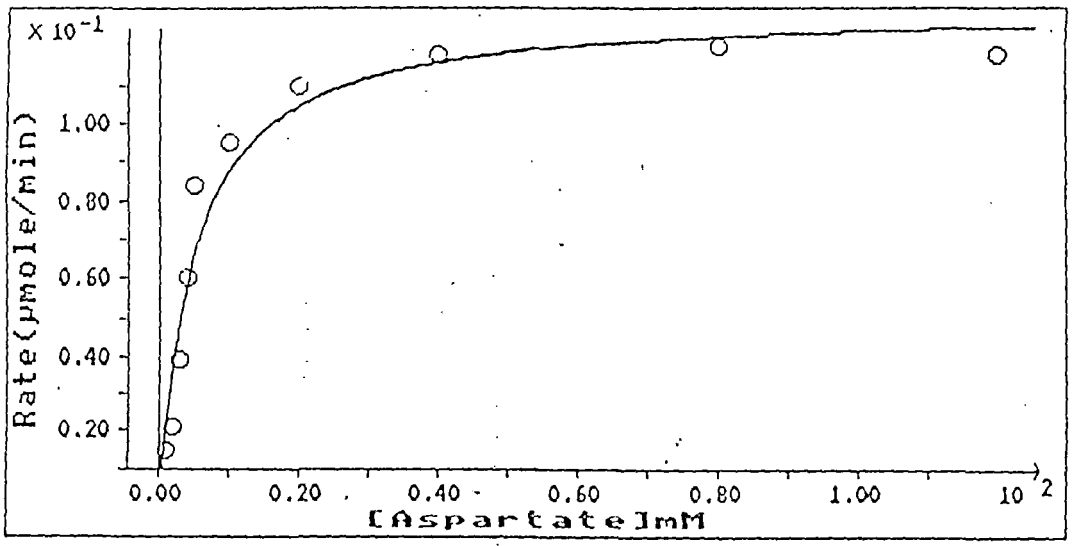


Figure 26 (A). Michaelis-Menten plot for cytosolic aspartate aminotransferase from the liver of 60-day old chicken with respect to aspartate as variable substrate. Data are computed and drawn using enzfitter programme of Sigma.

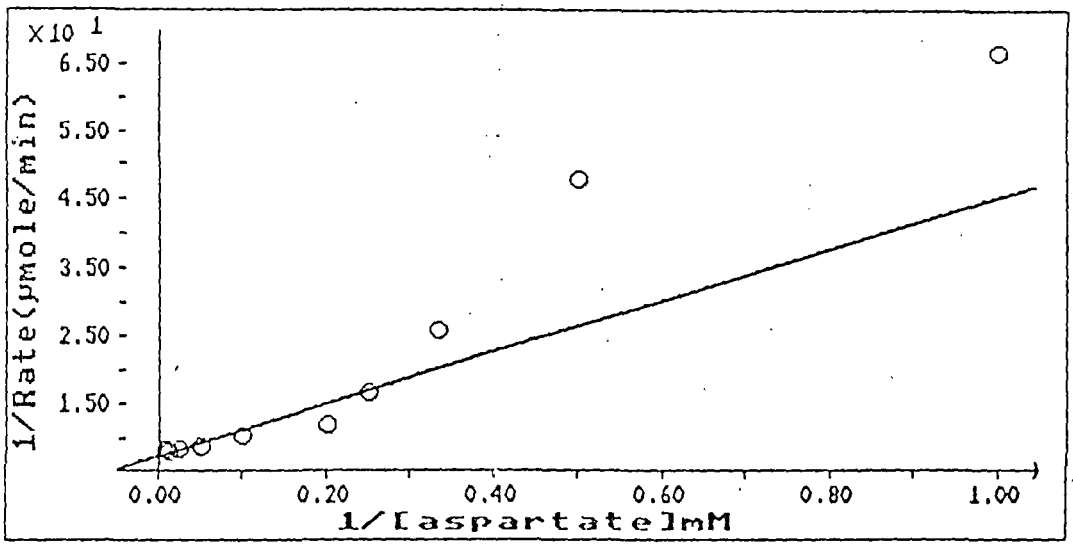


Figure 26 (B). Lineweaver-Burk plot of the same.

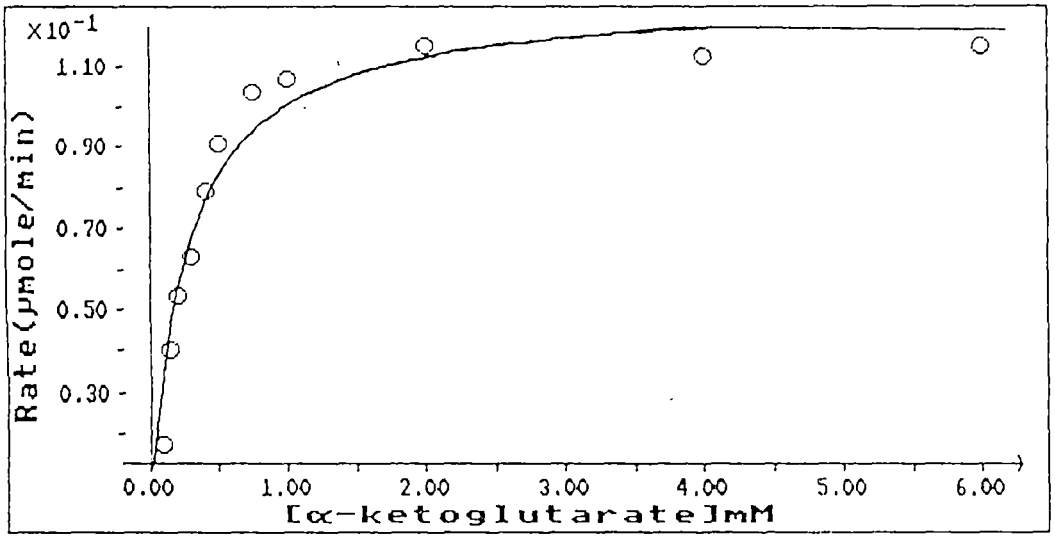


Figure 27 (A). Michaelis-Menten plot for cytosolic aspartate aminotransferase from the liver of 0-day old chicken with respect to α-ketoglutarate as variable substrate. Data are computed and drawn using enzfitter programme of Sigma.

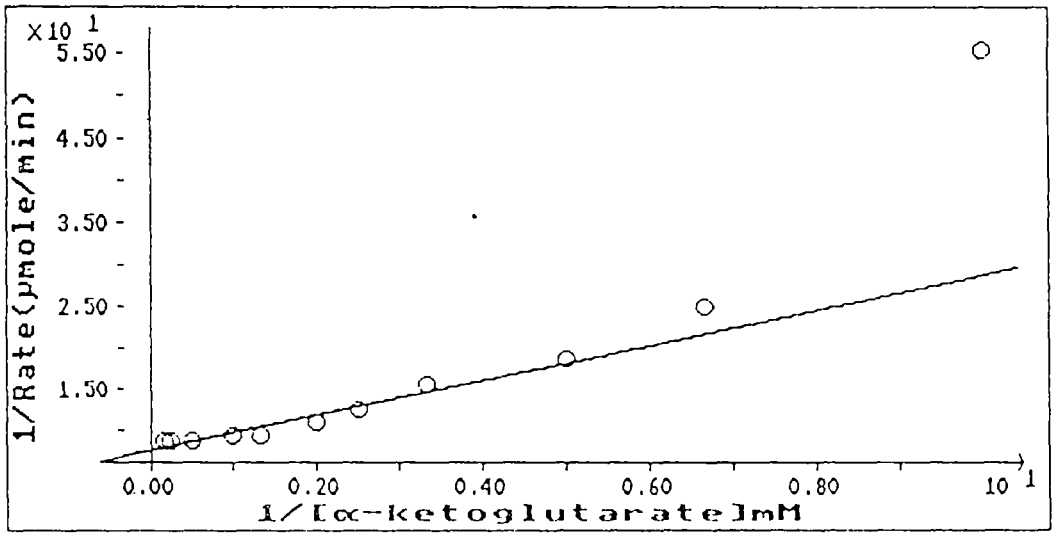


Figure 27 (B). Lineweaver-Burk plot of the same.

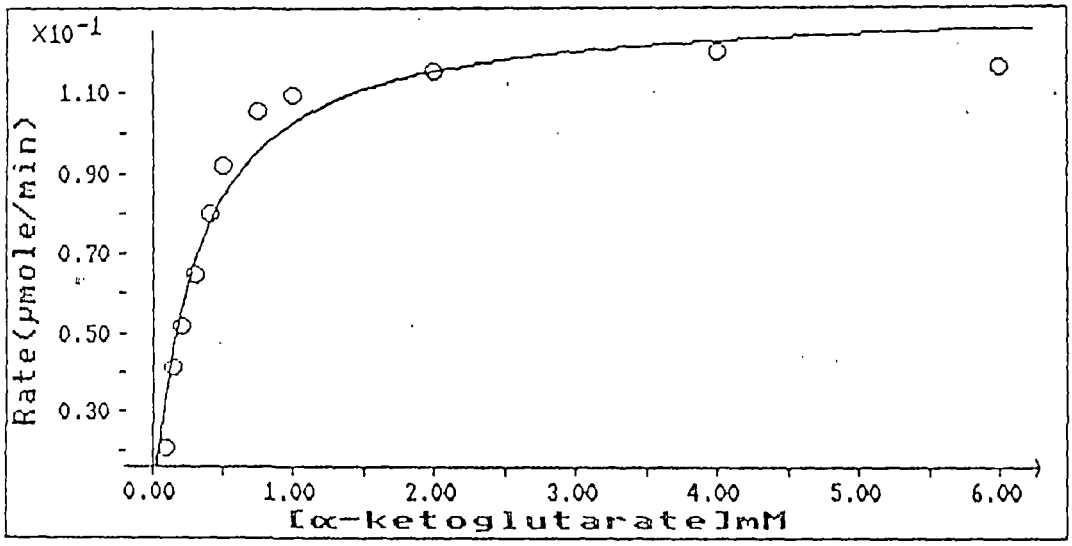


Figure 28 (A). Michaelis-Menten plot for cytosolic aspartate aminotransferase from the liver of 60-day old chicken with respect to α-ketoglutarate as variable substrate. Data are computed and drawn using enzfitter programme of Sigma.

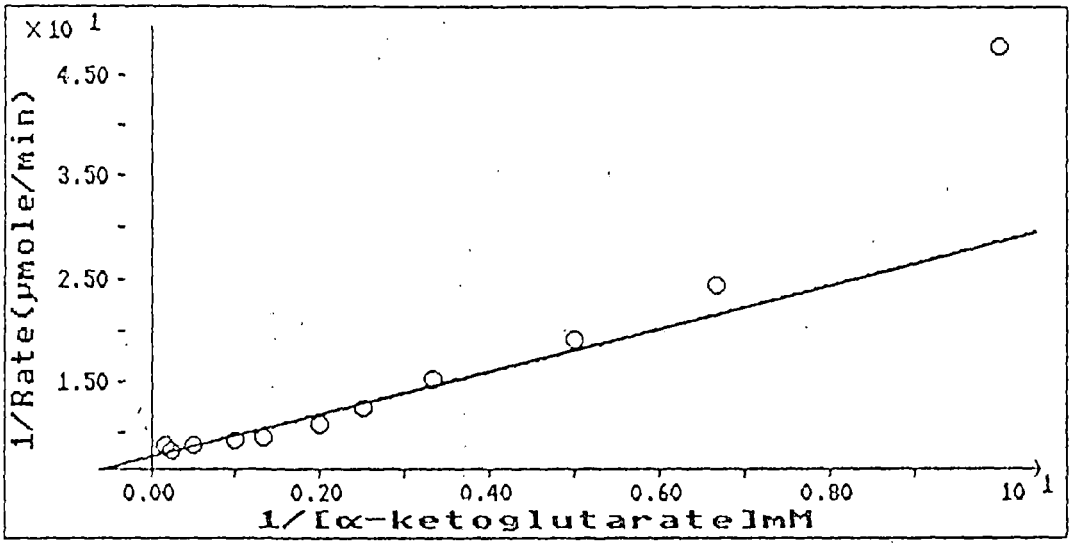


Figure 28 (B). Lineweaver-Burk plot of the same.

Table 11. Kinetic data of purified cytosolic aspartate aminotransferase from two different ages.

AGE (Days)	PARAMETERS	ASPARTATE (substrate)	α-KETOGLUTARATE (substrate)
0	K_m (mM)	4.590	0.268
	V_{max} (μmole/min)	0.125	0.120
	K_{cat} (sec^{-1})	0.129	0.127
60	K_m (mM)	4.900	0.275
	V_{max} (μmole/min)	0.126	0.124
	K_{cat} (sec^{-1})	0.130	0.130

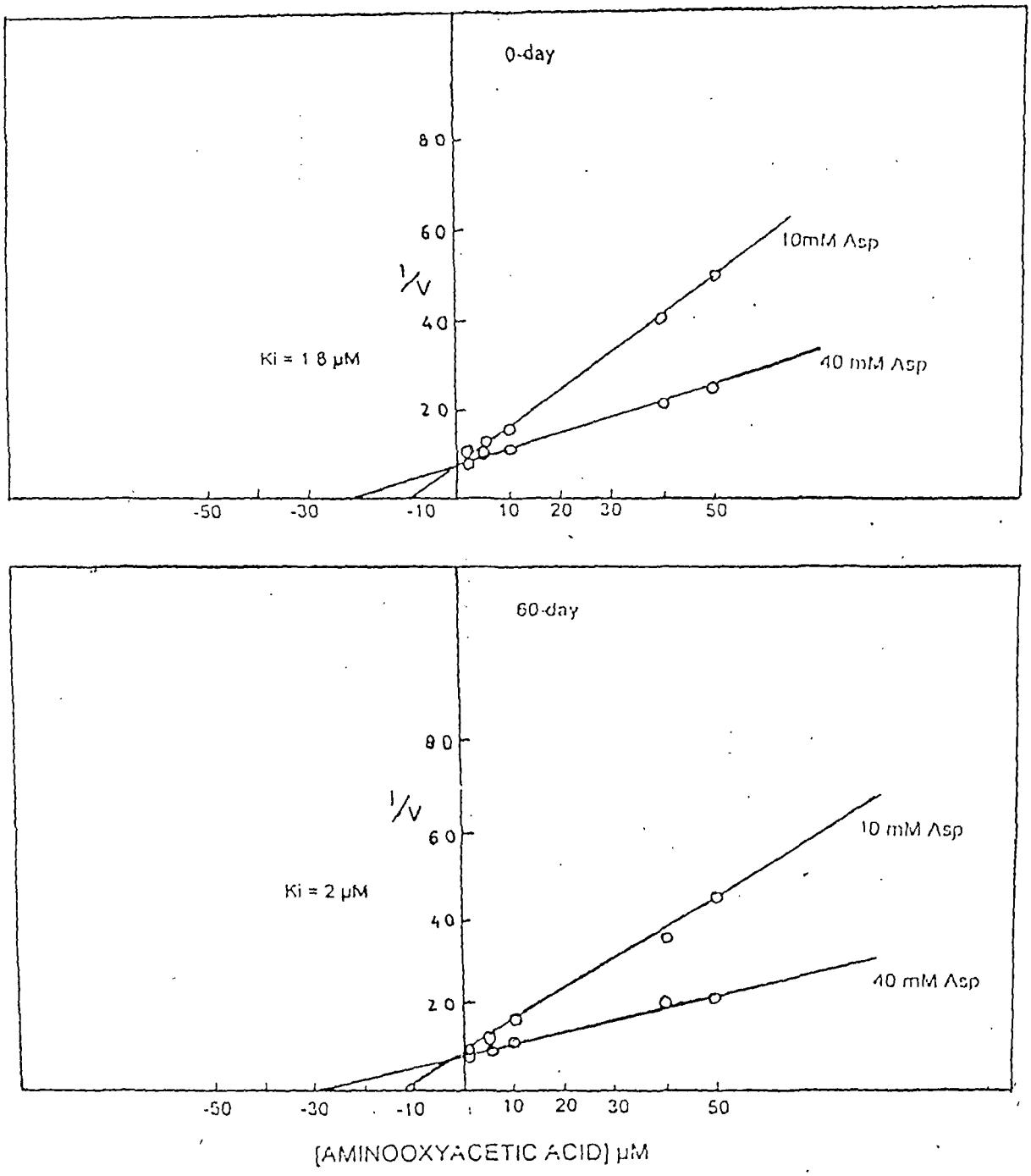


Figure 29. Inhibition of cytosolic aspartate aminotransferase from the liver of 0-day and 60-day male chicken by aminooxyacetic acid with respect to aspartate. (DIXON'S PLOT)

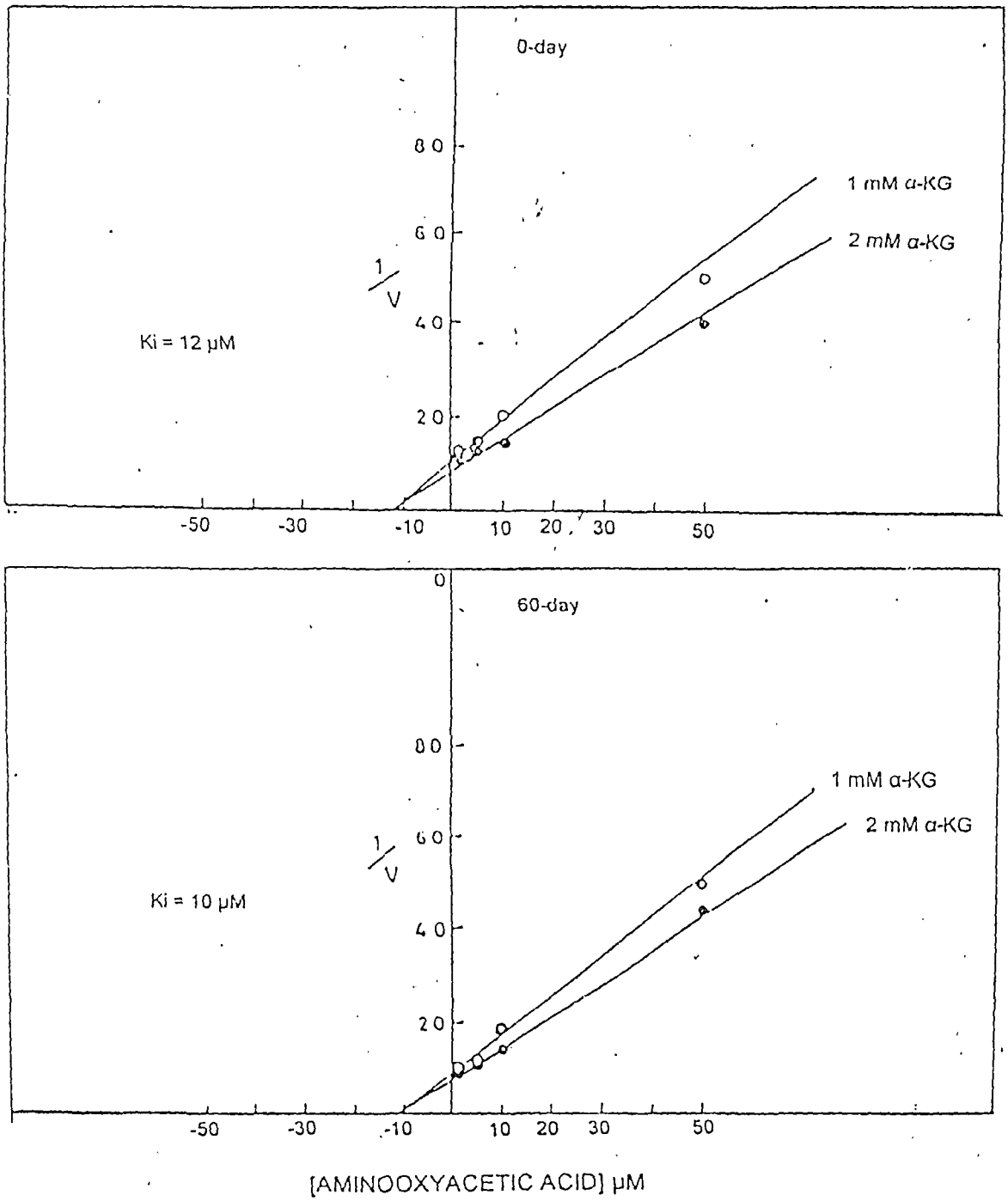


Figure 30. Inhibition of cytosolic aspartate aminotransferase from the liver of 0-day and 60-day male chicken by aminooxyacetic acid with respect to α-ketoglutarate. (DIXON'S PLOT)

Table 12. Kinetic properties of purified cytosolic aspartate aminotransferase from two different ages showing inhibition by aminooxyacetic acid (AoAA).

AGE (Days)	SUBSTRATES	K_m(mM)	K_I (μM)
0	L-aspartate	4.590	1.80
	α -ketoglutarate	0.268	12.0
60	L-aspartate	4.9	2.0
	α -ketoglutarate	0.275	10.0

are 1.8 μM (for 0-day old) and 2 μM (for 60-day old) with respect to L-aspartate and 12 μM (for 0-day old) and 10 μM (for 60-day old) with respect to α-ketoglutarate.

Studies on urea denaturation of purified c-AsAT:

Comparisons of the result of inactivation studies using different concentrations of urea on the purified liver c-AsAT of 0-day and 60-day old chicken were performed. c-AsAT isoenzymes from both 0-day and 60-day old chicken achieved 50% inactivation (IC_{50}) at 2 M urea indicating that there is no apparent difference between the ages for urea denaturation of cAsAT(Fig. 31).

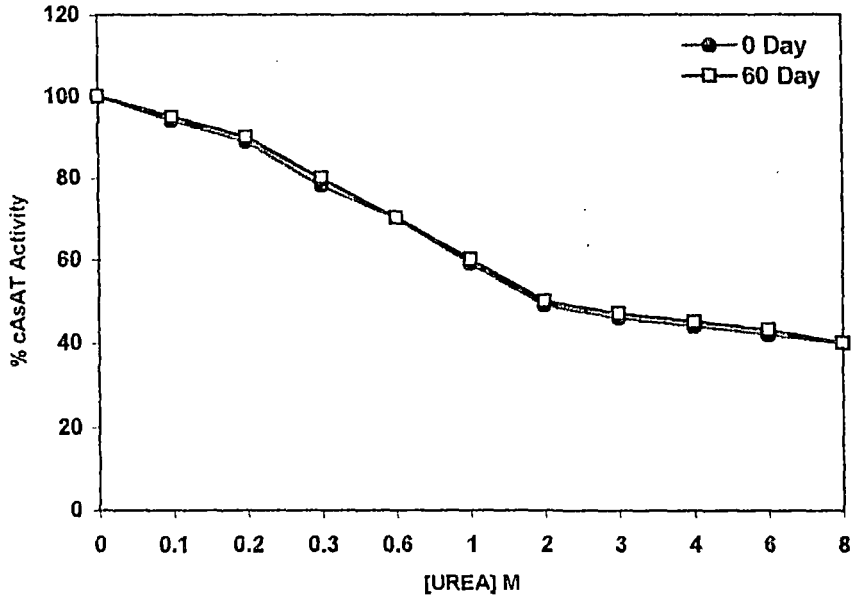


Figure 31. Inactivation profile for cytosolic aspartate aminotransferase from the liver of 0-day and 60-day old chicken, using varying concentration of urea. Values are expressed in per cent residual activity.

DISCUSSION

Intricate regulatory mechanisms have evolved in living cells to control the concentration of various enzymes that catalyze critical reactions. Enzymes are specific proteins that catalyze chemical reactions in biological systems. Enzymes are the direct phenotypic expression of their genes. The feature which characterizes all developing and aging populations is the progressive development and gradual impairment in the ability to adapt to the environmental changes. Adaptation at the biochemical level can be attributed to the alterations in the rates of synthesis and degradation of enzymes as well as changes in physiological activities. During development and aging, different metabolic adjustments take place as an adaptation in different tissues to the changing demands made upon them.

Several physiological and biochemical changes occur during development, growth, adulthood and senescence of an organism. The development includes an increase in the number and size of cells, their differentiation to perform specialized functions and formation of organs. The metabolic events that occur during development might influence the later part of life span. During development, several new proteins appear, indicating the expression of their cognate genes. The level of proteins changes as cells differentiate and organs formed, exhibiting changes in the expression of corresponding genes.

Study of all the enzymes of a particular metabolic pathway provides a complete profile of the biological functions as a function of age. Considerable amount of literature has developed concerning changes in enzyme level as a function of age and has been well reviewed (Wilson, 1972; Sharma, 1988, 1994). Keeping in view the importance of studying all the enzymes of a particular metabolic cycle, the work embodies in this thesis was planned to study the developmental and hormonal regulation of malate aspartate shuttle enzymes in different tissues of chicken during postnatal development. As there are set differences in the metabolic requirement of different groups of organisms, it was important to find out the malate aspartate shuttle regulation in chicken in order to compare and contrast with mammalian malate-aspartate shuttle.

The most active NADH shuttle for the movement of reducing equivalents (in the form of NADH) from the cytoplasm to the mitochondria in various tissues is the malate-aspartate

shuttle (McDonald, 1983). The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxalacetate to yield malate by the action of cytosolic malate dehydrogenase (c-MDH). The malate carrying reducing equivalents enters into mitochondrial matrix wherein it transfers them to the matrix NAD^+ regenerating oxalacetate by the action of mitochondrial malate dehydrogenase (m-MDH). The shuttle involves an influx of malate and glutamate and an efflux of aspartate and α-ketoglutarate from the mitochondria. Oxalacetate is a physiologically important intermediate of several metabolic pathways. These pathways may either be catabolic (Krebs cycle) or anabolic (gluconeogenesis) in nature. Since oxalacetate is impermeable to mitochondrial membrane, the malate-aspartate shuttle appears to be the primary mode of clearance of NADH reducing equivalents from the cytosol to mitochondria and vice-versa.

The enzymes of malate-aspartate shuttle are the malate dehydrogenase and the aspartate aminotransferase constituting of two homologous and genetically independent isoenzymes localized in the cytosol (c-) and mitosol (m-) fraction of several animal tissues (Boyd, 1961; Braunstein, 1973). The cytosolic isoenzymes of both MDH and AsAT are also implicated in gluconeogenesis, since the former converts malate and the later aspartate to oxalacetate, which is then converted to phosphoenolpyruvate. The functional significance of malate-aspartate shuttle also unfolds the degree of control points for glycolysis, gluconeogenesis and Krebs cycle. Although gluconeogenic reactions are similar in all organisms, the metabolic context and the regulations of the pathway differ from organism to organism, and from tissue to tissue (Lehninger et al., 1993). The major gluconeogenic precursor in chicken liver is lactate instead of pyruvate. The crucial gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK), found predominantly as a cytosolic enzyme in rats and mice, is localized in mitochondria of chicken liver and kidney (Ogata et al., 1982). Oxalacetate produced in mitochondria is converted directly to phosphoenolpyruvate (PEP) by a mitochondrial PEPCK. The PEP is then transported out of mitochondria and serves onto the gluconeogenic pathway. On the other hand, development encompasses programmed processes which occur by sequential activation and repression of genes. The programme of sequential activation and repression of genes, which are responsible for differentiation and development may continue after maturity and regulate the form and function of the organism. Enzymes are known to constitute a regulatory mechanism which is necessary to coordinate a complex series of reactions in the body. The internal milieu within the cell may change during the course of development and aging; contiguous with this change, the activities of several enzymes may also undergo physical or functional alteration.

Keeping in view the differences in metabolic make up in different groups of organisms, the present thesis describes the regulatory changes in the enzymes of malate-aspartate shuttle during postnatal development to get insight into such metabolic cycle as a function of age. In order to study the regulatory changes in the shuttle, the findings are grouped as:

(i) the endogenous activity levels of shuttle enzymes in different postnatal ages and their tissue-specific patterns.

(ii) Regulation of shuttle enzymes by various hormones such as glucocorticoid and thyroid hormone during postnatal development of chicken

(iii) Reconstitution studies of the malate-aspartate shuttle system to confirm the endogenous level and hormonal regulation of enzymes, and

(iv) Chemical and kinetic properties of one of the shuttle isoenzymes (cytosolic aspartate aminotransferase) from the liver of two different ages (0- and 60-day) to find out change, if any, in such properties as a funtion of age.

Endogenous activities of malate-aspartate shuttle isoenzymes:

The activity of isoenzymes of malate dehydrogenase and aspartate amiotransferase in the male chicken exhibited tissue specificity as well as age-related changes. The activity (U/mg protein) was observed to be highest in the liver and kidney followed by a lower activity in the heart and brain tissues. The high activity of malate-aspartate shuttle enzymes in the liver and kidney tissue, compared to others may be ascribed to the fact that liver and kidney metabolic functions especially at these ages are not only dictated by local needs but also directed towards the control of other systems, therefore requiring higher inputs. Secondly, development and growth in general will require additional enhanced biosynthetic turnover. In avian system, hatching is followed by a prodigious growth rate (30-50 times their hatching weight) which is not exhibited by any other class of vertebrates and is induced and regulated by growth hormone (Ricklefs, 1983). Growth hormone (GH) level which is highest between 4-8 weeks and decreases by the 12^{th} week has earlier been reported in chickens (Harvey et al., 1977,'79). Fat levels are reported to increase during the growth period in aves, but there are marked differences between species. Greater activity of shuttle enzymes in the liver and kidney of chicken clearly indicate the higher role of malate-aspartate shuttle in these tissues compared to heart and brain tissues (Lyngdoh and Sharma, 2001). As compared to mammalian

shuttle, the chicken shuttle enzymes exhibit lower activity, indicating lower role of this shuttle in chicken for the transfer of reducing equivalents from the cytosol to mitochondria .

The endogenous activities of isoenzymes of malate dehydrogenase and aspartate aminotransferase show a significant change during postnatal development of chicken. The activities of cytosolic malate dehydrogenase (c-MDH) was significantly higher in the liver of day 30 as compared to day 0 and 60. In contrast, mitochondrial malate dehydrogenase (m-MDH) activity was higher at day 0 and 30 in the liver. However, both c- and m- MDH had significantly lower activities at day 0, which increased sharply at day 30 and 60 in the kidney. It however, did not exhibit any significant change in the heart and brain tissues at different postnatal ages studied, except a gradual increase in the cMDH activity in the heart tissue only. On the other hand, activity of both cytosolic and mitochondrial aspartate aminotransferase (c- and m- AsAT) showed a peak value at day 30 in both the liver and kidney. In the heart and brain, however, both the isoenzymes showed a steady decrease during postnatal development except c-AsAT of heart which increased in level at day 30 and 60 as compared to day 0. The higher level of cAsAT in the liver, kidney and heart at day 30 may be correlated with a higher degree of transamination during these phases in the life span of chicken. These findings indicate not much role of this shuttle at day 0 and implicate the involvement of malate-aspartate shuttle at a relatively advance age in the transfer of reducing equivalents to compensate the metabolic demands in these tissues of growing chicken . MDH isoenzymes showed a differential pattern of activity in the kidney as compared to liver at day 30 and 60 of postnatal ages studied. This is in agreement with the previous report that kidney has a unique role in gluconeogenesis in chicken (Watford et al., 1981). The MDH isoenzymes also showed a pattern of activity expression like that of asparate aminotransferase isoenzymes in all tissues studied. Earlier reports have shown that the rates of the mitochondrial and cytoplasmic enzyme must be equal for the steady operation of this shuttle (Wiseman et al., 1991). Sharma et al., (1992) have also reported an early expression of malate aspartate shuttle activity and its enzymes in the liver of mice as compared to kidney. The findings on chicken shuttle enzymes corroborate the metabolic differences in the chicken as compared to rats and mice. It also manifests an age- and tissue-specificity with respect to shuttle activity.

Hormonal regulation of shuttle enzymes:

Alterations in the level of enzymes and their inducibility by certain hormones are age related phenomenon (Wilson, 1972; Adelman, 1975; Kanungo, 1980; Sharma, 1988) Much interest focusing on the mechanisms of hormonal regulation involving feedback control, synergistic, reciprocal interactions and cross-talk in signal transduction appeared in recent reviews (Wada et al., 1990; Sharma, 1993). It must be emphasized that a change in the activity cannot be directly equated with a change in enzyme concentration and a number of other possible mechanisms exist to affect enzyme induction and/or repression (Walker, 1983; Sharma, 1994). During development, strong influence of growth hormones (GH) in aves has been well established and reviewed (Scannes et al.,1984; Harvey, 1990). Further, in aves, it has been shown that amongst the plethora of GH-responsive system, only changes in endocrine function appear to provide inhibitory feedback in GH regulation. The hormones involved are insulin, glucagon, corticosterone, somatostatin and triiodothyronine, exhibiting either synergistic and/or antagonistic interaction (Wada et al., 1990).

The results on hormonal studies indicated that there exists differential response to these hormones viv-a-vis age and tissue of chicken. At these ages (0-, 30- and 60-day) used in this study, avian tissues undergo marked growth spurt and are under complex hormonal control, with many hormones influencing development of different organs and tissues. The influence of GH, T_3 and steroid hormones in avian development and the morphological changes have been well documented (Freeman and Vince, 1974; Scannes et al., 1990; Harvey, 1990; Kuhn et al., 1991) together with their combinational effects. Further, the role of hormones in controlling growth and development and also in homeostasis has been envisaged (Scannes et al., 1990).

Administration of hydrocortisone (HC) increases the activity of c-MDH in the liver of chicken at all ages studied and increases the activity of m-MDH only at day 0 compared to other postnatal ages. The magnitude of increase of c-MDH at different postnatal ages is variable indicating that glucocorticoids do play a role in the regulation of this isoenzyme and the variability may be due to the endogenous level of glucocorticoid receptors and/or post-receptor events at different postnatal ages

(Bohme et al., 1986). It confirms earlier reports that injection of adrenal cortical extracts resulted in retardation of growth in chicken embryo is dependent on the stage of embryonic development. In kidney, however, hydrocortisone increased only the activity of m-MDH at day 30 compared to other postnatal ages, corroborating earlier reports in mammals that the same enzyme in different tissues of developing animals might be regulated differentially by the same physiological stimuli.The differential effect of hydrocortisone also takes place in different tissues of chicken during development. The gene responsible for the synthesis of c- and m-MDH are reported to be different (Whitt, 1970). The variation in inducibility may be due to differential responsiveness of genes of c- and m-MDH isoenzymes towards hydrocortisone. On the other hand, hydrocortisone administration does not seem to be involved in the regulation of AsAT isoenzymes in liver and kidney of chicken at all postnatal ages studied, indicating that AsAT may not be responsive to glucocorticoids in chicken. This is in contrast to the glucocorticoid regulation of c-AsAT in rats and mice (Sharma and Patnaik, 1982). It also reflects that the role of glucocorticoid in avian and mammalian systems may be different due to variability in their metabolic context (Lehninger, 1993). Non-inducibility of cAsAT isoenzyme by hydrocortisone a gluconeogenic inducer, in chicken liver and kidney indicate that this isoenzyme may not be involved in gluconeogenesis so much as in case of mammalian system. In rats and mice, cAsAT is induced at the transcriptional level by glucocorticoids as well as at enzyme level (Santa and Sharma, 1997). It is well documented that major gluconeogenic precursor in chicken is the lactate and not the pyruvate.

Thyroid and pituitary hormones are known to influence growth in chicken (Scanes et al.,1983). However, there are variable reports on attempts to stimulate growth in intact chicks with exogenous T_3 or T_4. For instance, growth of broiler chicken was not affected by T_4 administration in the diet, while growth was decreased by dietary T_3 (May, 1980, 1982; Scanes et al., 1983). In addition, studies made by feeding a dietary supplement of T_3 was observed to interact genotypically and combination of T_3 and growth hormone exerts variable growth promoting effects in different tissues (Marsh et al., 1984 a&b). Studies on growing broilers using long term thyroid hormone supplement resulted in the decrease of both growth and fat deposition, with T_3 being more effective than T_4 (Kuhn et al., 1991).

Results on the hormonal regulation by triiodothyronine (T_3) of shuttle enzymes demonstrate that T_3 increased the activity of both isoenzymes of MDH in both the tissues studied at different postnatal ages of chicken. Our findings also exhibit that the magnitude of increase in the activity of both isoenzymes of MDH is significantly higher at day 30 and 60 as compared to day 0 in both liver and kidney. This is in agreement with the earlier reports that the effects of thyroid hormones at the cellular level are slow to occur in chicken at early stage of life span (Hazelwood, 1986). The effect, however, does not influence isoenzymes of AsAT in liver and kidney except for an increase in m-AsAT level in 0-day and 60-day old chicken liver, corroborating that both the isoenzymes of AsAT are also genetically independent differing from one another even in different tissues for their response towards triiodothyronine. These studies indicate that factors like hormones and their adaptive responses to enzyme exhibit a tissue- and age-related patterns depending on the metabolic state of the concerned tissue at that phase of organism's life span.

Shuttle reconstitution studies:

In order to confirm the differential expression of malate-aspartate shuttle enzymes in the liver and kidney of chicken, the shuttle activity in a reconstituted system was studied. *In vitro* reconstitution of malate-aspartate shuttle showed a higher activity in the liver of 30-day old chicken compared with that in 0-day chicken. A similar pattern could also be seen in the kidney of 30-day old compared to that in 0-day chicks indicating that the shuttle activity corresponds similarly to the expression of enzymatic activities in the liver and kidney of developing chicken, exhibiting higher level at day 30 in both the tissues of chicken. This also indicates that the role of this shuttle may be at a slower pace in 0-day old chicks and boosts to a higher level during later part of postnatal development of chicken.

The activity of malate-aspartate shuttle in the liver of 0-day and 30-day old chicken also showed a higher activity in T_3-treated chicken as compared with control, untreated chicken. An identical pattern was also seen in the kidney of 0-day and 30-day reconstituted system confirming that the shuttle activity is induced upon T_3 administration in parallel with induction of enzymatic activities by T_3 treatment during postnatal development of chicken. These reconstitution studies confirm a

similar pattern of endogenous and hormonel treated activities of malate-aspartate shuttle enzymes in developing chicken in a similar way as seen in mice (Sharma et al., 1992).

Chemical and Kinetic properties:

In order to determine the change, if any, as a function of age in the chemical and kinetic properties of one of the shuttle enzymes, cytosolic aspartate aminotransferase was isolated and purified from the liver of chicken of two selected postnatal ages. One of the age (0-day) was selected as immature and the other as mature (60-day). The enzyme preparations from both the ages were passed through the CM-Cellulose column. Elution profile of the specific activities of this isoenzyme from the liver of two ages of chicken exhibited the requirement of the same ionic strength (0.11 M) of sodium acetate buffer. This indicates that there is no difference in the charge content of the enzyme molecule between the two different age groups. The degree and the yield of purification achieved are approximately similar for both the ages. It was further confirmed by non-denaturing polyacrylamide gel electrophoresis.

Using non-denaturing (Native) polyacrylamide slab gel of 7.5% cross-linking, the preparation representing the immature (0-day) and mature (60-day) cytosolic aspartate aminotransferase showed the presence of one major and one or two minor bands in both the cases, when the gels were stained for general proteins. However, when the gels were stained specifically for this enzyme, they showed the presence of a single band for both the ages of chicken. Further, this band corresponds to the major band obtained after staining for the general proteins. The mobility of this enzyme of both 0- and 60-day old chicken on acrylamide gel is similar. These observations also indicate that the net charges on the enzyme molecule of the liver does not alter as a function of age of chicken. It also corroborates with the earlier reports that the number of bands varied from tissue to tissue but the electrophoretic mobilities of a given form in all tissues were analogous (Imperial et al., 1989).

Kinetic studies on the purified c-AsAT, of the liver of two age groups (0-day and 60-day old) were carried out to elucidate kinetic changes, if any, which occur in the active site of enzyme molecule as a function of age. Both the 0- and 60-day old

chicken liver c-AsAT showed a hyperbolic curve when the velocity of cytosolic aspartate aminotransferase reaction was plotted against varying concentrations of both the substrates, that is, L-aspartate and α-ketoglutarate by using enzfitter programme (Perella, 1988). The figures were drawn using the Michaelis-Menten equation and the insets of these figures were drawn using the Lineweaver-Burk transformation. The plots indicate that these substrates do not exhibit allosteric effects on the enzyme activity. Analysis of data indicates no significance difference between the K_m, V_{max} and K_{cat} values of this enzyme for both the substrates in 0-day and 60-day old chicken liver. Albeit, the Km values were higher for α-ketoglutarate than for aspartate indicating that the affinity for L-aspartate is much more than for α-ketoglutarate at either ages. The c-AsAT from the liver of chicken of both the ages are competitively inhibited by amino-oxyacetic acid (AoAA) with respect to L-aspartate and non-competitively with respect to α-ketoglutarate. The K_i values of this enzyme for AoAA at 0- and 60-day old chicken are also similar. It is known that AoAA inhibits c-AsAT competitively with respect to its amino acid substrate and noncompetitively with respect to its keto acid sustrate in various groups of animals (Braunstein, 1973; Rej, 1977; Sharma and Patnaik, 1982). The aminotransferase inhibitor, AoAA, completely inhibited gluconeogenesis from lactate in the perfused rat liver and to a small extend in the perfused chicken liver. In chicken liver, the highest rate of glucose production was seen with lactate, followed by fructose, pyruvate, and glycerol (Sugano et al., 1982). Excess cytosolic reducing equivalents generated by the oxidation of lactate to pyruvate are transferred from the cytosol to the mitochondria by the malate-aspartate shuttle. Amino-oxyacetic acid inhibits the shuttle and, consequently, glucose synthesis for want of pyruvate (Ochs and Harris, 1980).

The kinetic parameters of a number of enzymes have been measured as a function of age. Studies on pyruvate kinase (Chainy and Kanungo, 1978) of the brain, myosin ATPase and aldolase of skeletal muscle, cytosolic alanine aminotransferase of liver, cytosolic aspartate aminotransferase of rat liver (Sharma and Patnaik, 1982) showed that, in general, there is no significant difference between K_m, K_i and molecular weight from young and old rats. Differences in the kinetic parameters of enzymes seen in older animals may be due to post translational modifications. This kinetic difference in the V_{max} and K_{cat} has been reported in immature and mature chicken liver inorganic pyrophosphatase (Syiem,

1996). The kinetic differences in the catalytic efficiency of enzyme without affecting the affinities for substrate have been attributed to adaptational significance depending on the age-specific metabolic demand in animal's tissues.

The present findings provide firm support to the view that the enzymes synthesized in 60-day old chicken are structurally similar to those of 0-day old chicken. Hence, the genes coding for these enzymes do not undergo any structural alterations during such period of chicken's life span. Therefore, the alterations in the levels and differential induction of these enzymes that occur as a function of age may be due to the changes in the template activities of the corresponding genes which are brought about by various modulators (Kanungo, 1980, '94).

Unfolding and inactivation of c-AsAT:

A comparison of the result of unfolding and inactivation studies using different concentrations of urea on the purified liver c-AsAT of immature and mature ages of chicken was performed. c-AsAT from the liver of both the ages depict no differential folded structure since urea requirement for 50% inactivation remained the same. These findings indicate that the molecular structure of this isoenzyme does not change at these two postnatal ages of chicken.

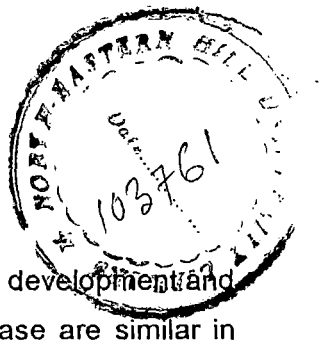
Taken together, it can be concluded that the malate-aspartate shuttle enzymes exhibit age- and tissue-specificity with respect to its activity expression and further the shuttle enzymes are also subjected to hormonal regulation in an age- and tissue-specific manner. Purification and kinetic analyses of cytosolic aspartate aminotransferase of the liver of 0-day and 60-day old chicken reveal that the structure of the enzyme molecule remains unaltered during such phases of chicken's life span. These findings indicate that factors like hormones and their adaptive responses to enzymes depend on the metabolic state of the concerned tissue at that phase of the organism's life span regulated by the level of hormones, their receptor/post-receptor events and tissue-specific factors needed for the expression of cognate genes in an organism-specific manner brought about by various extrinsic or intrinsic factors.

SUMMARY

Several complex regulatory mechanisms have been evolved in living cells to control the concentration of various enzymes that catalyse critical reactions. The feature which most probably characterizes all developing and aging populations is the progressive development and gradual impairment in the ability to adapt to the environmental changes. Adaptation at the biochemical level can be expressed as the alterations in the rates of synthesis and degradation of enzymes as well as changes in physiological activities. During development and aging, different metabolic adjustments take place as an adaptation in different tissues to the changing demand made upon them. Study of all the enzymes of a particular metabolic pathway provides a complete profile of the biological functions as a function of age. Keeping in view the importance of studying all the enzymes of a particular metabolic cycle, the work embodies in this thesis was planned to study the developmental and hormonal regulation of malate-aspartate shuttle enzymes.

Eukaryotic cells have evolved a number of shuttle systems that harvest electrons of cytosolic NADH for delivery to mitochondria without actually transporting NADH across the inner mitochondrial membrane. Various mechanisms of such transfer were proposed, of which, glycerol-phosphate and malate-aspartate shuttles are the most important. Malate-aspartate shuttle differs from glycerol-phosphate shuttle in that it is reversible and operates only if the $NADH/NAD^+$ ratio in the cytosol is higher. The shuttle system feeds electrons of cytosolic NADH into the electron transport chain present in the inner mitochondrial membrane. The malate-aspartate shuttle involves an influx of malate and glutamate, and an efflux of aspartate and α-ketoglutarate from mitochondria. The main enzymes of malate-aspartate shuttle are malate dehydrogenase (MDH) and aspartate aminotransferase (AsAT). Both these enzymes have two homologous and genetically independent isoenzymes: one in the cytosol and the other in the mitochondria.

Enzymes are specific proteins that catalyse chemical reactions in biological systems. Changes in the level and properties of these enzymes, particularly the enzyme



activity, may alter the functional ability of an organism, specially during development and aging. In aves, the cytosolic and mitochondrial malate dehydrogenase are similar in molecular weights, but are different in amino acid composition and immunological properties. The isoenzymes of aspartate aminotransferase also differ from one another in chemical, physical, catalytic and immunological properties. The cytosolic enzymes are important for gluconeogenesis, whereas the mitochondrial isoenzymes are involved in bioenergetics. Thus, this shuttle evolves the degree of control points for glycolysis, gluconeogenesis and krebs cycle.

The present study aims :

(a) to assess the endogenous activity level of shuttle enzymes in different tissues of chicken at various postnatal ages.

(b) regulation of shuttle enzymes by various hormones such as glucocorticoid and thyroid hormone during postnatal development of chicken

(c) reconstitution studies of the malate aspartate shuttle system to confirm the endogenous level and hormonal regulation of enzymes, and

(d) chemical and kinetic properties of one of the shuttle isoenzymes (cytosolic aspartate aminotransferase) from the liver of two different ages (0- and 60-day) to find out change, if any, in such properties as a funtion of age.

Endogenous level of shuttle enzymes :

The endogenous activities of isoenzymes of malate dehydrogenase and aspartate amonotransferase show a significant change during postnatal development of chicken. The activities of cytosolic malate dehydrogenase (c-MDH) was significantly higher in the liver of day 30 as compared to day 0 and 60. In contrast, mitochondrial malate dehydrogenase (m-MDH) activity decreased at day 60 in the liver. However, both c- and m-MDH had significantly lower activities at day 0, which increased sharply at day 30 and 60 in the kidney. It, however, did not show any significant change in the heart and brain tissues at different postnatal ages studied. On the other hand, activity of both cytosolic and mitochondrial aspartate aminotransferse (c- and m-AsAT) showed a peak value at day 30 in both the liver and kidney. In the heart and brain, however, it showed a decrease in the activity of mitochondrial aspartate aminotransferase but a slight increase in the cytosolic isoenzyme of the heart, with no significant change as compared to liver

and kidney. These findings indicate a late developmental expression of the shuttle enzymes, which implicate the involvement of malate- aspartate shuttle at a relatively advance age in the transfer of reducing equivalents to compensate the metabolic demands in these tissues of growing chicken. It also manifests an age and tissue specificity with respect to shuttle activity.

Hormonal regulation of shuttle enzymes:

Hormone administration to different posthatch chicken indicates that the shuttle enzymes may be hormonally regulated in an age- and tissue-specific manner. Administration of hydrocortisone increases the activity of c-MDH in the liver of chicken at all ages studied and increases the activity of m-MDH only at day 0 compared to other postnatal ages studied. The magnitude of increase of c-MDH at different postnatal ages is variable indicating that glucocorticoids do play a role in the regulation of this isoenzyme and the variability may be due to the endogenous level of glucocorticoid receptors and/or post-receptor events at different postnatal ages. Hydrocortisone administration in the kidney, however, increases only the activity of m-MDH at day 30 compared to other postnatal ages, corroborating that the same enzyme in different tissues of developing animals might be regulated differentially by the same physiological stimuli. The variation between c- and m-MDH may be due to the differential responsiveness of genes of c- and m-MDH isoenzymes in regulation by hydrocortisone. On the other hand, hydrocortisone administration does not seem to be involved in the regulation of AsAT isoenzymes in the liver and kidney of chicken at all postnatal ages studied, indicating that AsAT may not be responsive to glucocorticoids in chicken.

It is also seen that administration of triiodothyronine (T_3), which is a potent thyroid hormone, increased the activity of both isoenzymes of MDH in both the tissues studied at different postnatal ages of chicken. The magnitude of increase in the activity of both isoenzymes of MDH is significantly higher at day 30 and day 60 as compared to day 0 in both the liver and kidney indicating that the effects of thyroid hormones at the cellular level are slow to occur in chicken at early stage of lifespan. This hormone, however, does not influence isoenzymes of AsAT in the liver and kidney except for a slight increase in m-AsAT level in 0-day and 60-day old chicken liver, corroborating that both

the isoenzymes of AsAT are also genetically independent differing from one another even in different tissues for their responses towards triiodothyronine.

Shuttle reconstitution studies :

In order to confirm the differential expression of malate-aspartate shuttle enzymes in the liver and kidney of chicken, the shuttle activity in a reconstituted system was studied. *In vitro* reconstitution of malate-aspartate shuttle showed a higher activity in the liver of 30-day old chicken compared with that in 0-day chicken. A similar pattern could also be seen in the kidney of 30-day old compared to that in 0-day chicks indicating that the shuttle activity corresponds similarly to the expression of enzymatic activities in the liver and kidney of developing chicken.

The activity of malate-aspartate shuttle in the liver of 0-day and 30-day old chicken also showed a higher activity in treated chicken as compared with control, untreated chicken. An identical pattern was also seen in the kidney of 0 day and 30 day reconstituted system confirming that the shuttle activity also exhibits a similar pattern to the induction of enzymatic activities by hormonal treatment during postnatal development of chicken.

Chemical and kinetic properties:

In order to determine the change, if any, as a function of age, in the physico-chemical and kinetic properties, one of the shuttle isoenzymes, that is, cytosolic aspartate aminotransferasem (c-AsAT) was isolated and purified from the liver of chicken of two selected ages (0-day and 60-day) using similar experimental conditions. The isoenzyme from liver of both 0-day and 60-day old chicken was eluted from the CM-Cellulose column at the same ionic strength (0.11 M) of sodium acetate buffer. It appears that there is no difference in the charge content of the enzyme molecule between 0-day and 60-day old chicken. The degree and yield of purification achieved are approximately similar for both the ages. It was further confirmed by subjecting enzyme preparation for polyacrylamide gel electrophoresis and staining the gels with general and specific stains. The banding patterns of purified cAsAT showed similar

mobility at these two ages studied. Kinetic analyses show a hyperbolic curve when the velocity of cytosolic aspartate aminotransferase reaction was plotted against varying concentrations of both the substrates, that is, L-aspartate and α-ketoglutarate indicating that these substrates do not exhibit allosteric effects on the enzyme activity. K_m values of this enzyme show no significant difference for both the substrates in 0-day and 60-day old chicken. The liver cAsAT of chicken from both the ages are competitively inhibited by amino-oxyacetic acid (AOAA) with respect to L-asparate and non-competitively with respect to α-ketoglutarate. The K_i values of this enzyme for amino-oxyacetic acid of both the 0-day and 60-day old chicken are also similar. Inactivation studies of the enzymes from both the ages also depict no differential folded structure since urea requirement for 50% inactivation remained the same. Thus, these findings indicate that the molecular structure of this isoenzyme does not change at these two postnatal ages of chicken.

In conclusion, present study on chicken malate-aspartate shuttle enzymes revealed that the shuttle enzymes exhibit age- and tissue-specificity with respect to its activity expression and further the shuttle enzyme are also subjected to hormonal regulation in an age- and tissue –specific manner. Purification and kinetic analyses of purified cytosolic aspartate aminotransferase of the liver of 0-day and 60-day old chicken reveal that the structure of the enzyme molecule remains unaltered during such phases of chicken's life span. These findings indicate that factors like hormones and their adaptive responses to enzymes depend on the metabolic state of the concerned tissue at that phase of the organism's lifespan regulated by the level of hormones, their receptor/ post-receptor events and tissue-specific factors needed for the expression of cognate genes in an organism-specific manner brought about by various extrinsic or intrinsic factors.

REFERENCES

Adamo M, Simon J, Rosebrough RW, McMurtry JP, Steele NC and Leroith D (1987) Characterization of the chicken muscle insulin receptor. **Gen. Comp. Endocrinol.** 68, 456-465.

Adelman RC (1975) Enzyme Induction (Parke DV ed.) pp 303-311, **Plenum Press**, New York.

Adelman RC (1981) Handbook of Biochemistry (Florini JR ed.) **CRC Press**, Boca Raton, Florida.

Allan GF, Tsai SY, O'Malley BW and Tsai M (1991) Hormone responsive interaction by ligand-receptor complexes. **J. Bioassays** 13, 73-78.

Arrey LB (1966) Developmental anatomy, **W B Saunders & Co**. Philadelphia.

Bailey GS, Cocks GT and Wilson AC (1969) Aspartate aminotransferase isoenzymes. **Biochem. Biophys. Res. Commun.** 34, 605-612.

Banaszak L and Bradshaw RA (1975) Malate dehydrogenases. The Enzymes Vol 11, (Boyer PD ed.) pp 369-396, **Acad. Press**, New York.

Banks BCE, Doonan S, Lawrence AJ and Vernon CA (1968) The molecular weight and other properties of aspartate aminotransferase from pig heart muscle. **Eur. J. Biochem.** 5, 528-539.

Barra D, Bossa F, Doonan S, Fahmy HMA, Martini F, and Hughes GJ (1976) Large-scale purification and some properties of the mitochondrial aspartate aminotransferase from pig heart. **Eur. J. Biochem.** 64, 519-526.

Barron JT, Gu L and Parrillo JE (1998) Malate-aspartate shuttle, cytoplasmic NADH redox potential, and energetics in vascular smooth muscle. **J. Mol. Cell. Cardiol.** 30, 1571-1579.

Baumber ME and Doonan S (1976) A quantitative study of the subcellular distribution of aspartate aminotransferase isoenzymes in rat liver. **Int. J. Biochem.** 7, 119-124.

Beato M (1989) GRE modulators influence metabolic responses. **Cell** 56, 335-344.

Behra R, Christen P and Sonderegger P (1981) Independent quantitation of the mitochondrial and the cytosolic isoenzyme of aspartate aminotransferase in chicken tissues by radioimmunoassays. **J. Biol. Chem.** 256, 3381-3384.

Benzi G, Arrigoni E, Dagani F, Marzatico R, Curti D, Polgatti M and Villa RF (1980) Drug interference on the age-dependent modification of the cerebral enzymatic activities related to energy transduction. **Aging** 13, 113-117.

Benzo CA and De La Haba G (1972) Insulin affect maturation of hepatocytes. **J. Cell. Physiol.** 79, 53-64.

Berstein LH, Grinsham MB, Cole KD and Everse J (1978) Substrate inhibition with pig heart malate dehydrogenase isoenzymes differs from lactate dehydrogenase in chicken. **J. Biol. Chem.** 253, 8697-8701.

Bertland LH and Kaplan N O (1970) Studies on the conformations of the multiple forms of chicken heart aspartate aminotransferase. **Biochemistry** 9, 2653-2665.

Bhargava MM and Sreenivasan A (1966) On the origin of increased serum aspartate aminotransferase in hepatic damage. **Enzymologia** 31, 245-247.

Bisbis S, Taouis M, Derouet M, Chevatier B and Simon J (1994a) Corticosterone is the principal glucocorticoid. **Gen. Comp. Endocrinol.** 96, 370-377.

Bisbis S, Derouet M and Simon J (1994b) Characterization of insulin receptors in chicken kidneys : effect of nutritionl status. **Gen. Comp. Endocrinol.** 96, 37-49.

Bjorksten J (1964) Cross-linking theory. **Chemistry** 37, 6-11.

Blazguez E, Montoya E and Quijada C L (1970) Insulin drops precipitously at birth in rats. **J. Endocrinol.** 48, 553-561.

Bobhuiya MA and Sharma R (1995a) Regulation of hepatic glucocorticoid receptor during development of mice. **Biochem. Mol. Biol. Intnl.** 37, 645-652.

Bobhuiya MA and Sharma R (1995b) Postnatal changes in kidney glucocorticoid receptor of mice. **Ind. J. Biochem. Biophys.** 32, 125-129.

Bohme HJ, Belay, Dettmer D, Goltzseh W, Hoffmann E, Lange L, Schubert C, Schulze E, Sperman G and Weiss E (1986) interaction of adrenal and pancreatic hormones in the control of hepatic enzymes during development. **Adv. Enz. Regul.** 26, 31-61.

Borisov VV, Borisova SN, Kachalova GL, Torchinsky, Yu M and Braunstein AE (1978) Three dimensional structure at 5^0A resolution of cytosolic aspartate transaminase from chicken heart. **J. Mol. Biol.** 125, 275-292.

Borst P and Poters EM (1961) Intracellular localization of aspartate aminotransferases in heart. **Biochim. Biophys. Acta** 54, 188-189.

Borst P (1963) **Funktionelle and Morphologishen organization der zelle**, pp 137-158, Springer Berlin.

Boyd JW (1961) The intracellular distribution, latency and electrophoretic mobility of L-glutamate-oxalacetate transaminases from rat liver. **Biochem. J.** 81, 434-441.

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal. Biochem.** 72, 248-254.

Braunstein AE (1973) The Enzymes (Boyer PD ed.) pp 379-481, **Acad. Press**, New York.

Brizee KR, Cacilla PA, Sherwood N and Timiras PS (1969) Aging pigments constitute lipofuscin. **J. Gerontol.** 24, 127-135.

Brizee KR, Harkin JC, Ordy JM and Kerack B (1975) Lipofuscin has been correlated with loss of neurons. **Aging** 1, 39-78.

Cake MH, Yeoh G and Litwack G (1979) Developmental changes in glucocorticoid induction of hepatic tyrosine aminotransfarase. Adv. in Biosciences 25; (Kaye AM and Kaye M eds.) **Pergamon Press**, Oxford.

Caliendo AM (1981) Increase insulin receptor in rats increases blood insulin before birth. **Fed. Proc.** 39, 1877-1881.

Cederbaum AI, Lieber CS, Beattic DS and Rubin E (1973) Characterisation of shuttle mechanism for the transport of reducing equivalents into mitochondria. **Arch. Biochem. Biophys.** 158, 263-281.

Chainy GB and Kanungo MS (1978) Differential expression of pyruvate kinase. **Biochem. Biophys. Res. Commun.** 104, 335-337.

Cheeseman AJ and Clark JB (1988) Influence of the malate-aspartate shuttle on oxidative metabolism in synaptosomes. **J. Neurochem.** 50, 1559-1565.

Chen SH and Giblett (1971) Cytoplasmic alanine aminotransferase subunits have two separate genes. **Science** 173, 148-149.

Christen P and Metzler D (1985) Transaminases, pp 542-544, 553-555 **Wiley-Interscience publcation** .

Cohen A (1973) Glucocorticoid expression differs with age and metabolic responses. **Horm. Metabol. Res.** 5, 66-70.

Cohen A, Hellen H and Kulka RG (1972) Exogenous hydrocortisone induces several enzymes. **Dev. Biol.** 29, 293-306.

Cohn M, Monod J, Pollock MR, Spiegelman S and Stainer RY (1953) Enzyme activity induction brought by change in substrate concentration or hormone action. **Nature** 172, 1096-1100.

Cremel G, Filliol D and Waksman A (1985) Simultaneous purification by affinity chromatography of rat liver mitochondrial aspartate aminotransferase and malate dehydrogenase and electrophoretic properties. **Anal. Biochem.** 150, 332-336.

Crow KE, Braggins TJ, Batt RD and Hardman MJ (1982) Rat liver cytosolic malate dehydrogenase: purification, kinetic poperties, role in control of free cytosolic NADH concentration. **J. Biol. Chem.** 10: 257, 14217-14225.

Cutler RG (1984) Aging and Cell function, (Johnson JE Jr. edn.) , pp 116-129 **Plenum Press**, New York.

Darras VM, Visser TJ, Berghman LR and Kuhn ER (1992) Ontogeny of type I and type III deiodinase activities in embryonic and posthatch chicks: relationship with changes in plasma *triiodothyronine and growth hormone levels*. **Comp. Biochem. Physiol.** 103A, 131-136.

Davidson RG and Cartner JA (1967) Malate-aspartate shuttle enzymes have two homologous and genetically independent isoenzymes. **Sci.** 157, 1569-1571.

Davis, BJ (1964) Native polyacryamide gel electrophoresis. **Ann. N Y Acad. Sci.** 121, 404-427

Dawson AG (1982) Rapid oxidation of NADH via the reconstituted malate-aspartate shuttle in system containing mitochondrial and soluble fractions of rat liver: Implications for ethanol metabolism. **Biochem. Pharmacol.** 31, 2733-2738.

Decker LF and Rau EM (1963) Multiple forms of glutamic-oxalacetic transaminase in tissues. **Proc. Soc. Exp. Biol. Med.** 112, 144-148.

Di Cola D, Polidoro G, Di Ilio C, Del BE, Moriggi M, Politi L and Scandurra R (1976) Simultaneous purification of mitochondrial and cytoplasmic isoenzymes of aspartate aminotransferase from beef kidney. **Acta Vitam. Enzymol.** 30, 28-37.

Dickerson JWT and Basu TK (1975) Enzyme induction in the process of development, (Parke DV ed.) pp 27-77, **Plenum Press**, New York.

DiMarco PN, Ghisalberti AV, Martin CE and Oliver IL (1978) Insulin-glucagon ratio decreases at hypoglyceamia. **Eur. J. Biochem** .87, 243-247.

Domenech C, Mazo A, Artigas R, Cortes A and Bozal J (1986) Malate dehydrogenase species in the cytosolic fraction of chicken liver. **Biol. Chem. Hoppe-Seyler** 367, 1069-1076.

Doonan S, Barra D, Bossa F, Porter PB and Wilkinson SM (1981) Interspecies comparisons of aspartate aminotransferases based on amino acid compositions. **Comp. Biochem. Physiol.** 69B, 747-752.

Edgar RS and Wood WB (1977) Gene expression during development and aging processes. **Sci.** 198, 1285-1286.

Eichel HJ and Bukovsky J (1961) Intracellular distribution pattern of rat liver glutamic-oxalacetic transaminases. **Nature New Biol.** 191, 243-245.

Eichele G, Ford GC and Jansonius JN (1979) Crystallization of pig mitochondrial aspartate aminotransferase by seeding with crystals of the chicken mitochondrial isoenzyme. **J. Mol. Biol.** 135, 513-516.

Elduque A, Casudo F, Cortes A and Bozal J (1982) Intramitochondrial location of the molecular forms of chicken liver mitochondrial malate dehydrogenase. **Int. J. Biochem.** 14, 221-229.

Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Aizawa T, Akanuma Y, Aizawa S, Kasai H, Yazaki Y and Kadowaki T (1999) Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. **Sci.** 283, 981-985.

Fahien LA and Strmecki M (1969) Gluconeogenic mitochondrial enzymes. Conversion of oxalacetate to fumarate by bovine liver malate dehydrogenase and fumarase. **Archs. Biochem. Biophys.** 130, 478-483.

Ferre P and Williamson DH (1978) Evidence for the participation of aspartate aminotransferase in hepatic glucose synthesis in the suckling newborn rat. **Biochem. J.** 176, 335-338.

Feldman D (1974) Glucocorticoid receptor concentration increases right after birth. **Endocrinol.** 95, 1219-1227.

Fleisher GA, Potter CS and Walkim KG (1960) Separation of 2 glutamic-oxalacetic transaminases by paper electrophoresis. **Proc. Soc. Exp. Biol. Med.** 103, 229-231.

Foa PP, Melli M, Berger CK, Billinger D and Guidotti GG (1965) Insulin enhances lipogenesis. **Fed. Proc. Am. Soc. Exp. Biol.** 24, 1046-1050.

Ford GC, Eichele G and Jansonius JN (1980) Mitochondrial aspartate aminotransferase subunit domain contains pyridoxal 5' phosphate. **Proc. Natl. Acad. Sci.** 79, 2559-2563.

Freeman BM and Manning ACC (1971) Adenylate cyclase andyclic-AMP is immature in aves during incubation period. **Comp. Gen. Pharm.** 2, 198-204.

Freeman BM and Vince MA (1974) Development of Avian Embryo;Adrenal hormones suspected to be involved in development. pp 62-83, 208-236, **Chapman & Hall Publn.** London.

Frolkis VV (1982) Aging and Life Prolonging Processes, **Springer-Verlog**, New York.

Garreth RH and Grisham CM (1999) Biochemistry, **Saunders College Publishing**.

Gehring H and Christen P (1978) Coenzyme of aspartate aminotransferase shuttles between pyridoxal and pyridoxamine p-forms. **J. Biol. Chem.** 253, 3158-3163.

Gelpi JL, Dorbal A, Mountserrat J, Mazo A and Cortes A (1992) Kinetic studies of the regulation of mitochondrial malate dehydrogenase by citrate. **Biochem. J.** 1, 283, 289-297.

Gerl M, Rudolph R and Jaenicke R (1985) Mechanism and specificity of reconstitution of dimeric lactate dehydrogenase from *Limulus poliphemus* **Biol. Chem. Hoppe-Seyler** 366, 447-454.

Ghisalberti AV, Steele JE, Cake MH, McGrath MC and Oliver IL (1980) Correlation of gluconeogenic enzymes with glucagons. **Biochem. J.** 190, 688-690.

Giannopoulos G (1975) Onset of birth increases glucocorticoid receptors. **J. Biol. Chem.** 250, 5847-5851.

Gil M, Cascante M, Cortes A and Bozal J (1987) Intramitochondrial location and some characteristics of chicken liver aspartate aminotransferase. **Int. J. Biochem.** 19, 355-363.

Gillies SD, Morrison SL, Oi VT and Tonegawa SF (1983) Enhancer elements are tissue specific. **Cell** 33, 717-728.

Girard JR, Cuendet GS, Marliss EB, Kervran A, Rieutori M and Assan R (1973) Glucagon concentration increases during the first hour after birth. **J. Clin. Invest.** 52, 3190-3200.

Glatthaar BE, Barbarash GR, Noyes BE and Banaszak LJ (1974) The preparation of cytosolic and mitochondrial forms of malate dehydrogenase and aspartate aminotransferase from pig heart by a single procedure. **Anal. Biochem.** 57, 432-451.

Goodridge AG, Crish JF, Hillgartner FB and Wilson SB (1989) Nutitional and hormonal regulation of the gene for avian malic enzyme. **J. Nutr.** 119, 299-308.

Grimm FC and Doherty DG (1961) Malate dehydrogenase is an enzyme of malate-aspartate shuttle. **J. Biol. Chem.** 236, 1980-1985.

Hagele E, Neef J and Mecke D (1978) The malate dehydrogenase isoenzymes of *Saccaromyces cerevisiae*. Purification, characterization and studies on their regulation. **Eur. J. Biochem.** 83, 67-76.

Hamilton WD (1966) Special pleiotropic gene is linked to development and causes aging. **J. Theor. Biol.** 12, 12-16.

Hanawalt PC (1987) Modern Biological Theories of Aging (warner HR ed.) pp 183-198 **Raven Press**, New York.

Harman D (1986) Free radical Theory of Aging, Aging and Degenerative Diseases (Johnson JE, Walford R, Harman D and Miquel J eds.) , pp 3-50 **Alan Liss Inc.** , New York.

Harvey S (1990) Thyroidal inhibition of growth hormone secretion: negative feedback? Endocrinology of birds: Molecular to behavioural (Wada M, et al ed.) pp 111-127. **Japan Sci. Soc. Press.** Tokyo.

Harvey S and Scanes CG (1977) Purification and radioimmunoassay of chicken growth hormone. **J. Endocrinol.** 73, 321-329.

Harvey S, Davison TF and Chadwick A (1979) Ontogeny of growth hormone and prolactin secretion in the domestic fowl (*Gallus domesticus*). **Gen. Comp. Endocrinol.** 39, 270-273.

Hazelwood RL (1986) Carbohydrate metabolism. In 'Avian Physiology" 4th edn. (P. D. Sturkie Ed.) pp 303-325 **Springer–Verlag**, New York.

Heide DV and Ende-Visser MD (1980) Thyroid hormones implicated in the appearance of glucokinase and malic enzyme. **Acta Endocrinol.** 93, 448-454.

Herzfeld A and Greengard O (1971) Aspartate aminotransferase in rat tissues: changes with growth and hormones. **Biochim. Biophys. Acta** 237, 88-98.

Imperial S, Busquets M, Cortes A and Bozal J (1989) Cytosolic aspartate aminotransferases from different chicken tissues: purification and characterization of their multiple forms. **Prep. Biochem.** 19, 113-127.

Imperial S, Quiroga C, Busquets M and Cortes A (1991) Heterogeneity of chicken liver cytosolic aspartate aminotransferase. **Biochim. Biophys. Acta** 1077, 72-78.

Jaussi R, Behra R, Giannottasio S, Flura T and Christen P (1987) Expression of cDNAs encoding the precursor and the mature form of chicken mitochondrial aspartate aminotransferase in Escherichia coli. **J. Biol. Chem.** 262, 1234-12437.

Juretic N, Mattes U, Ziak M, Christen P and Jaussi R (1990) Structure of the genes of two homologous intracellularly heterotopic isoenzymes. **Eur. J. Biochem.** 192, 119-126.

Kagamiyama H and Wada H (1975) Amino acid sequence of fifty two residues from the N-terminus of mitochondrial aspartate aminotransferase. **Biochim. Biophys. Res. Commun.** 64, 425-430.

Kalimi M (1984) Glucocorticoid receptors: from development to aging. A review, **Mech. Ageing. Dev.** 24, 129-138..

Kanungo MS (1980) Changes in enzymes during ageing. **Biochemistry of Aging,** pp 79-126, **Academic Press**, London.

Kanungo MS (1994) Genes and Aging, **Cambridge University Press**, pp 1-42 and 115-180.

Kanungo MS and Gandhi BS (1972) Induction of malate dehydrogenase isoenzymes in livers of young and old rats. **Proc. Natl. Acad. Sci.**, 69, 2035-2038.

Kanungo MS, and Patnaik SK (1975) Regulation of growth and differentiated function in Eukaryotic cells (Talwar GP ed.) pp 479-490 **Raven Press**, New York.

Karmen A (1955) A note on the spectrophotometric assay of glutamic oxalacetic transaminase in human blood serum. **J. Clin. Invest.** 34, 131-133.

Karstrom H (1936) **Proc. 2nd Intnl. Conf. Microbiol.** (London) pp 473-474.

Kauppinen RA, Sihra TS and Nicholls DG (1983) Aminooxyacetic acid inhibits the malate-aspartate shuttle in isolated nerve terminals and prevents the mitochondria from utilizing glycolytic substrates. **Biochim. Biophys. Acta** 930, 173-178.

Keast D, Nguyen T and Newsholme EA (1989) Maximal activities of glutaminase, citrate synthase, hexokinase, phosphofructokinase and lactate dehydrogenase in skin of rats and mice at different ages. **FEBS lett.** 247, 132-134.

King DB and King CR (1973) Thyroidal influence on early muscle growth of chickens. **Gen. Comp. Endocrinol.** 21, 517-529.

King DB and Scanes CG (1986) Hypophysectomy reduces growth while replacement therapy with mammalian growth hormone partially restores growth rate. **Proc. Soc. Exp. Biol. Med.** 182, 201-207.

Kitto B (1969) Methods in Enzymol. (Lowenstein JM ed.) V 13 pp 106-107, **Acad. Press**, New York.

Kohler E, Seville M, Jager J, Fotheringham I, Hunter M, Edwards M, Jansonius JN and Kirschner K (1994) Significant improvement to the catalytic properties of aspartate aminotransferase : role of hydrophobic and charged residues in the substrate binding pocket. **Biochemistry** 33, 90-97.

Kopelovich L, Sweetman L and Nisselbaum JS (1970) Time-dependent inhibition of aspartate aminotransferase isoenzymes by DL-glyceraldehyde 3-phosphate. **J. Biol. Chem.** 245, 2011-2017.

Krebs HA, Gascotne T and Notton BM (1967) Generation of extramitochondrial reducing power in gluconeogenesis. **Biochem. J.** 102, 275-282.

Kuan KN, Jones GL and Vestling CS (1979) Rapid preparation for malate dehydrogenase from rat liver and heart. **Biochemistry** 18, 4366-4373.

Kuhn ER, Vanderpooten A, Dewil E, Darras VM, Huybrechts LM, Berghman L and Decuypere E (1991) Ontogeny and control of GH and T_3 receptors in the chicken. **Proceedings of 1st World Congress of Cellular and Molecular Biology**, Paris 1-7 Sept., 1991.

Kuramitsu S, Inoue K, Kondo K, Aki K, Kagamiyama H (1985) Amino terminal amino acid sequence of rabbit liver aspartate aminotransferase isoenzymes show differences from other animals. **J. Biochem.** (Tokyo) 97, 1337-1345.

Landriscina C, Papa S, Coratelli P, Mazzarella L and Quagliarriello E (1970) Enzymatic activities of the matrix and inner membrane of pigeon-liver mitochondria. **Biochim. Biophys. Acta** 205, 136-147.

Lardy HA, Poetkau V and Walter P (1965) Paths of carbon in gluconeogenesis and lipogenesis: the role of mitochondria in supplying precursors of phosphoenolpyruvate. **Proc. Natl. Acad. Sci.**, 53, 1410-1415.

Larry HB and Johannes (1978) Malate dehydrogenase mechanism of action is closely related to that of lactate dehydrogenase. **J. Biol. Chem.** 253, 8702-8727.

Lauber JK, Vriend J and Oishi T (1987) Plasma corticosterone in chicks reared under several lighting schedules. **Comp. Biochem. Physiol.** 86 A, 73-78.

Lehninger AL (1951) Intact mitochondria are impermeable to externally added NADH. **J. Biol. Chem.** 190, 345-359.

Lehninger AL, Nelson DL and Cox MM (1993) Principles of Biochemistry, pp 599-607, **CBS Publn.**, Delhi.

Levi G and Lattes MG (1969) Insulin enhance uptake of neutral amino acids. **Brain Res.** 13, 579-594.

Lewin B (2000) Genes VII, **Oxford University Press Inc.**, New York.

Libby DA, Meites J and Schaible PJ (1955) Growth hormone effects in chickens. **Poultry Sci.** 34, 1329-1331.

Lu RB , Lebenthal E and Lee PC (1987) Developmental changes of glucocorticoid receptors in the rat pancreas. **J. Steroid Biochem.** 26, 213-218.

Lyngdoh H G and Sharma R (2001) Hydrocortisone and triiodothyronine regulation of malate-aspartate shuttle enzymes during postnatal development of chicken. **Ind. J. Biochem. Biophys.** 38, 170-175.

Magee SC and Phillips AT (1971) Molecular properties of the multiple asparate aminotransferases purified from rat brain. **Biochemistry** 10, 3397-3405.

Malashkevich VN, Toney MD andJansonius JN (1993) Crystal structure of stable, closed complexed chicken mitochondrial aspartate aminotransferase. **Biochemistry** 32, 13451-13462.

Malik P, McKenna MC and Tildon JT (1993) Regulation of malate dehydrogenases from neonatal, adolescent, and mature rat brain. **Neurochem. Res.** March 18, 247-257.

Markert CL and Moller F (1959) Lactate dehydrogenase isoenzyme is tissue and age-specific. **Proc. Natl. Acad. Sci.** 45, 753-762.

Markert CL and Ursprung (1962) M-type LDH converts pyruvate to lactate. **Dev. Biol.** 5, 363-381.

Marra E, Doonan S, Saccone C and Quagliarriello E (1977) Selective permeability of rat liver mitochondria to purified aspartate aminotransferases in vitro. **Biochem. J.** 164, 685-691.

Marsh JA, Gause WC, Sandu S and Scanes CG (1984 a) Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. **Proc.Soc. Exp. Biol.** 175, 351-360.

Marsh JA, Lauterio TJ and Scanes CG (1984 b) Effects of triiodothyronine treatments on body and organ growth and the development of immune function in dwarf chickens. **Proc. Soc. Exp. Biol.** 117, 82-91.

Martin CE, Cake MH, Hartman PE and Cook IF (1977) Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. **Acta Endocr. Copenh.** 84, 167-176.

Martinez-Carrion M, Turano C, Chiancone E, Bossa F, Giartosio A, Riva F and Fasella P (1967) Isolation and characterization of multiple forms of aspartate aminotransferase from pig heart. **J. Biol. Chem.** 241, 2661-2664.

May JD (1980) Effect of dietary thyroid hormone on growth and feed efficiency of broilers. **Poultry Sci.** 59, 888-892.

May JD (1982) Effect of dietary thyroid hormones on serum hormone concentration, growth and body composition of chickens. Aspects of Avian Endocrinology: Practical and Theoretical Implication (Scanes CG et al. eds.) pp 185-189 **Texas Tech. Press**, Lubbock, Texas.

McDonald MJ (1983) Evidence for malate-aspartate shuttle in pancreatic islets. **Arch. Biochem. Biophys.**. 213, 643-649.

McNabb FMA (1988) Peripheral thyroid hormone dynamics in precocial and altricial avian development. **Am. Zool.** 28, 427-440.

Medawar PB (1957) The Uniqueness of the Individual, **Methuen and Co. Inc.** , London.

Meijer AJ and Van Dam K (1974) The metabolic significance of anion transport in mitochondria. **Biochem. Biophys. Acta** 346, 213-244.

McLoughlin DJ and Howell MM (1987) Bilirubin inhibition of enzymes involved in the mitochondrial malate-aspartate shuttle. **Biochim. Biophys. Acta** 893, 7-12.

Metzler DE and Snell EE (1952) AsAT effects the reversible transfer of an amino group from L-aspartate or L-glutamate to α-ketoglutarate and oxalacetate. **J. Am. Chem.** 253, 3158-3163.

Miquel J, Lundgren PR and Johnson JE (1978) Loss of neurons during aging. **J. Gerontol.** 33, 5-19.

Morgunov Igor and Srere Paul A (1998) Interaction between citrate synthase and malate dehydrogenase. **J. Biol. Chem.** 273, 29540-29544.

Morino Y, Kagamiyama H and Wada H (1964) Immunochemical and kinetic properties of anionic and cationic glutamic-oxalacetic transaminases separated from human heart and human liver. **J. Biol. Chem.** 239, 943-947.

Muller J, Manent MF and Pfleiderer G (1983) Importance of tyrosine for structure and function of mitochondrial malate dehydrogenases. **Biochim. Biophys. Acta** 742, 189-196.

Muller J, Gorisch H and Parkhurst LJ (1984) Dissociation of mitochondrial malate dehydrogenase. **Biochim. Biophys. Acta** 787, 258-263.

Nagashima F, Tanase S, Fukumoto Y, Joh T, Nomujama H, Tsuzuki T, Shimada K, Kuramutsui S, Kagamiyama H and Morina Y (1989) cDNA cloning and expression of pig cytosolic aspartate aminotransferase in *Escherichia coli* : amino-terminal heterogeneity of expressed products and lack of its correlation with enzyme function. **Biochemistry** 28, 1153-1160.

Nakata Y, Svematsu T and Sakamoto Y (1964) Specific activity of c-AsAT is higher in the adult as compared to that of fetal liver. **J. Biochem.** 55, 199-201.

Nordenberg J, Heffetz D, Cohen TJ and Bectner R (1981) Glucose-1,6-diphosphate and carbohydrate metabolism in skeletal muscle of old rats. **Int. J. Biochem.** 13, 317-321.

O'Keef RJ, Puzas JE, Brand JS and Rosier RN (1988) Transforming growth factor-β effects on chondrocytes in chick leg long-bone. **Endocrinol.** 122, 2953-2961.

Ochs RS and Harris RA (1980) Aminooxyacetate inhibits gluconeogenesis by isolated chicken hepatocytes. **Biochim. Biophys. Acta** 632, 260-269.

Ogata K, Watford M, Brady LJ and Hanson RW (1982) Mitochondrial phosphoenolpyruvate carboxykinase (GTP) and the regulation of gluconeogenesis and ketogenesis in avian liver. **J. Biol. Chem.** 257, 5385-5391.

Okayabashi K and Nakano E (1984) Purification and properties of malate dehydrogenase from unfertilized eggs of the Sea Urchin *Anthocidaris crassipina*. **J. Biochem.** 57, 432-451.

Orgel LE (1963) The maintenance of the accuracy of protein synthesis and its relevance to ageing. **Proc. Natl. Acad. Sci.** 49, 517-521.

Pablo F de, Girbau M, Garvey JA, Hernandez E and Roth J (1985) Role of insulin in chick embryo growth and development. **Diabetes** 34, 1063-1066.

Patnaik SK and Kanungo MS (1976) Cytoplasmic alanine aminotransferase (c-AlAT) of the old rat liver revealed that sequential changes in the expression of proteins extent to old age. **Ind. J. Biochem. Biphys.** 13, 117-124.

Pearce J(1977) Some differences between avian and mammalian biochemistry. **Int. J. Biochem.** 8, 269-275.

Pease K, Shen H, Acres GS, Repnow JH and Dixon JE (1980) Thyroid releasing hormone (TRH) and thyroid stimulating hormone (TSH) increase in concentration in the blood precedes the increase in the concentration of thyroid hormone in the neonate **J. Endocrinol.** 85, 55-61.

Perrella FW (1988) EZ-FIT: a practical curve-fitting microcomputer program for the analysis of enzyme kinetic data on IBM-PC compatible computers. **Anal. Biochem.** 174, 437-447.

Pitot HC and Yatvin MB (1973) relationship of mammalian hormone, enzyme level and induction. **Physiol. Revs.** 53; 229

Price NC (1989) Fundamentals of Enzymology, pp13 **Oxford University Press**, Oxford.

Rej R (1977) Aminooxyacetate is not an adequate differential inhibitor of aspartate aminotransferase isoenzymes. **Clin. Chem.** 23,1508-1509.

Reichel W (1968) Lipofuscin accumulation during aging. **J. Gerontol.** 23,145-153.

Ricklefs RE (1983) Avian Biology (Farner DS, King JR and Parkes KC eds.)Vol II, pp 1-83, **Acad. Press**, New York.

Roderick SL and Banaszak LJ (1986) Crystalline porcine heart mitochondrial malate dehydrogenase by X-ray diffraction studies was determined. **J. Biol. Chem.** 261, 9461-9464.

Rodwell V W (1996) Harper's Biochemistry (Murray RK, Granner DK, Mayes PA and Rodwell V W eds.) **Prentice Hall International Inc.**, USA

Ross D, Silva P and Bullock S (1977) Role of malate-aspartate shuttle in renal sodium transport in the rat. **Clin. Sci.** 60,419-426.

Roth GS (1988) Mechanism of altered hormone and neurotransmitter action during aging: The role of impaired calcium mobilization. **Ann. New York Acad. Sci.** 521,170-176.

Russel RL (1987) Modern Biological Theories of Aging (Werner HR ed.) pp 35-61, **Raven Press**, New York.

Sacher GA (1977) Handbook of the Biology of the Aging (Finch CE and Hayflick L eds.) **Van Nostrand, Reinhold**, New York.

Santa D and Sharma R (1997) Malate -aspartate shuttle enzymes activity in the liver and kidney of mice was differentially expressed in an age- and tissue-specific manner. **Ind. J. Biochem. Biophys.** 35, 224-227.

Scanes CG, Aramburo C and Campbell RM (1990) Hormonal Involvement in Avian growth and development: growth hormone and insulin-like growth factor I. Endocrinology of birds: Molecular to behavioural (M. Wada et al. eds.) pp 85-91. **Japan Sci. Soc. Press.** Tokyo.

Scanes CG and Johnson AL (1984) Growth hormone plasma concentration show no circulating sex steroid concentrations. **Gen. Comp. Endocrinol.** 53, 398-401.

Scanes CG, Marsh JA, Decuypere E and Rudas P (1983) Abnormalities in the plasma concentration of thyroxine, triiodothyronine and growth hormone in sex-linked dwarf and autosomal dwarf White Leghorn domestic fowl (*Gallus domesticus*). **J. Endocrinol.** 97, 127-135.

Scanes CG, Harvey S, Marsh JA and King DB (1984) Hormones and growth in poultry. **Poultry Sci.** 63, 2062-2074.

Serano J, Scavo L and Pablo FD (1990) The ontogeny of insulin gene expression. Endocrinology of birds: Molecular to behavioural (M. Wada et al. eds.) pp 85-91. **Japan Sci. Soc. Press.** Tokyo.

Sharma R and Patnaik SK (1982) Induction of rat liver cytosolic aspartate aminotransferase by hydrocortisone. **Biochem.Int.** 5, 561-566.

Sharma R and Timiras PS (1987) Age-dependent activation of glucocorticoid receptors in the cerebral hemispheres of male rats. **Dev. Brain Res.** 36, 285-267.

Sharma R and Timiras PS (1987) Regulatory changes in glucocorticoid receptors in the skeletal muscle of immature and mature male rats. **Mech. Ageing Dev.** 37, 249-256.

Sharma R, Dey S and Verma R (1992) Age-specific development of malate-aspartate shuttle in the liver and kidney of mice. **Biochem. Int.** 27, 1059-1066.

Sharma R and Timiras PS (1987) Age-dependent regulation of glucocorticoid receptors in the liver of male rats. **Biochim. Biophys. Acta** 930, 237-243.

Sharma R (1988) Physiological Basis of Aging and Geriatrics (Timiras PS ed.) pp 43-58, **MacMillan Inc.**, New York.

Sharma R (1991) Glucocorticoid actions and biomodulators: An integrated biological control. **Ind. J. Biochem. Biophys.** 28, 159-163.

Sharma R (1994) Physiological Basis of Aging and Geriatrics (Timiras PS ed.) pp 37-46, **CRC Press**, Boka Raton, Florida.

Sharma R (1993) Cross-talk in signal transduction. **Current Science** 65, 342-347.

Sheid B and Roth JS (1965) Isoenzymes of AsAT are involved in the process of gluconeogenesis and it is the cytosolic isoenzyme which is regulated. **Adv. Enzy. Regul.** 3, 335-350.

Shock NW (1979) Overall performance of an animal is by control mechanisms that regulate the interplay between different organs and tissues Systems Physiology and Aging, **Fed. Proc.** 38, 161-165.

Shrawder EJ and Martinez-Carrion M (1973) Simultaneous isolation and characterization of chicken supernatant and mitochondrial isoenzymes of aspartate transaminase. **J. Biol. Chem.** 248, 2140-2146.

Simon J (1984) Corticosterone treatment in chickens impairs growth and induces fattening and insulin resistance. **Diabete & Metabolisme** 10, 211-217.

Simon J and Leroith D (1986) Insulin receptors of chicken liver and brain. **Eur. J. Biochem.** 158, 125-132.

Singer S and Litwack G (1971) Effects of age and sex on ^{3}H-cortisol uptake, binding and metabolism in liver and on enzyme induction capacity. **Endocrinol.** 83, 1448-1455.

Singh SN and Kanungo MS (1968) Lower proportion of M_4-LDH may cause a decrease in the ability of the tissue to cope with anaerobic conditions **J. Biol. Chem.** 243, 4526-4529.

Snell EE and DiMari SJ (1970) The Enzymes (Boyer PD ed.) pp 335-370 **Acad. Press**, New York.

Sondergger P, Gehring H and Christen P (1977) Interspecies comparison of cytosolic and mitochondrial aspartate amnotransferases **J. Biol. Chem.** 252, 609-612.

Sonderegger P, Jaussi R and Christen P (1980) Cell free synthesis of a putative precursor of mitochocndrial aspartate aminotransferase with higher molecular weight. **Biochem. Biophys. Res. Commun.** 94, 1256-1260.

Strehler BL (1964) A marked change occurs in cell composition during aging. **Adv. Gerontol. Res.** 1, 343-384.

Strhler BL, Hirsch G, Gusseck B, Johnson R and Bick M (1971) Codon restriction theoy of ageing and development. **J. Theo. Biol.** 33, 429-474.

Sugano T, Shiota M, Khono H and Shimada M (1982) Intracellular redox state and control of gluconeogenesis in perfused chicken liver. **J. Biochem.** 91, 1917-1929.

Sugano T, Nishimura K, Sogabe N, Shiota M, Oyama N, Noda S and Ohta M (1988) Ca^{2+}-dependent actvation of the malate-aspartate shuttle by norepinephrine and vasopressin in perfused rat liver. **Arch. Biochem. Biophys.** 264, 144-154.

Sugano T, Ochla T, Torui A and Miyamee Y (1986) Effects of alanine on malate-aspartate shuttle in perfused livers from cold exposed rats. **Am. J. Physiol.** 251, E385-E392.

Sulstan JE and White JG (1980) Gene expression. **Dev. Biol.** 78, 577-597.

Syiem D and Sharma R (1996) Purification and kinetic characterization of chicken liver inorganic pyrophosphatase. **Ind. J. Biochem. Biophys.** 33,363-370.

Szilard L (1959) Somatic mutation theory. **Proc. Natl. Acad. Sci.** 45, 30-33.

Taouis M, Derouet M, Chevalier B and Simon J (1993) Corticosterone effect on insulin receptor number and kinase activity in chicken muscle and liver. **Gen. Comp. Endocrinol.** 89, 167-175.

Timiras PS (1994) Physiological Basis of Aging and Geriatrics, pp 7-21 **CRC Press, Boca Raton,** Florida.

Toth SE (1968) Cell composition during aging. **Expl. Gerontol.** 3, 19-30.

Van Heyningen V, Craig IW and Badmer WF (1974) The Biogenesis of Mitochondria (Kroon AM and Saccone C eds.) pp 231-244 **Acad Press.**, New York.

Vogel HJ (1957) Enzyme repression from exposure of cell to given substrate. **Proc. Natl. Acad. Sci.** 43, 491-496.

Wada M, Ishii S and Scanes CG (1990) Endocrinology of Birds, **Japan Sc. Soc. Press**, Tokyo.

Wada H and Morino Y (1964) Organ- or tissue-specific forms have not been detected for either asparate amnotransferase isoenzyme. **Vitamins and Hormones** 22, 411-444.

Wahli Wand Martinez E (1991) Steroid and thyroid receptor-ligand complexes interact with definite cis-acting sequences of genes and expressed in a tissue-specific manner **FASEB J.** 5, 2243-2249.

Walford RL (1969) The Immunological Theory of Aging, **Murkgaard Publn.**, Copenhagen.

Watford M, Hod Y, Chiao Y, Utter MF and Hanson R W (1981) The Unique role of the kidney in gluconeogenesis in the chicken. **J. Biol. Chem.** 256, 10023-10027.

Walker PR (1974)). Pyruvate kinase (PK II) isoenzyme is present in fetal rat liver but absent in adult tissue. **Life science** 15, 1507-1514.

Walker R (1983) The Molecular Biology of Enzyme Synthesis. Regulatory Mechanisms of Enzyme Adaptation, pp 263-354, **John Wiley and Sons**, New York.

Whitt GS (1970) Malate dehydrogenase is the main enzyme of malate-aspartate shuttle. **Experientia** 26, 734-736.

Williams GC (1957) Pleiotropic gene expression linked to development. **Evolution** 11, 398-402.

Williamson JR, Jacob A and Refino C (1971) Control of the removal of reducing equivalents from the cytosol in perfused rat liver. **J. Biol. Chem.** 246, 7632-7641.

Wilson PD (1972) Lactate dehydrogenase activity increases in liver of male. **Gerontologia** 18, 36-54.

Wilson PD and Franks LM (1971) Glucose-6-phosphate dehydrogenases increases in kidney of aging male. **Gerontologia** 17, 16-32.

Wise PM and Frye BE (1973) Corticosterone is known to increase during the early stages of development with a marked rise at about the 15th day. **J. Exp. Zool.**, 185, 277-292.

Wiseman MS, McKay D, Crow KE and Hardman MJ (1991) Rat liver mitochondrial malate dhydrogenase : purification, kinetic properties and role in ethanol metabolism. **Arch. Biochem. Biophys.** 290, 191-196.

Wood DC, Hodges CT and Harrison JH (1978) The relation of the pH and concentration-dependent dissociation of porcine mitochondrial malate dehydrogenase. **Biochem. Biophys. Res. Commun.** 82, 943-950.

Yagi T, Kagamiyama H, Motosugi K, Nozaki M and Soda K (1979) Crystallization and properties of aspartate aminotransferase from *Escherichia coli*. **FEBS Lett.** 100, 81-84.

Yamamoto KRA (1985) Steroid receptor regulated transcription of specific genes and gene networks. **Ann. Rev. Genet.** 19, 209-252.

Yarnell GR, Nelson PA and Wagle SR (1966) Carbohydrate is primarily utilized within the first 7 days of the 21-day incubation period in chicken development. **Arch. Biochem. Biophys.** 114, 539-542.

Yeung D and Oliver IL (1968) Glucagon increase has been correlated to the appearance of gluconeogenic enzymes such as PEPCK. **Biochem. J.** 108, 325-331.

Zuckerkandle E (1965) Sequential activation and repression of haemoglobin genes during development. **Sci. Am.** 212, 110-118.

BIO-DATA

Name	:	**HERBERT GOLDFIELD LYNGDOH**
Address	:	Greens' View, Professors Para, Malki, Shillong-793001.
Date of Birth	:	17th December, 1972

Educational Qualifications:

M. Sc_{NEHU} (Biochemistry) (1996)	2nd Class	57.22%
B. Sc_{NEHU} (Botany) (1994)	2nd Class	57.75%

Research Field : Enzymology, Aging

Research experience: Five years

Present Position : Lecturer, department of Biochemistry,
St. Anthony's College, Shillong.

Research Publication:

Lyngdoh HG and Sharma R (2001) Hydrocortisone and triiodothyronine regulation of malate–aspartate shuttle enzymes during postnatal development in chicken. **Indian Journal of Biochemistry & Biophysics.** 38, 170-175.

Conference/ symposia/ seminars:

1. Attended the **International conference on Radiation Biology : DNA damage, Repair & Carcinogenesis & Indo-German Satellite Symposium on Molecular Biology of Radiation, Damage & Repair**, 7th –10th April, 1998, Deptt. Biochemistry, NEHU, Shillong, India
2. Attended and presented a paper titled " Endogenous activity level of malate-aspartate shuttle isoenzymes in chicken", in the **68th Annual Meeting of Biological Chemists (India) & Symposium on Current Trends in Biology**, held at the Indian Institute of Science, Bangalore, from 27th-29th December 1999.

Member of scientific body:

Society of Biological Chemists (SBC), Bangalore, India