Indian Journal of Biochemistry & Biophysics
Vol. 38, June 2001, pp. 170-175

Hydrocortisone and triiodothyronine regulation of malate-aspartate shuttle enzymes during postnatal development of chicken

Herbert G Lyngdoh and Ramesh Sharma*
Department of Biochemistry, North Eastern Hill University, Shillong 793 022, India

Received 18 July 2000; revised and accepted 13 March 2001

The normal endogenous level of malate-aspartate shuttle enzymes and its regulation by hydrocortisone and triiodothyronine were studied in the liver and kidney of 0-, 30- and 60-day old male Rhode Island Red (RIR) chicken. The endogenous activity 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. On the other hand, activity of both cytosolic and mitochondrial aspartate aminotransferase (c- and m-AsAT) showed peak value at day 30 in both liver and kidney. Hydrocortisone administration induced c-MDH in the liver at all the ages studied, but did not influence the activity of the isoenzymes in the kidney whereas, it induced m-MDH in the liver at day 0 and in kidney at day 30. Administration of hydrocortisone, however, did not influence AsAT isoenzymes (c- and m-AsAT) in either of the tissues at any of the postnatal ages. Triiodothyronine induced c-MDH in the liver at all the ages whereas kidney isoenzyme was induced only at day 60. In contrast, m-MDH was induced by triiodothyronine in both liver and kidney at day 30 and 60. Administration of triiodothyronine did not influence c-AsAT of liver and kidney at either of the ages, whereas it induced m-AsAT of only liver at day 0 and 60. These findings indicated a tissue- and age-specific expression of the malate-aspartate shuttle enzymes in chicken and difference in the regulation exerted by hydrocortisone and triiodothyronine during postnatal development of chicken.

The malate-aspartate shuttle serves as one of the primary mechanisms for the transfer of reducing equivalents from the cytosolic NADH to the mitochondria in many animal tissues[1,2]. It has been established that the inner mitochondrial membrane is impermeable to NADH. The NADH formed by glycolysis in the cytoplasm by the oxidation of glyceraldehyde-3-phosphate must be regenerated to NAD^+ for glycolysis to operate. The shuttle involves an influx of malate and glutamate and an efflux of aspartate and 2-oxoglutarate from mitochondria[3-5]. The main enzymes of the shuttle are malate dehydrogenase (MDH; EC 1.1.1.37) and aspartate aminotransferase (AsAT; EC 2.6.1.1). Both these enzymes have two homologous and genetically independent isoenzymes; one in the cytosolic (c-) and the other in the mitochondrial (m-) fractions[6].

In developing animals, metabolic adjustments take place in different tissues as an adaptation to the changing demands made upon them. Many studies have been done on individual enzymes as a function of age in different groups of animals[7,8], however, reports on the activity of all the enzymes of a particular metabolic pathway are scanty[9]. Alterations in the level of enzymes and their inducibility by certain hormones are age-related phenomenon, characterized by changes in the responsiveness of tissues and cells to these hormones[10]. Glucocorticoids play a crucial role in the development and aging of animals[11,12]. The effect of glucocorticoids in post-hatch chicken resembles those observed in mammals[13]. It depends on the level as well as on the physicochemical properties of its receptors. Therefore, age-dependent changes in the inducibility of enzymes by glucocorticoids are influenced by the level of receptors and also by the post-receptor events[14,15]. On the other hand, effects of thyroid hormones at the cellular level are slower in avian during early stages of development as is true in mammals[16]. Thus, thyroid hormones also influence the age-dependent changes in enzymes of adult birds[17]. Earlier work on the malate-aspartate shuttle has been done mostly on the mammalian system. However, information on the development and hormonal regulation of avian malate-aspartate shuttle are scanty. The present work describes the endogenous activity level of chicken malate-aspartate shuttle at three ages (0-, 30- and 60-day old) in the

*Author for correspondence

liver and kidney. In addition, the effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity of shuttle enzymes are also reported and their regulatory roles are discussed.

Materials and Methods

Animals and chemicals

Male chicken (Rhodes Island Red, RIR breed) were locally purchased from a veterinary farm. They were maintained at 25±2°C under normal laboratory conditions and fed with a chick mash diet (Priemier Hatchery Ltd, Shillong) and tap water *ad libitum*. Chicks of three different postnatal age groups (0-, 30- and 60-day) were used for the experiments. All the chemicals used were of analytical grade, and the biochemicals were purchased from Sigma Chemical Co., USA.

Tissue preparation and assay of shuttle enzymes

Chicken were sacrificed at a fixed time of the day (9:00 hr) by decapitation. Tissues (liver and kidney) were removed immediately, washed in ice-cold saline (0.9% NaCl) and blotted dry. Homogenates (25%, w/v) of the liver and kidney were prepared in ice-cold 0.25 *M* sucrose. Each homogenate was centrifuged at 800×*g* for 10 min at 2°C to sediment nuclei. The resulting supernatant was further centrifuged at 14,000×*g* for 30 min at 2°C to sediment mitochondria. The supernatant thus obtained was used for the assay of cytosolic malate dehydrogenase (MDH) and aspartate aminotransferase (AsAT). The mitochondrial pellet was washed twice, suspended in a solubilizing medium (0.25 *M* sucrose/10 m*M* potassium phosphate buffer, *p*H 7.5/0.5% Triton X-100) for 3 hr , and used for the assay of mitochondrial (m-) MDH and AsAT.

Both the isoenzymes of MDH[18], and AsAT[19] were assayed spectrophotometrically in a Hitachi U-2000 spectrophotometer as described earlier[20,21]. Protein contents of cytosolic and mitochondrial fractions were determined by the dye binding method of Bradford[22] using bovine serum albumin as reference standard. The activity of both the isoenzymes of MDH and AsAT was expressed as unit (µmole NADH oxidised per min) per mg protein. The data were statistically analysed[23] and the level of significance (*p*-values) between different sets of data was calculated according to paired Student's t-test.

Hormone treatment

Effects of hydrocortisone and triiodothyronine were 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). Trial experiments were undertaken to determine the time- and dose-response of the isoenzymes towards hydrocortisone and triiodothyronine. Maximum response of the isoenzymes was obtained with a repeated dose of 1 mg/100 g body weight for three days for hydrocortisone and 25 µg/100 g body weight for three days for triiodothyronine. All chicken received the same amount of 0.5 ml suspension medium (10% ethanol in 0.9% NaCl) administered intraperitoneally (i.p.) at a fixed time of the day (9:00 hr) for three days. Chicken were sacrificed after 6 hr of the last hormone injection, and their tissues (liver and kidney) were taken out, washed in ice-cold saline, blotted dry and stored at -35°C till the assay of MDH and AsAT.

Results

Endogenous activity level of the malate-aspartate shuttle enzymes

Our results show that the normal endogenous activity (units/mg protein) of cytosolic malate dehydrogenase (c-MDH) was significantly higher in the liver on day 30 as compared to day 0 (+53%) and day 60 (+38%) (Fig. 1A). Mitochondrial (m-) MDH, however, does not exhibit any significant change in the liver upto day 30 and declines significantly (-51%) at day 60 (Fig. 1A). In kidney, both c- and m-MDH show (Fig. 1B) a significantly lower activity at

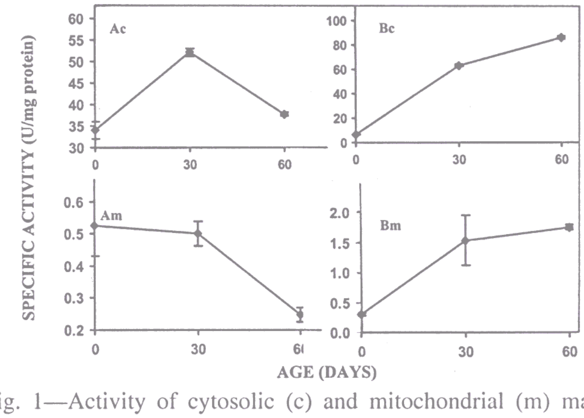

Fig. 1—Activity of cytosolic (c) and mitochondrial (m) malate dehydrogenase isoenzymes in the liver (A) and kidney (B) of normal male chicken at various postnatal ages. [Fractionation and assay conditions for respective isoenzymes are described in 'Materials and Methods' section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation].

day 0 and increase sharply at day 30 (8-fold and 4-fold, respectively) and at day 60 (+35% and +14%, respectively). On the other hand, activities of both cytosolic and mitochondrial aspartate aminotransferase (c- and m-AsAT) show a peak value at day 30 in both liver and kidney (Fig. 2A and B, respectively).

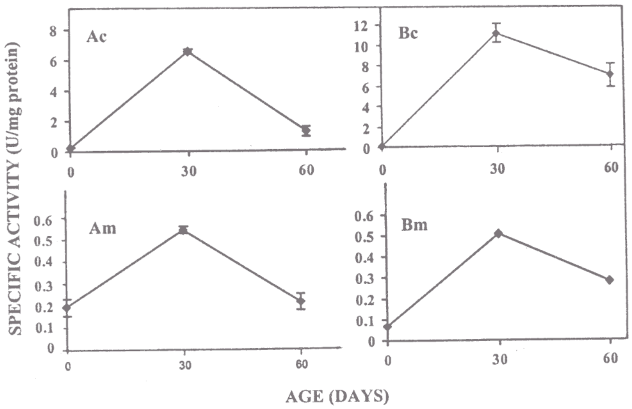

Fig. 2—Activity of cytosolic (c) and mitochondrial (m) aspartate aminotransferase isoenzymes in the liver (A) and kidney (B) of normal male chicken at various postnatal ages. [Fractionation and assay conditions for respective isoenzymes are described in 'Materials and Methods' section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation].

Hormonal regulation of malate dehydrogenase (MDH) isoenzymes

Hydrocortisone administration causes a significant increase in the activity (units/mg protein) of c-MDH in liver of chicken at day 30 (+23%) and day 60 (+32%) (Fig. 3A), while it does not influence the activity of this isoenzyme in the kidney of chicken (Fig. 4A). It however, significantly increases (+141%) the activity level of m-MDH at 0-day in liver and at day 30 (+102%) in kidney (Fig. 3B and Fig. 4B, respectively).

Administration of triiodothyronine, on the other hand, significantly increases the activity of liver c-MDH at 0-day (+61%) and 60-day (+87%) whereas, it influences moderately (+20%) the kidney isoenzyme only at day 60 (Fig. 3A and Fig. 4A, respectively).

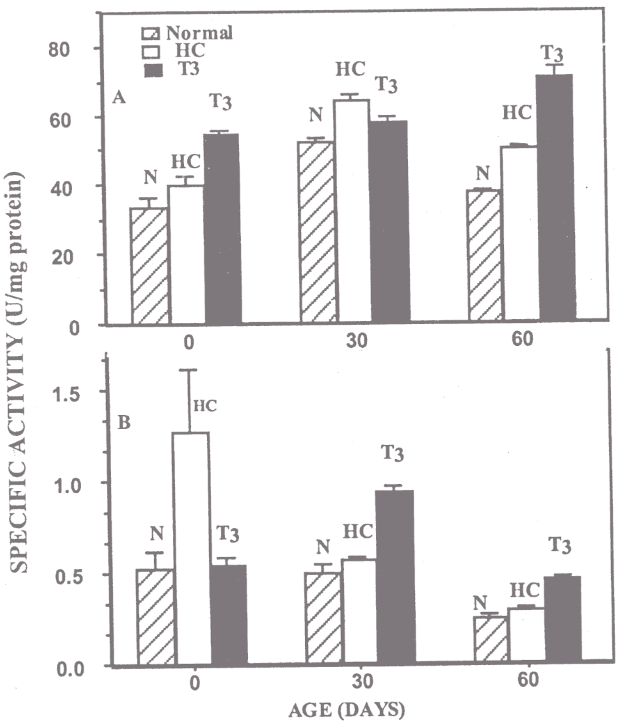

Fig. 3—Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity of cytosolic (A) and mitochondrial (B) malate dehydrogenase isoenzymes in the liver of male chicken of different postnatal ages. [N, normal; HC, hydrocortisone treated; T_3, triiodothyronine treated. Hormonal treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation].

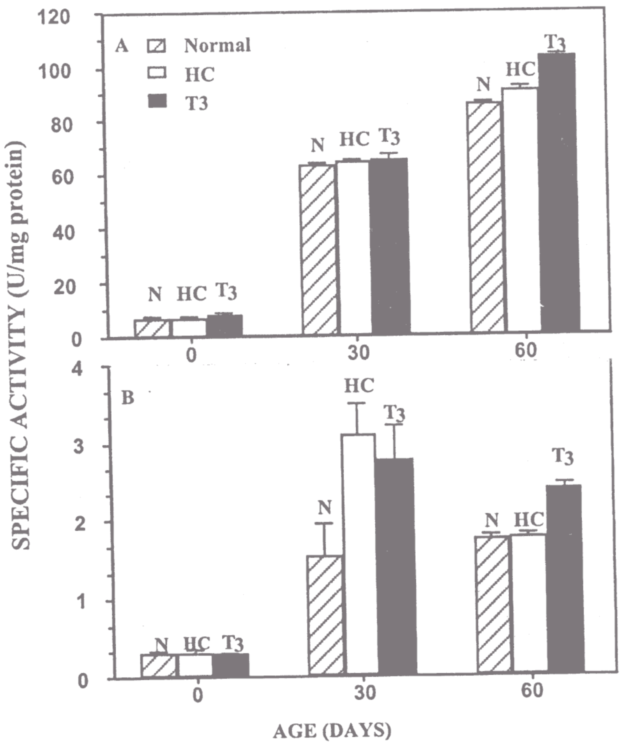

Fig. 4—Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity of cytosolic (A) and mitochondrial (B) malate dehydrogenase isoenzymes in the kidney of male chicken of different postnatal ages. [N, normal; HC, hydrocortisone treated; T_3, triiodothyronine treated. Hormonal treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation].

The activity of m-MDH is also increased significantly in the liver (+88% and +87%) and also in the kidney (+80% and +37%, respectively) at day 30 and day 60 (Fig. 3B and Fig. 4B).

Hormonal regulation of aspartate amino-transferase(AsAT) isoenzymes

Administration of hydrocortisone does not influence AsAT isoenzymes (c- and m-AsAT) in either liver or kidney at any of the postnatal ages studied (Fig. 5A,B and 6A,B). Triiodothyronine administration does not induce c-AsAT isoenzymes in both the tissues at these postnatal ages, however, it increases m-AsAT of liver at day 0 (+69%) and day 60 (+98%) (Fig. 5A, B and 6A, B).

Discussion

The malate-aspartate shuttle is mainly involved in the transfer of reducing equivalents from cytosolic NADH to the mitochondria in various tissues[4] . The cytosolic isoenzymes of both MDH and AsAT are also implicated in gluconeogenesis, since the former converts malate and the latter converts aspartate to oxaloacetate, which subsequently gets converted to phosphoenolpyruvate[11]. 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[24]. 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[25]. 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

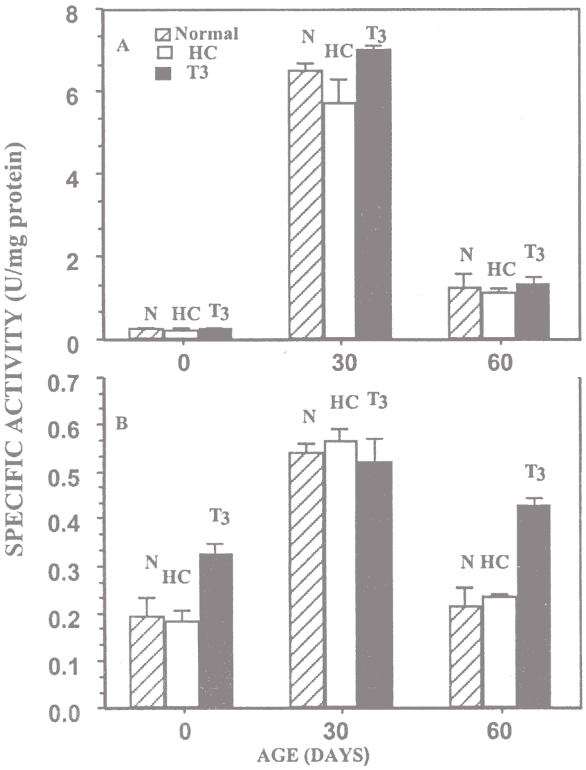

Fig. 5—Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity of cytosolic (A) and mitochondrial (B) aspartate aminotransferase isoenzymes in the liver of male chicken of different postnatal ages. [N, normal; HC, hydrocortisone treated); T_3, triiodothyronine treated. Hormonal treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation].

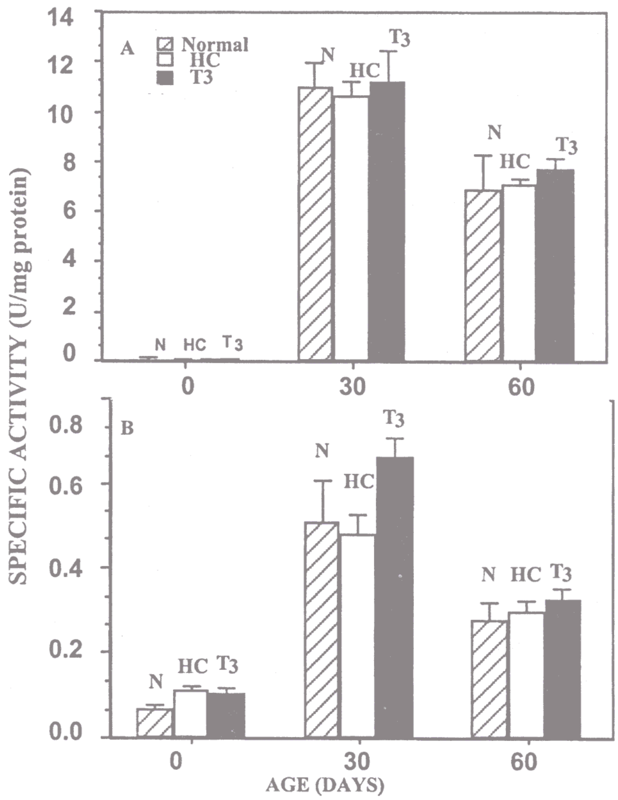

Fig. 6—Effects of hydrocortisone (HC) and triiodothyronine (T_3) on the activity of cytosolic (A) and mitochondrial (B) aspartate aminotransferase isoenzymes in the kidney of male chicken of different postnatal ages. [N, normal; HC, hydrocortisone treated; T_3, triiodothyronine treated. Hormonal treatments and other experimental conditions are described in methods section. Values are mean for 4-5 chicks of each age group. Bars represent standard deviation].

pathway. Keeping in view the differences in metabolic make up, we planned to study the endogenous level and hormonal regulation of malate-aspartate shuttle enzymes in the liver and kidney of chicken at various post-hatched ages.

The endogenous activity levels of both MDH and AsAT isoenzymes are significantly higher at day 30 in the liver and kidney of chicken indicating 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. 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[26]. We have previously reported an early expression of malate-aspartate shuttle activity and its enzymes in the liver of mice as compared to kidney[27]. The findings on chicken shuttle enzymes corroborate the metabolic differences in the chicken as compared to rats and mice.

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[28]. 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 gene responsible for the synthesis of c- and m-MDH are reported to be different[29,30]. 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. This is in difference to the glucocorticoid regulation of c-AsAT in rats and mice[14].

Results on the hormonal regulation by triiodothyronine of shuttle enzymes demonstrate that triiodothyronine 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 lifespan[16]. 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 responses towards triiodothyronine. These studies indicate that factors like hormones and their adaptive responses to enzymes exhibit a tissue- and age-related patterns depending on the metabolic state of the concerned tissue at that phase of organism's lifespan. Such adaptive responses may, in turn, be regulated by the level of hormones, their receptors/post-receptor events and tissue-specific factors needed for the expression of cognate genes in an organism-specific manner.

Acknowledgement

Fellowship support to HGL from the North Eastern Council, Goverment of India is gratefully acknowledged.

References

1 Cederbaum A I, Lieber C S, Beattie D S & Rubin E (1973) *Arch Biochem Biophys* 158, 763-781

2 McDonald M J (1983) *Arch Biochem Biophys* 213, 643-649

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

4 Lanoue K F, Bryla J & Basset D J P (1974) *J Biol Chem* 249, 7514-7521

5 Davis E J, Bremer J & Akerman K E (1980) *J Biol Chem* 255, 2277-2283

6 Braunstein A E (1973) in *The Enzymes* (Boyer P D ed) pp. 379-481, Vol IX, Third edition, Academic Press, New York

7 Kanungo M S (1980) in *Biochemistry of Aging*, pp. 79-126, Academic Press, London

8 DeAlmeida A F, Curi R & Newsholme E A (1989) *Int J Biochem* 21, 937-940

9 Sharma R (1988) in *Physiological Basis of Aging and Geriatrics* (Timiras P S ed) pp. 75-86, Macmillan Press, New York

10 Sharma R (1994) in *Physiological Basis of Aging and Geriatrics* (Timiras P S ed) pp. 50-52, CRC Press, Buca Raton, Florida

11 Cohen A (1973) *Horm Metab Res* 5, 66-78
12 Martin C E , Cake M H , Hartmann P E & Cook I F (1977) *Acta Endocrinol* 84, 167-176
13 Kuhn E R, Decuypere E Huybrechts L M & Darras V M (1990) in *Endocrinology of Birds : Molecular to Behavioural* (Wada M ed) pp. 129-142, Japan Sci. Soc. Press, Tokyo
14 Sharma R & Patnaik S K (1987) *Arch Gerontol Geriartr* 6, 27-32
15 Sharma R (1993) *Curr Sci* 65, 342-347
16 Hazelwood R L (1972) in *Avian Biology Vol II* (Farner D S ed) pp. 471-526, Academic Press, London
17 Harvey S (1990) in *Endocrinology of Birds: Molecular to Behavioural* (Wada M ed) pp. 129-142, Japan sci. Soc. Press, Tokyo
18 Kitto G B (1969) *Methods Enzymol* 13, 106-116
19 Karmen A (1955) *J Clin Invest* 34, 131-133
20 Herzfeld A & Greengard O (1971) *Biochim Biophys Acta* 237, 88-98
21 Dey S & Sharma R (1998) *Indian J Biochem Biophys* 35, 224-228
22 Bradford M (1976) *Anal Biochem* 72, 248-254
23 Garett H E (1966) *Statistics in Psychology and Education*, David Makey, New York
24 Lehninger A L, Nelson D L & Cox M M (1993) in *Principles of Biochemistry* (1st Indian ed) pp. 599-607, CBS Publ., Delhi
25 Ogata K, Watford M, Brady L J & Hanson R W (1982) *J Biol Chem*, 257, 5385-5391
26 Watford M, Hod Y, Chiao Y, Utter M F & Hanson R W (1981) *J Biol Chem* 256, 10023-10027
27 Sharma R, Dey S & Verma R (1992) *Biochem Int* 27, 1059-1066
28 Bohme H J, Belay D, Goltzseh W, Hofmann E, Lange L, Schubert C, Schulze E, Sparman G & Weiss E (1986) *Adv Enz Regul* 26, 31-61
29 Whitt G S (1971) *Experientia* 27, 647-648
30 Basaglia F (1989) *Comp Biochem Physiol* 91B, 213-226