COMPARATIVE STUDY OF HORMONAL REGULATION OF THE OXIDATIVE METABOLISM IN HIBERNATING AND NON-HIBERNATING AMPHIBIANS

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I hereby certify that the thesis entitled "Comparative Study of Hormonal Regulation of the Oxidative Metabolism in Hibernating and Non-hibernating Amphibians" by Ms. Anju Mahanta for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong (Meghalaya) embodies the record of original investigation carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph. D. degree. This work has not been submitted for any Degree of any University.

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Preface

During the course of evolution, animals developed a large number of systems (e.g. respiratory, circulatory, excretory, reproductive, nervous and endocrine systems etc.) and metabolic pathways (e.g. intermediary, oxidative, anabolic and catabolic pathways) for their successful survival and propagation/continuity. The nervous system and the neuroendocrine system were developed for regulation of proper functioning of various other systems and also for co-ordination among different inter-related systems and /or metabolic pathways.

The neuroendocrine system plays a major role in adaptation of vertebrates against their environment. Hormonal regulation of oxidative/energy metabolism is one of the major roles played by endocrine glands for successful survival of vertebrates in ever changing environment. The oxidative metabolism generates chemical energy in the form of ATP which is used by each and every system in living organisms for various purposes. The oxidation of food substances for energy production is controlled by a large number of oxidative enzymes, and hormones influence the oxidative metabolism by influencing activities of the oxidative enzymes. Since the energy requirement of different systems increases or decreases with changes in environment and activity, thus, the temporal role of various hormones involved in energy production is also altered in a way best suited to the organism.

There is a large body of information regarding the role of various hormones in the regulation of the oxidative metabolism in mammals, birds and reptiles. Now it is well established that while thyroid hormones are mainly responsible for the energy metabolism in homeotherms, the calorigenic role of these hormones is doubtful in poikilothermic vertebrates. In reptiles, hormones of gonads and adrenal are reportedly very important for the regulation of the oxidative metabolism. Preliminary studies indicate that adrenal and gonadal hormones might also be involved in the oxidative metabolism of amphibians. Since amphibians significantly differ from terrestrial vertebrates (mammals, birds and reptiles) in habits and habitats, a different endocrine mechanism is expected to be involved in the regulation of the energy metabolism. However, there is scarcity of information on the role of hormones in the oxidative metabolism of amphibians in general and of tropical/sub-tropical amphibians in particular. Therefore, it was thought worthwhile to undertake a comprehensive study of the role of a number of metabolic hormones in the regulation of the amphibian oxidative metabolism at different ambient temperatures under natural climatic conditions at Shillong. The experiments were conducted on adult males of two species, namely Indian streaked frog, Rana limnocharis (hibernating species) and Indian skipper frog, Rana cyanophlyctis (non-hibernating species).

The experimental studies incorporated in the thesis are divided into 7 chapters. The details of the chapters are given below:-

Chapter 1 : Materials and Methods

In this chapter details of materials and methods used in the present study are described.

Chapter 2 : Annual Variations in the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

This chapter deals with annual variations in the rate of oxygen consumption of different tissues (liver, skeletal muscle and kidney).

Chapter 3 : Role of Thyroid Hormones in the Regulation of the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

This chapter deals with experiments regarding effects of exogenous L-T_3, L-T_4 and PTU on the rate of oxygen consumption of different tissues during summer/rainy and winter months.

Chapter 4 : Role of Testicular Hormones in the Regulation of the oxidative metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

This chapter incorporates experimental studies regarding effects of testosterone and cyproterone acetate on tissues respiration during summer/rainy and winter months.

Chapter 5 : Role of Adrenal Hormones in the Regulation of the oxidative metabolism in *Rana limnocharis* and *Rana cyanophlyctis*..

This chapter deals with the experimental studies regarding the calorigenic action of epinephrine, norepinephrine, corticosterone, cortisol and metapyrone in tissues during summer/rainy and winter months. Experiments involving alpha- and beta-adrenergic agonists and antagonists are also included in the Chapter.

Chapter 6 : Effect of Melatonin in the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

In this chapter experimental studies regarding the effects of melatonin on the rate of tissues respiration during summer/rainy and winter months have been included.

Chapter 7 : Summary and Conclusions

This chapter contains general summary and conclusions which are based on experimental findings discussed in earlier chapters.

It is expected that the experimental findings of the present study shall provide a complete picture of hormonal regulation of the oxidative metabolism in the two amphibian species. These findings are expected to bridge the gap of information and throw light on the evolution of hormonal control mechanism involved in the regulation of the oxidative metabolism.

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CONTENTS

INTRODUCTION

All living organisms require a constant supply of energy to sustain life. In animals, production of energy is achieved by the oxidation of food. Oxygen is a prerequisite for the oxidation of food. The oxidative metabolism involves a chain of biochemical reactions responsible for production and storage of energy, mainly as ATP which is used for providing energy to maintain various processes like active transport, oxidation of fat and carbohydrate, protein synthesis, muscle contraction, sodium pump, formation of high energy phosphate bonds, calcium pump etc. The energy released as heat is used for thermoregulation.

The history of oxidative metabolism goes back to the days of Levoisier (1780). The rate of oxygen consumption is not merely an index of activity of energy production, but also a standard of overall measure of metabolic processes (Bishop, 1952). Metabolic rate of vertebrates is influenced by a number of factors both internal (hormones, age, sex, feeding status etc.) and external (temperature, photoperiod, seasons/months,availability of food, nature of food etc.). Among internal factors, hormones secreted from Adrenal, Thyroid, Gonads and Pineal are actively involved in the regulation of the oxidative metabolism. Notwithstanding a large body of information on hormonal regulation of oxidative metabolism in homeotherms, information on poikilotherms is sparse and scanty. It is well established that thyroid hormones (thyroxine and triiodothyronine) play a major role in the regulation of energy yielding metabolic processes in homeotherms. However, the calorigenic effects of thyroid hormones in poikilotherms are doubtful. In reptiles, gonadal hormones are reported to play a critical role in the regulation of oxygen consumption at low temperature, where thyroid hormones are calorigenically ineffective. Therefore, it seems that during the course of evolution, homeotherms and poikilotherms have developed different sets of endocrinological/hormonal mechanism(s) for the regulation of energy metabolism to meet their energy demands. A brief review of literature on hormonal regulation of the oxidative metabolism in different groups of vertebrates is given below.

Hormonal Regulation Of The Oxidative Metabolism In Homeotherms

Homeotherms maintain their body temperature constant (within a narrow range) irrespective of wide variations in environmental temperature. In order to maintain a constant body temperature, a high metabolic rate is maintained by mammals and birds. Unlike in homeotherms, body temperature in poikilotherms conforms to that of the environment. In general, the metabolic rate of homeotherms is found to be higher than poikilotherms kept at the same temperature.

Hormonal Regulation of Oxidative metabolism in Mammals:

Magnus Levy (1895) was first to report that thyroid hyperactivity increased and hypoactivity decreased the rate of oxygen consumption in man. Extensive studies on several mammalian species have clearly established that thyroid hormones (T_3 and T_4) increase and thyroidectomy decreases the rate of whole body oxygen consumption. Administration of desiccated thyroid increased the rate of oxygen consumption in liver, muscle and kidney in rat (Rohrer, 1924). Further, thyroidectomy depressed oxygen consumption of diaphragm of rats and addition of T_4 reduced the decline in oxygen consumption of the kidney cortex of thyroidectomized rats in culture medium (Barker, 1957). Now the role of thyroid hormones in calorigenesis in mammals is well established (Pitt-Rivers and Tata, 1959; Barrington, 1964; DeGroot and Stanbury, 1975; Bentley, 1976; Oppenheimer, 1979). Catabolic doses of T_3 were found to induce immediate increase in the basal metabolic rate and mitochondrial respiration (Tata et al., 1963). Thyroid hormones, when administered in vivo or in vitro, stimulated mitochondrial protein synthesis which paralleled the increase in oxygen consumption (Brown, 1966; Hoeh, 1968). Thyroid hormones increase the rate of oxygen consumption of a number of vital tissues. Most of the in vivo studies in rats confirmed that the oxidative metabolism is stimulated by thyroid hormone(s) in all tissues except mature brain, testes and spleen (Oppenheimer, 1979). However, T_4 increased oxygen consumption of neonatal rat brain (Oppenheimer, 1979; Rajan and Katyare, 1982). Biphasic actions of thyroid hormones on oxidative metabolism are also reported in mammals. A large dose of thyroxine generally produces catabolic action with increased oxygen uptake while a small dose in normal or hypothyroidic animal produces anabolic action (Tata, 1964).

Environmental factors also influence calorigenic role of thyroid hormones in mammals. Increased oxygen consumption accompanied with thyroid hyperplasia has been observed in rats exposed to prolonged cold. Hypothyroidic animals, in contrast, showed decreased capacity to adapt against low temperature. It seems that the presence of thyroid hormones and increased metabolic rate are essential for the successful survival of mammals at low temperatures (Turner and Bagnara, 1976). Thyroid hormones directly regulate the activity of the oxidative enzyme. a-Glycerophosphate dehydrogenase (a-GPDH) in liver (Shambaugh,1978; Bernal and DeGroot,1980). a-GPDH plays an important role in the regulation of basal metabolic rate (Lee and Lardy, 1965). The stimulation of a-GPDH activity by thyroid hormones (Oppenheimer, 1975, 1979; Somjen et al., 1981). The stimulatory effect of thyroid hormones on the metabolic rate was blocked by inhibitors of protein synthesis (Lee et al.,1959; Dillmann,et al, 1977). Hepatic mitochondrial a-6-GPDH activity was increased in hyperthyroidic and decreased in hypothyroidic rats (Hamada Satoshi and Yasunao-Yoshimasa. 1983) and rabbits (Ablaev et al., 1979). An age-related decrease in the hepatic mitochondrial L-GPDH activity and its stimulation by T_4 treatment was observed in rats (Kaliman,et al., 1981). Thyroid hormones are reported to have special affinity for liver mitochondrial respiratory enzymes in mammals (Tata, 1980).

There has been substantial progress in defining the molecular basis of thyroid hormone action at cellular level. On the basis of recent studies, it has been concluded that in mammals T_3 is the active hormone and T_4 serves as a precursor hormone (Braverman et al.. 1970; Schwartzetal., 1971). The nucleus has been proposed to be the target for thyroid hormone action (Tata etal., 1963: Tata, 1964, 1967; Tata and Widnell, 1966). Thyroid hormone receptors have been identified in the nucleus, mitochondria, cytoplasm and plasma membrane of target cells (Oppenheimer *et al.*, 1975, Oppenheimer, 1979; Sterling, 1979; Tata, 1980). However, Ismail-Beigi and Edelman (1970, 1971) have reported that thyroid hormones primarily stimulate $Na^+ - K^+$-ATPase (Sodium pump activity) leading to increased availability of ADP (P/O ratio) which in turn increases mitochondrial oxygen consumption. The well known effect of thyroid hormones on protein synthesis can be blocked by inhibitors (Actinomycin-D and Chloramphenicol) of transcription (Gorbman *et al.*,1983a). Further, mitochondrial biogenesis is reportedly due to the direct action of thyroid hormones on the nucleus and mitochondrial genomes (Nelson *et al.*, 1987). These findings strongly suggest that thyroid hormones influence the process of genomic transcription (Seelig *et al.*, 1981, 1982).

Recent investigations suggest that T_4 is also deoidinated to 3, 3',5'-triiodothyronine which is also called as r-T_3 (reverse T_3). The formation of r-T_3 is reported in man, rats, sheep, lambs and other mammals (Wray *et al.*, 1980; Vybiral *et al.*, 1985; Norman and Litwack,1987). However, the exact role of rT_3 in mammals is not established (Norman and Litwack, 1987). In rats, rT_3 seems to be involved in the regulation of BMR. When rT_3 is administered to rats, it counteracts the calorigenic action of adrenaline (Vybiral, *et al.*, 1985). Further, rT_3 has been reported to inhibit the metabolic action of T_3 in lambs (Lynch *et al.*, 1985). rT_3 is also reported to be involved in the regulation of T_3 formation. Increased formation of rT_3 inhibits formation of T_3 due to deiodination of T_4 (Boye, 1986; Obregon *et al.*, 1986; Norman and Litwack, 1987). Fasting is reported to increase rT_3 production and decrease T_3 receptors (Schussler and Orlando, 1978). Therefore, it seems that low BMR in fasting mammals might be due to decreased T_3 receptors and T_3 production and also due to increased production of rT_3. These evidences suggest that increased production of rT_3 might be responsible for decreased metabolic rate in mammmals as reported in Birds (Vybiral _et al._, 1985; Lynch, _et al._,1985; Abdel-Fattah _et al._, 1990).

There is scarcity of information on effects of sex steroids on the oxidative metabolism of mammmals. In experimentally hypothyroidic rats, 17B-estradiol reduced basal metabolic rate (Sherwood, 1941). However, administration of testosterone has been reported to increase the rate of oxygen consumption in the ventral prostrate of normal and castrated dogs (Barron and Huggins, 1944). Some studies indicate involvement of pituitary-gonad axis in the regulation of the oxidative metabolism of mammalian brain (Mas and Salis, 1977).

Catecholamines (Nor-epinephrine and epinephrine) are also very important as calorigenic hormones in mammals. Large doses of epinephrine and Norepinephrine reduce oxygen consumption transiently and then increase the metabolic rate significantly in mammalian species (Karlberg _et al._, 1962; Muller and Krake, 1963; Ellis, 1965). The ability to increase the metabolic rate might be one of the most important functions of these hormones (Brodie _et al._, 1966). Adrenaline is found to be more potent than norepinephrine in stimulating the rate of oxygen consumption in mammals (Hsieh and Carlson, 1957; Gorbman and Bern, 1962; Hagen and Hagen, 1964; Frieden and

Lipner, 1971). Moreover, increased secretion of adrenaline from nerve endings and /or Adrenal medulla is seen when mammals are exposed to severe and chronic cold stress (Himms Hagen, 1975; Joels, 1975). Adrenaline secretion is supposed to be the last defensive mechanism against cold and other stresses (Cannon, 1928; Robinson et al., 1972).

Adrenocorticoids are reported to increase blood glucose and gluconeogenesis and deposition of glycogen in liver (Gorbman et al., 1983b). Glucocorticoids affect glucose metabolism through gluconeogenesis (Exton and Park, 1972). Hydrocortisone is reported to act as a regulatory factor in governing selective permeability of mitochondrial membrane and thereby in tissue metabolism. This hormone inhibits oxidative metabolism without having any effects on the oxidative phosphorylation in rat (Gallahger, 1960). The oxidative phosphorylation in hepatic mitochondria of rat was inhibited by corticosterone (Kerppola, 1960). However, a single injection of hydrocortisone resulted in marked increase in oxygen uptake by rat liver homogenate (Goetsch and McDonald, 1962). Further, corticosterone treatment has also been reported to increase metabolic rate of rat liver and ATP production (Bottoms and Goetsch, 1968). These reports seem to suggest that corticosteroids might also be involved in the regulation of the oxidative metabolism of mammals.

It has been suggested that photoperiod acting via pineal gland is capable of influencing energy metabolism and thermoregulation in mammals (Heldmaier et al., 1989). Short photoperiod and administration of melatonin increased the behavioral thermoregulation as well as the non-shivering thermogenesis and also improved the thermogenic capacity of mammals and birds (Ralph *et al*., 1979a; Heldmaier *et al*., 1981; Hall and Lynch, 1985; Holtorf *et al*., 1985; Heldmaier and Lynch, 1986; McElroy and Wade, 1986; Saarela and Heldmaier, 1987; Puchalski *et al*., 1988; Heldmaier *et al*., 1989). There are few indications that melatonin can influence metabolic rate of mammals also by affecting thyroid activity (Lewinsky *et al*., 1987)

Hormonal Regulation of the Oxidative Metabolism in Birds:-

As reported in mammals, thyroid hormones are actively involved in the regulation of the oxidative metabolism of birds also. Thyroidectomy reportedly decreased and thyroxine increased oxygen uptake in liver, heart and skeletal muscle of chickens and pigeons (Haarman,1936). A significant decline in the whole body oxygen consumption after thyroidectomy has been observed in goose (Lee and Lee, 1937), chick (Winchester, 1939), pigeon (Marvin and Smith, 1943), spotted munia, (Thapliyal *et al*., 1977,1983a). Myna and redheaded bunting (Thapliyal,*et al*., 1983b). Thyroxine and triiodothyronine and combination of both the hormones in chick (Singh,*et al*., 1968) and thyroxine in spotted munia have been shown to stimulate the rate of oxygen consumption (Thapliyal, 1980a;Thapliyal,*et al*. 1981). Thyroidectomy inhibited and administration of low doses of T_4 stimulated oxidative metabolism in isolated liver tissue and skeletal muscle of spotted munia, whereas high doses were found to be ineffective in spotted munia (Thapliyal *et al*., 1983a). A direct relationship between the production of T_3 and level of energy intake in chicken has also been reported (Sharp and Klandorf, 1985). Thyroxine induces a significant increase in the activities of certain glycolytic, enzymes in Japanese quail, *Coturnix coturnix* at different temperatures (Konecka and Majewska, 1980). There is very little information on the formation of rT_3 in birds and its physiological role remains to be investigated. Recent reports indicate that, as in mammals, rT_3 also acts as a hypometabolic hormone and depresses metabolic rate in birds. Abdel-Fattah, *et al.*, (1990) have reported that rT_3 administration depresses whole body oxygen consumption of chickens. When rT_3 is injected in combination with T_3, it delays the stimulatory effects of T_3 on oxygen consumption (Abdel-Fattah *et al.*, 1990).

Hypometabolic action of rT_3 in both birds (Abdel-Fattah ,1990) and mammals (Lynch *et al.*, 1985) strongly suggest that rT_3 is actively involved in the regulation of the metabolic rate in homeothermic vertebrates. There are two possibilities for the hypometabolic action of rT_3 : (i) rT_3 might be occupying the rT_3 receptors, and thereby, it might be inhibiting the metabolic action of T_3 (Chopra, 1977), and (ii) rT_3 might be decreasing the formation of T_3 by influencing the process of deiodination of T_4 (Boye, 1986; Obregon *et al* 1986; Norman and Litwack, 1987). Involvement of rT_3 in energy metabolism is evident from the reports that rT_3 formation is increased in chickens exposed to higher temperature(s) (35 dgree C) and also after fasting (Rudas and Pethes, 1984a; Sechman *et al.*, 1989). Increased level of rT_3 in mammals under emotional stress or in hyperthyroidic mammals (Chopra, 1981; Robbins, 1981; Bobek *et al.*, 1986;

Niezgoda, et al., 1987) also indicates the involvement of reverse T_3 in calorigenesis. Circulating level of rT_3 is reportedly increased under physiological conditions which are associated with decreased metabolic rate, i.e., high ambient temperature, fasting, low level of T_3 etc. (Schussler and Orlando, 1978; Danforth, 1983; Rudas and Pethes, 1984b; Sechman et al., 1989). rT_3 is also reported to inhibit the thermogenic action of adrenaline in rats (Vybiral et al., 1985) and calorigenic action of T_3 in lambs (Lynch et al., 1985). These findings seem to suggest that rT_3 plays a very important role in decreasing the metabolic rate in homeotherms under certain physiological conditions. Thus, rT_3 formation might be an adaptation for keeping the metabolic rate at low level to conserve energy under unfavorable conditions. Further, increase and decrease in the oxidative metabolism might be governed by respective increase and decrease in the ratio of T_3 and rT_3. However, these suggestions remain to be supported by experimental findings.

In birds, very little is known about the role of gonadal hormones in energy metabolism (Assenmecher, 1973). Energy metabolism is reported to be decreased significantly in castrated quails and the reduction in the metabolism is not affected by administration of testosterone (Hanssler and Prinzinger, 1979). Recently it has been reported that, in addition to thyroid hormones, the oxidative metabolism of photosensitive redheaded bunting is also influenced by gonadal hormones (Thapliyal, et al., 1983b).

There is practically no evidence in favor of calorigenic role of catecholamines in birds. Administration of catecholamines could not alter the oxidative metabolism in pigeons (Hart, 1962) and gulls (Palonkangas _et al_., 1971). Functional differences of corticoids among non-mammalian vertebrates are reported. Glucocorticoids cause hyperglycemia and glycogen deposition in birds (Snedecor, _et al._, 1963). Further, administration of corticosterone in a bird, (_Parus major_) stimulated the rate of oxygen consumption (Hissa and Palonkangas, 1970). These findings suggest that while catecholamines are not involved in energy metabolism, corticoids are capable of stimulating the metabolic rate of birds.

The Pineal gland, which regulates/synchronizes a number of circadian and circannual rhythms, also affects endocrine glands and metabolism. It has been suggested that photoperiod, acting via pineal gland, is capable of influencing energy metabolism and thermoregulation in avian species (Heldmaier, _et al._, 1989). Short photoperiod and melatonin administration increase the behavioral thermoregulation as well as non-shivering thermogenesis, and also improve the thermogenic capacity of homeotherms (McElroy, _et al._, 1986; Viswanathan, _et al._, 1986; Heldmaier, _et al._, 1989). Melatonin treatment in pinealectomized chicken (melatonin added to food) increased the body weight, food intake and energy retention. These responses are accompanied with an increase in plasma T_3 and T_4 (Cogburn and Harrison, 1980; Osei et al.,1989). These findings indicate that the pineal is also, directly or indirectly, involved in energy metabolism in birds. At present, the mechanism of action and its biological significance are not well explored.

Hormonal Regulation Of the Oxidative metabolism in Poikilotherms

The oxidative metabolism of Poikilothermic vertebrates is regulated by a complex neuroendocrine mechanism (Gupta and Thapliyal, 1991). Hormones of thyroid, gonads, adrenal and pineal are actively involved in the regulatory process. Unlike homeotherms, poikilotherms seem to have evolved different mechanism(s) for the regulation of their oxidative metabolism. A critical review of literature on the role of hormones in energy metabolism of poikilothermic vertebrates is given below.

Hormonal regulation of oxidative metabolism in fish

Numerous attempts have been made to establish the calorigenic action of thyroid hormones in ectothermic vertebrates. Several investigators have reported stimulation of the metabolic rate following administration of thyroid hormones and decrease in the respiratory rate following thyroidectomy (chemical/surgical/radiological) in a number of piscine species (Smith, 1930; Haarman, 1936; Smith, and Mathews, 1947; 1948; Jones, *et al.*, 1951; Osborn, 1951; Zaks and Zamkova, 1952; Muller, 1953; Chawin and Rossmoore, 1956; Hopper, 1959; Mohsen and Godet, 1960; Pritchard and Gorbman, 1960; Wolf and Wolf , 1964; Sage, 1965; 1968; Massey and Smith, 1968; Ruhland, 1969, 1970; Leray *et al.*, 1970; Gabos, *et al.*, 1973; Pandey and Munshi, 1976; Peter and Oommen, 1987, 1988a, 1989c,a). In contrast to these reports, an equally large number of scientists could not find any effects of thyroxine administration in fishes like *Lebistes reliculatus* (Drexler and Von Issekutz, 1935), *Opsanustau* (Root and Etkin, 1937), *Carassius auratus* (Etkin *et al.*, 1940; Hasler and Meyer, 1942), *Carassius auratus* and *Rhodeus amatus* (Punt and Jungbloed, 1945), *Carassius auratus* (Chavin and Rossmoore, 1956), Carassius sp. at 20 ^{0}C (Hoar, 1958), *Scyllium canicula* (Matty, 1954), *Salmo gairdneri* at 16.2-17.5 ^{0}C (Fromme and Reinke, 1956). It is reported that as in mammals, in fish also T_4 acts only after its conversion into T_3 which represents the active thyroid hormone at the target cells level (Darling, *et al.*, 1982). In a recent study, *in vivo* and *in vitro* administration of T_3 and T_4 did not stimulate the respiratory rate of liver and skeletal muscle tissues of an air breathing fish (*Clarius batrachus*) maintained at 16 ^{0}C (Gupta, 1988). This report again confirms the ineffectiveness of thyroid hormones at low temperature.

There are few reports regarding the stimulatory effect of thyroid hormones on the oxidative enzymes in a number of tissues (skeletal muscle, cardiac muscle, liver, gills, brain, kidney etc) of fishes (Massey and Smith, 1968; Leray *et al.*, 1970; Shivkumar and Jayaraman, 1984; Leloup and Luze, 1985). In *Anabas testudineus*, administration of a physiological dose of T_3 significantly stimulated the activities of cytochrome oxidase and a-GPDH while it inhibited activities of mitochondrial Adenosine triphosphase, glucose-6-phosphate dehydrogenase and cytosolic malate dehydrogenase (Peter and Oommen, 1987). Thyroid hormones are also reported to be involved in the regulation of the intermediary metabolism in fishes (Narayan Singh and Eales, 1975; Eales, 1979; Plisetskaky *et al.*, 1983). Involvement of these hormones in both intermediary and oxidative metabolism seems to suggest that even the metabolic function of the thyroid gland has been conserved to a greater extent during the complex course of evolution (Gupta and Thapliyal, 1991).

Gonadal hormones play a major role in the development, growth, maturation and maintainance of the primary and secondary sex characters in all vertebrates. Hasler and Meyer (1942) pointed out the importance of sex steroids in the acceleration of oxygen consumption in gold fish, *Carassius auratus*. Testosterene administration significantly stimulated oxygen consumption of gold fish at 20^0C (Hoar, 1958). It is also observed that administration of testosterone in males and estradiol in females is followed by stimulation of respiratory rate in a number of fish species (Raffy and Fountaine. 1930: Stanley and Tescher. 1931: Mann. 1939: Gupta. 1988).

In some of the fishes. hypophysectomy resulted in decreased metabolic rate. presumably due to decreased levels of gonadal hormones (Chavin and Rossmoore. 1956: Hanson and Stanley. 1970: Johanson and Gomery. 1973: Chan and Woo. 1978a). Recent reports strongly suggest direct involvement of gonadal hormones in regulation of the oxidative metabolism of amphibians and reptiles (Gupta. 1982: Thapliyal *et al.*. 1974a,c,1975a,d; Chakravartty, 1990: Gupta and Thapliyal, 1991). Estrogen administration, both *in vivo* and *in vitro*. has recently been reported to regulate the activities of hepatic oxidative enzymes in a teleost *Anabas testndineus* (Peter and Oommen. 1989b.d).

Adrenaline produced marked hyperglycemia in many fishes (Patent. 1970). while noradrenaline acted at a slower rate (Young and chavin . 1965; Matty and Lone. 1983; Ottolenghi *et al.*. 1984). Since hyperglycemia is generally associated with glucose oxidation. Adrenaline and Noradrenaline also seem to be involved in the oxidative metabolism of fish. Adrenaline administration has been reported to increase blood glucose. blood lactate and plasma free fatty acids in *Anguilla anguilla* (Larsson, 1973), northern pike, *Esox lucius* (Thorpe and Ince, 1974), in rainbow trout. *Samo gairdneri* (Moratta *et al.*.

1982), and *Ictalurus melas* (Ottolenghi *et al.*, 1984). Glucogen breakdown followed by an increase in glucose-6-phosphatase activity by adrenaline is reported in *Anabas testudineus* (Jameela and Oommen, 1988). Further, adrenaline is also reported to increase the activities of oxidative enzymes like cytochrome oxidase, ATPase, a-GPDH, SDH, mitochondrial and cytosolic MDH in *Anabas testudineus* (Ignatius and Oommen, 1987). However, notwithstanding these reports, adrenaline inhibited the rate of oxygen consumption in *Girella nigricans* (Smith and Mathews, 1942), *Tilapia mossambica* (Banerjee and Joshi, 1981) and *Sarotherodon mossambica* (Akbarsha, 1984).

Corticoid hormones also seem to be involved in the energy metabolism of fish. It is reported that oxygen consumption in Japanese eel, *Anguilla japonica* was stimulated by cortisol (Chan and Woo, 1978 a). Corticosterone has been shown to stimulate the respiratory rate of liver and muscle tissue of *clarius batrachus* (Gupta, 1988). Glucocorticoids are also reported to stimulate oxidative enzymes in *Anabas testudineus* (Ignatius and Oommen, 1990). Corticoids are also reported to be involved in the intermediary metabolism of many fish species. Cortisol produces hyperglycemia and stimulates gluconeogenesis (Butler, 1973). Cortisol stimulates gluconeogenesis and lipolysis in *Anguilla anguilla* (Lidman *et al.*, 1979). Liver and muscle glycogen was increased following cortisol administration in many fish species (Robertson *et al.*, 1963; Chidambaram *et al.*,1973). Effects of corti

-coid hormones on carbohydrate metabolism indirectly indicate the involvement of these hormones in energy metabolism of fish. However, unlike in other vertebrates, there is no information about the role of melatonin in the energy metabolism of any fish species (Gupta and Thapliyal, 1991).

Regulation of the Oxidative Metabolism in Reptiles

Influence of hormones on the oxidative metabolism of reptiles was practically not known till late fifties. Maher and Levedahl (1959) observed that the lizard, *Anolis carolinensis*, maintained at room temperature (22°-25°C) did not show any change in the rate of oxygen consumption following thyroidectomy and thyroxine or thyrotropin administration. However, when these animals were maintained at 30°C, the rate of oxygen consumption decreased after thyroidectomy and increased following thyroxine and thyrotropin injections. Earlier, thyroid feeding was found to have no effect on the rate of oxygen consumption in a lizard (Drexler and Issekutz, 1935). Later, temperature-dependent effects of thyroid hormones and thyroidectomy on the oxidative metabolism was confirmed in *Lacerta muralis* (Maher, 1961), *Eumeces obsoletus* (Maher, 1964), *Sceloporus cyanogenys* (Wilhoft, 1966), and *Natrix rhombifera* (Turner and Tipton, 1972). Similarly. Thapliyal and co-workers also reported temperature-dependent stimulatory effects of thyroid hormones on the rate of oxygen consumption of whole body. liver and skeletal muscle of four tropical reptiles. i.e. .*Calotes versicolor*,*Hemidactylas flaviviridis*,*Natrix piscator*.and *Ptyas mucosus* (Chandola.*et al.*. 1973;1974b: Thapliyal.*et al.*. 1974b: Kumar.*et al.*. 1974: Gupta. 1982: Gupta and Thapliyal. 1985a: Kar.*et al.*. 1989: Chandola-Saklani and Kar. 1990). Thyroidectomy significantly decreased the rate of oxygen consumption of the whole body. liver. muscle. kidney and brain of *C. versicolor* and the declined rate of oxygen consumption followed by thyroidectomy was inhibited by L-T_4 (Gupta and Thapliyal. 1985a) throughout the year except during hybernation when thyroid hormones are absent or present in trace quantities (Thapliyal and Chandola. 1973). *In vitro* stimulation of oxygen uptake of various tissues of reptiles following administration of thyroid hormones suggests that thyroid hormones stimulate oxidative process by acting directly at cellular and/or at sub-cellular levels (Thapliyal and Gupta. 1983: Gupta and Thapliyal. 1985a. 1991). This suggestion is further supported by a number of recent findings indicating that both T_3 and T_4 stimulate a wide range of mitochondrial enzymes in a number of tissues (skeletal muscle. cardiac muscle. liver. gills. brain. kidney etc) of reptiles (John-Alder. 1983. 1984a.b. 1986. 1988. 1990a,b: Oommen and Sreedeviamma. 1988: Joos and John-Alder. 1990: John-Alder and Joos. 1991). Thyroid hormones are also actively involved in the regulation of the intermediary metabolism of many reptilian species (Lynn. 1970: Thapliyal *et al.*. 1974b: Thapliyal and Gupta. 1984: Gupta *et al.*. 1975: Thapliyal. 1980b: Chiu *et al.*. 1986: Chiu and Woo. 1988: Eales. 1990. Jacob and Oommen. 1990). Involvement of these hormones in both intermediary and oxidative metabolism of poikilotherms (fish and reptiles) seems to suggest that the metabolic function of the thyroid gland has been conserved to a greater extent during the complex course of evolution (Gupta and Thapliyal. 1991).

Gonadal hormones. in addition to their reproductive functions. are also reported to influence reptilian oxidative metabolism (Thapliyal *et al.*. 1974a.c: Thapliyal and Gupta. 1983. 1984). In a series of *in vivo* and *in vitro* experiments. Thapliyal and co-workers have reported significant increase in respiratory rates of vital tissues following administration of gonadal hormones and significant decrease in the respiratory rates following castration/ovariectomy in a number of reptilian species (Chandola *et al.*. 1974a: Thapliyal *et al.*. 1974a: Thapliyal *et al.*. 1974c: Thapliyal *et al.*. 1975a.d: Oommen. 1980. 1981: Thapliyal and Gupta. 1983. 1984: Oommen and Sreedeviamma. 1988). Castration in *Calotes versicolor* caused significant decrease in the rate of respiration of whole body and tissues. Administration of testosterone always reversed the depressive effect of castration and also further increased the rate of respiration of the whole body (Gupta and Thapliyal, 1985a). Thapliyal et al. (1974a) were the first to report the calorigenic action of gonadal steroids in reptiles during winter months. Detailed studies on the effect of temperature and photoperiod on gonadal activity show that the calorigenic action of male hormone is independent of these external factors (Gupta and Thapliyal, 1985a). Testosterone was found to be calorigenic even at simulated low temperature (15^0 C) where thyroxine failed in stimulating the oxygen uptake rate (Thapliyal and Gupta, 1984). Calorigenic effect of gonadal hormones seems to be determined by the energy demand of various tissues. Recently, testosterone has been reported to stimulate a number of mitochondrial enzymes in the liver of indian garden lizard (Oommen and sreedeviamma, 1988). The metabolic rate in Chalcides ocellatus has been found to be more sensitive to gonadal hormones (testosterone and estradiol) at 15^0 C and below (Al Sadoon and El Banaa, 1986). The sensivity towards gonadal hormones was reduced when the animals approached their prefered body temperature (28^0-37^0 C). Reduction in the sensivity might be a measure of conservation of energy (Al-Sadoon and Spellberg, 1985). The calorigenic action of gonadal hormones in lizards at lower temperature could have an adaptive and ecological significance since it may help the lizards to meet energy demands during winter months when thyroid hormones are calorigenically ineffective. Recently it is has been reported that Testosterone significantly increased the whole body oxygen consumption in the gonadectomized male lizards *Chalcides ocellatus* and estradiol significantly increased oxygen consumption in gonadectomized females (Al Sadoon, *et al.*, 1990).

Gonadal hormones have also been reported to exert significant effect on the intermediary metabolism and erythropoiesis of reptiles and other poikilothermic vertebrates (Follet and Redshaw, 1968; Chandola, *et al.*, 1974a; Kaur and Thapliyal, 1975; Thapliyal, *et al.*, 1975a,d; Wiegand and Peter, 1980; Lone and Matty, 1980; Sinha, 1982; Pati and Thapliyal, 1984; Sreedeviamma and Oommen, 1987). Involvement of gonadal steroids in the oxidative metabolism of lower vertebrates, especially at low ambient temperatures, might be of great significance for the survival and successful breeding of these cold blooded animals under diverse climatic conditions (Gupta and Thapliyal, 1985a, 1991).

In addition to thyroidal and gonadal hormones, catecholamines are also reported to stimulate the oxidative metabolism of reptiles (Gupta and Thapliyal, 1983; Thapliyal and

Gupta, 1984; Gupta and Thapliyal, 1985b). In the Indian garden lizard, intramuscular injection of adrenaline was found to be calorigenically more potent than noradrenaline in the euthyroidic lizards. However, both the hormones were equipotent in the thyroidectomized animals (Gupta and Thapliyal, 1985b). Further, the presence of thyroid is a prerequisite for the calorigenic action of adrenaline in the garden lizard during the breeding phase (Gupta and Thapliyal, 1983). Administration of epinephrine and norpinephrine have been reported to inhibit the resting metabolic rate (RMR) of Alligator mississipiens for about two hours and thereafter to stimulate the RMR which remains increased for many hours (Coulson and Hernandez, 1979). It is noteworthy that the calorigenic action of catecholamines in reptiles is independent of ambient temperature and needs very short lag period (Coulson and Hernandez, 1979; Gupta and Thapliyal, 1983). Due to their temperature-independent and instant calorigenic action, catecholamines are supposed to act as emergency hormones for the regulation of the energy metabolism in poikilothermic vertebrates (Thapliyal and Gupta, 1983; Gupta and Thapliyal, 1985b, Gupta, 1987, 1988; Gupta and Chakravarty, 1990; Gupta and Thapliyal, 1991). There is scarcity of information regarding the calorigenic function of corticosteroid hormones in lower vertebrates (Hanke, 1990). In the Indian garden lizard, corticosterone administration had no effect on the whole body oxygen consumption in any season. However. depending on the season (activity phase) and thyroidal status. it has been found to stimulate. inhibit or produce no effect on respiratory rate of liver. muscle. kidney and brain (Gupta and Thapliyal,1983). Hydrocortisone reportedly stimulates cytochrome oxidase activity in the liver of *Calotes versicolor* in a dose-dependent manner (Prasanna- kumar and Oommen. 1988: Jacob and Oommen. 1992). Though the calorigenic effects of corticosteroids are not prominent. there are many reports which suggest that adrenocortical hormones play an important role in the regulation of intermediary metabolism in cold-blooded vertebrates (Inui and Yokote. 1975: Callard and Callard. 1976: Chan and Woo. 1978: Leach and Taylor. 1982: Barton *et al.*. 1985: Vijayan and Leatherland. 1989: Hanke.*et al.*. 1990). In *Calotes versicolor*. corticosterone and cortisone significantly increased the concentration of blood glucose and urea. the specific activities of glucose 6-phosphates and glutamic oxaloacetic transaminase (Jacob and Oommen. 1992).

There is scarcity of information on the role of Pineal and melatonin in the energy metabolism of Reptiles. The pineal complex, however, seems to be involved in the thermoregulation of lizards (Hutchinson and Koch . 1974: Engbretson and Hutchinson. 1976: Barthalomew. 1982). In general, parietalectomy is reported to increase and pinealectomy to decrease the body temperature of lizards

(Stebbins, 1960; Roth and Ralph, 1976; Ralph *et al.*, 1979a; Firth, *et al.*, 1988). In the lizards, *L. viridis*, short photoperiod and administration of melatonin reduced body temperature (Rismiller and Heldmaier, 1982, 1985, 1987 and 1988). The precise role of melatonin action in the regulation of reptilian energy metabolism remains practically unknown (Gupta and Thapliyal, 1991).

On the basis of the above mentioned literature, it can be concluded that hormones of thyroid, gonad, adrenal and pineal are actively involved in the regulation of the oxidative metabolism of Reptiles. Gonadal hormones and catecholamine hormones seem to be very important for the regulation of the metabolic rate at low temperatures.

Hormonal Regulation Of the Oxidative Metabolism in Amphibia

During the course of evolution, amphibians were the first group of vertebrates which ventured terrestrial as well as aquatic life. Due to their crucial phylogenetic positions in the animal kingdom, amphibians possess some characters/physiology which are also present in fish (aquatic animals) and/or in reptiles (terrestrial animals). Since the neuroendocrine system has been evolved in respect to the habit and habitat, the hormonal mechanism for the regulation of the oxidative metabolism in amphibians might be different from that of fish and reptiles.

Attempts have been made to investigate the role of hormones in energy metabolism of amphibians. In the first few decades, a large number of investigators studied the effects of experimentally induced hypo- and hyper-thyroidism on the metabolic rate of a large number of amphibian species. Many investigators like Euler (1933), Warren (1940), Donoso (1960), Matty and Green (1963), Maher (1967), McNabb (1969), Packard *et al.*,(1974), and Packard and Packard (1975) reported stimulatory effect of thyroid hormones on energy metabolism in amphibians. A few investigators have also reported inhibitory effect of thyroid hormones in a few amphibian species (Taylor, 1939; Jankowsky, 1960). However, many scientists did not find any effects of thyroid hormones and thyroidectomy on the metabolic rate of amphibian species like *Rana esculanta Rana pipiens* and *Rana somibifrons* (Gayda, 1922; Henschel and stauber, 1935; Galton and Ingbar, 1962; Taylor and Barker, 1967; Packard and Packard, 1975).

In the above mentioned studies, contradictory findings are reported. These contradictions might be due to differences in the acclimation temperature used in different studies. Administration of thyroxine induced significant increase in oxygen consumption of liver tissue in *Rana pipiens* acclimated at 25^{0}C but not at 15^{0}C (Packard and Packard, 1975). These findings indicate that the metabolic effect of the thyroid hormones might be temperature dependent. The inhibitory effect in *Rana pipiens* and in other species might be due to very high/toxic doses of thyroid hormones used in these studies. Further. in metamorphosing amphibian tadpoles. endogeneous thyroid hormones reportedly have no effect on the standard metabolic rate (SMR) (Etkin. 1934: Fletcher and Mayant. 1959: Lewis and Frieden. 1959: Marussic.*et al.*. 1966: Funkhouser and Mills. 1969).

A critical analysis of the above mentioned reports indicates that the earlier studies were not carefully planned. Most of these investigations were not carried out under controlled/defined laboratory conditions and no importance was given to the acclimation/ambient temperature (Rosenkilde. 1981: Gupta and Thapliyal. 1991). In a recent study.*in vivo* and *in vitro* treatments with T_3 and T_4 did not stimulate the oxygen consumption rate of liver and skeletal muscle of the Indian streaked frog.*R. limnocharis* exposed to low natural temperature (minimum 6.7^{0}. maximum 16^{0}C) during winter and at comparatively higher natural temperature (minimum 13^{0} and maximum 21^{0}C) during summer/rainy seasons (Gupta and Chakravartty. 1990). However. both T_3 and T_4 stimulated tissue respiration of the toad.*Bufo melanostictus* maintained at natural temperature (minimum 14^{0}C and maximum 25^{0}C) during summer/rainy seasons but not during winter (minimum 5^{0}C and maximum 20^{0}C) (Deka-Borah. 1989). These findings seem to suggest that probably calorigenic effect of the thyroid hormones might be species dependent and/or temperature dependent (Gupta and Thapliyal, 1991). *In vitro* stimulation of oxygen uptake of various tissues of amphibians at high temperatures (at or above 25^{0}C) strongly suggests that thyroid hormones might be capable of stimulating metabolic rate by acting directly at cellular/subcellular levels (Deka-Borah, 1989). This suggestion is further supported by recent reports that both T_3 and T_4 stimulate a wide range of mitochondrial oxidative enzymes like cytochrome oxidase, L-GPDH, cytosolic and mitochondrial malate dehydrogenase in a number of tissues (skeletal muscle, liver, kidney) of amphibians (Lagersptez *et al.*, 1974; Brucker and Cohen, 1976; Lagerspetz, 1977; Goto *et al.*, 1982; Sutharam and Oommen, 1989; Sutharam *et al.*, 1990).

Notwithstanding a large number of confusing reports regarding the calorigenic /metabolic action of thyroid hormones in amphibians, no attempt has been made so far to investigate in detail the role of these hormones in the regulation of the oxidative metabolism with special reference to acclimation temperature, natural ambient temperature, hibernation, and physiological doses of the hormones. Fasting/starvation has been reported to alter the receptors of thyroid hormones. But there is complete lack of information regarding the impact of feeding/fasting on the calorigenic action of thyroid hormones in amphibians and other poikilothermic vertebrates. Further, so far no attempt has been made to study the synergistic action of adrenal hormones (especially catecholamine hormones) and thyroid hormones under natural climatic conditions during different months/seasons (Gupta and Thapliyal,1991).

Preliminary investigations suggest that, as in fish and reptiles, gonadal hormones might also be involved in the regulation of the metabolic rate of amphibians (Gupta and Chakravartty, 1990; Gupta and Thapliyal, 1991). Administrations of testosterone and estradiol in low doses have been reported to stimulate respiratory rate of liver and skeletal muscle tissues in both the sexes of the Indian streaked frog,R. limnocharis during both hibernation and non-hibernation phases (Gupta and Chakravartty, 1990). *However, testosterone and estradiol have been found to be more potent in males* and females respectively. Further, estradiol has also been found to be calorigenic in liver and muscle tissues of the female toad,Bufo melanostictus following both in vivo and in vitro treatments irrespective of seasons/activity-phases (Deka-Borah, 1989). Testosterone and its metabolites are also reported to have a definite role in the regulation of hepatic oxidative metabolism of an apodan amphibian,G. carnosus (Sutharam et al., 1991).

Analysis of the above mentioned reports regarding the role of gonadal hormones in the oxidative metabolism in amhibians shows that in earlier studies there is no information on effects of castration or anti-androgenic drugs on the oxidative metabolism. There is also complete lack of information on time-dependent action of gonadal hormones in amphibians and other poikilotherms. Further, at present there is practically no information regarding the mechanism of action of gonadal steroids in energy metabolism of poikilothermic vertebrates (Gupta and Thapliyal, 1991).

In addition, attempts have also been made to investigate the possible involvement of adrenal hormones in amphibian energy metabolism. Ahlgren (1925) reported that oxygen consumption of minced muscle tissue of frogs was stimulated by adrenaline. Harri and Hadenstam (1972) reported that administration of adrenaline and nor-adrenaline stimulated the oxidative metabolism in frog. Administration of adrenaline is also reported to stimulate the oxidative metabolism in*Rana pipiens* (Farrar and Frye, 1977). Adrenaline stimulated the metabolic rate of axolotle *Ambystoma maxicanum* in a dose-dependent manner (Janssens,*et al.*, 1983). Catecholamines (epinephrine and norepinephrine) have also been reported to stimulate the rate of tissue oxygen consumption in *Rana limnocharis* (Gupta and Chakravartty, 1990). In amphibians, the relative potencies of epinephrine and norepinephrine may vary with the species, tissues and seasons (Gupta and Thapliyal, 1991). Adrenaline is also reported to stimulate the activities of lactate dehydrogenase, cytosolic and mitochondrial malate dehydrogenase, succinate dehydrogenase, L-GPDH, and cytochrome oxidase in an apodan, _G. carnosus_ (Josekumar and Oommen, 1988b).

Catecholamines (both epinephrine and norepinephrine) also play a significant role in the regulation of intermediary metabolism of amphibians (Danforth _et al._, 1962; Hermann, 1977; Farrar and Frye, 1979; Janssens _et al._, 1983; Janssens and Griggs, 1984; Janssens _et al._, 1986).

In most of the above mentioned studies regarding the calorigenic effects of catecholamines, experiments were conducted without considering the sex of the animals, acclimation/ambient temperatures, responses under natural climatic conditions, and the doses of the hormones. There is also complete lack of information regarding the synergistic action of these hormones with thyroid hormones. So far no attempts has been made to study the time dependent effects of catecholamine hormones on the metabolic rate of amphibians.

Corticosteroids are generally involved in the energy dependent osmoregulatory mechanism. In amphibians (the clawed toad, axolotle and _Rana temporaria_), corticosterone influences the metabolic reactions and increases the activities of malic enzymes and the G-6P-

DH/6-PG-DH enzyme system (Hanke. 1990). Corticosterone also stimulates respira-tory rate of liver and muscle tissues of a frog.Rana limnocharis and a toad.Bufo melanostictus (Gupta. 1988: Deka-Borah. 1989: Gupta and Chakravarty. 1990). Administration of corticosterone (low doses) increased the activities of some oxidative enzymes like lactate dehydrogenase. succinic dehydrogenase. cytochrome oxidase and catalase in G. carnosus (Josekumar and Oommen. 1988a).

Corticosterone induced increase in the respiratory rate might be associated with energy consuming anabolic processes. mobilization and oxidation of free fatty acids and osmoregulation (Gupta and Thapliyal. 1991). Administration of Glucocorticoids has been reported to induce hyperglycemia and glycogenesis in some amphibian species (Hanke and Neumann. 1972: Hanke. 1974: Woof and Janseens. 1978: Woody and Jaffe.1985).

There is scarcity of information on the role of melatonin in the energy metabolism of amphibians. Effects of parietalectomy in the frog.Rana temporaria at different temperatures is reported to be inconsistent (Kashbohm. 1967: Chugunov and Kispoev. 1969). Some of the findings suggest that interaction between temperature and photoperiod. depending on the circannual phase. produces varied effects on the amphibian metabolic rate (Kashbohm. 1967: Dunlop.1989). However. there is no information regarding the calorigenic action of melatonin with special reference to ambient temperature and/or month/season of the year. There is also scarcity of information regarding the synergistic action of melatonin and thyroid hormones in amphibians.

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