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

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THESIS SUBMITTED FOR THE DEGREE OF
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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

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: Qsborn, 1951: Zaks and Zamkova. 1952: Muller. 1953: Chavin 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 $16.2\text{-}17.5^{0}$ 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^{0} C (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..

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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, 1978a).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 (22^0-25^{0}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^{0}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 <u>Chalcides</u> <u>ocellatus</u> and estradiol significantly increased oxygen consumption in gonadectomized females (Al Sadoon, <u>et</u> <u>al.</u>, 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,<u>et</u> <u>al.</u>, 1974a; Kaur and Thapliyal, 1975; Thapliyal,<u>et</u> <u>al.</u>, 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 °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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CHAPTER 1

Chapter 1

Materials and Methods

Introduction

Amphibians are of particular interest to zoologists (Endocrinologists/physiologists) as representatives of animals which bridge the true-aquatic and true-terrestrial vertebrates. Noble (1931) commented that the amphibians were the first vertebrates to develop voice and break silence on the earth . Approximately $3x10^9$ years ago, the ancestral amphibians were the first animals to emerge from the aquatic environment and to crawl on the land (Cochran,1961). However, many aspects of amphibian life remain to be investigated. As it is clear from the review of Literature presented in "Introduction" that the role of endocrine glands and their hormones in the energy metabolism of amphibians remains to be established. Keeping in view the scarcity of information, we decided to investigate in detail the role of the major metabolic hormones secreted by thyroid, adrenal (Chromaffin and Interrenal tissues), gonads and Pineal in the regulation of the oxidative metabolism in amphibians. For the proposed study , two amphibian species were chosen. The Indian streaked frog, *Rana limnocharis* was selected as a hibernating and the Indian skipper frog, *Rana cyanophlyctis* as a non-hibernating species of frogs. A brief account of materials and methods used for this dissertation is given below.

Animals:

All the experiments were conducted on adult male *Rana limnocharis* and *Rana cyanophlyctis*.

Rana limnocharis

The Indian streaked frog,*Rana limnocharis* is a small frog (Snout to vent length in adult male: 31-33 mm and adult females : 43-45 mm). It is very similar in appearance to the Indian bull frog,*Rana tigerina*. Dorsal skin is coarsely granular with broken ridges and belly is pale white. Color of the dorsum plate is gray and brown with a conspicuous mid-dorsal white line. *Rana limnocharis* is found in and around Shillong throughout the year (Latitude : 25°30' N; Longitude : 91°52' E, Altitude 1450 ASL; Minimum temperature 1.4°C; Maximum temperature 25°C). *Rana limnocharis* inhabits paddy fields, ponds, marshy places and near banks of streams and water pools. It hibernates under soil or stones in nature as well as under laboratory during winter months (November, December, January). In nature, *Rana* limnocharis breeds during the months of April to August. During hibernating phase (winter), Rana limnocharis develops anorexia and becomes lethargic. Even under laboratory condition, it does not eat any feed (eg., live earthworm) provided ad libitum. When the gut content was examined during winter, no food (undigested/semi-digested) was found in Rana limnocharis . The annual life cycle of Rana limnocharis can be divided into following 4 phases:

1. Pre-hibernation phase (September, October)
2. Hibernation phase (November, December and January)
3. Post-hibernation phase (February-March)
4. Breeding phase (April to August)

Breeding biology of this frog in Meghalaya and in other parts of India is well studied (Sahu, 1981; Saidapur, 1989).

Rana cyanophlyctis:

The skipper frog, Rana cyanophlyctis is an aquatic frog, which is available in Shillong throughout the year. This species is identified by equal size of first and second fingers, slightly dilated and completely webbed toes and warty skin with pores and tubercles. As in Rana limnocharis, males are smaller and lighter than females (snout to vent length = adult male : 35-39 mm and adult female : 48-51 mm). This species is slightly bigger than *Rana limnocharis*. The coloration of the dorsal side of the head and body varies from brownish to dirty green, dotted with numerous olive-dark markings of irregular shape. The mid-dorsal line is absent in *Rana cyanophlyctis*. It remains active throughout the year. Even during winter months, when the temperature touches near 0^{0} C, this frog is seen to relax on water surface and basks in bright sun during morning hours. This species is known to be reproductively active throughout the year (Saidapur, 1989; Sahu, 1981). It is presumed that this species does not hibernate (cold torpor) during winter months. However, activity level is slightly decreased during winter months as compared to other seasons. When gut content was examined during winter, remains of undigested/semi-digested food materials (aquatic insects?) were found throughout the alimentary canal. The presence of food in gut seems to suggest that this frog feeds even during winter months and does not hibernate.

Hormonal Treatments:

Hormonal treatments were given under both *in vivo* and *in vitro* conditions in both the species. A brief account of the mode of the hormonal treatments is given below.

IN VIVO Experiments :

In vivo experiments were conducted on both the species during hibernation/winter months and active phases(summer/rainy seasons). For *in vivo* experiments,during hibernation phase, adult male *Rana limnocharis* (body weight,8-10 g) and adult male *Rana cyanophlyctis* (body weight,10-12 g) were collected (December,January) and caged in wire-netted plastic cages (size : 35x25x15 cm). The cages were maintained under natural environmental conditions. During the course of acclimatization and hormonal treatments, frogs were provided with live earth-worms *ad libitum*. After seven days of acclimatization, the frogs were divided into different groups (of five each) for different types of hormonal/inhibitors/agonists/antagonists treatments. During the treatments, minimum and maximum ambient temperatures and relative humidity were recorded. Hormones were administered intramuscularly on alternate legs daily for four days.

Collection of tissues:

Frogs were decapitated, tissues (Liver, skeletal muscle and kidney) were rapidly removed, rinsed in ice-cold frog Ringer's solution and stored in a refrigerator at - 6 ^{0}C to -8 ^{0}C. Tissues were processed within 10-15 days. When tissues were stored in a refrigerator, no significant alteration was found in the rate of tissue respiration upto one month.

During active phase (July and August), adult male Rana limnocharis and Rana cyanophlyctis were collected. During the treatment, ambient temperature(maximum and minimum) and relative humidity were recorded (max.temperature 23^{0},min.temperature 12^{0} C and R.H. 90%). The procedures, regimes and doses of hormonal treatments were kept the same as in the case of *in vivo* experiments conducted during the hibernation .

IN VITRO experiments:

In vitro effects of the hormones on the respiratory rate of tissues were also studied during the hibernation (Winter) and active phases (Summer/Rainy season) of the annual activity cycle. During hibernation phase,Rana limnocharis were found to be lethargic and anorexic as mentioned earlier. However, Rana cyanophlyctis were found to be active during Winter, but the level of activity was found to be slightly low during as compared to the active phase (Summer/Rainy season). For *in vitro* experiments during winter months, adult male Rana limnocharis and Rana cyanophlyctis were collected locally during the months of December and January (minimum 4^{0} C ; maximum 12^{0} C and relative humidity 75-80% on the day of collection). Immediately after collection, four males of each species were first weighed and then decapitated. Tissues (Liver and skeletal muscle) were rapidly removed separately, rinsed in ice cold frog Ringer's solution and stored in a refrigerator at -6^{0} C to -8^{0} C. For <u>in vitro</u> treatments, the tissues were blotted, weighed and homogenized in a loose fitting all-glass homogenizer in ice-cold frog Ringer-phosphate buffer solution (pH:7.4). One ml of homogenate, 3.9 ml of frog Ringer-phosphate buffer and 0.1 ml solution of hormone (having the desired concentrations of hormones) were added to the incubation chamber for measuring the respiration. L-T_3 & L-T_4 and Testosterone were added to the tissue homogenates and incubated at 4^{0} C for 1 hour in a refrigerator prior to the measurement of oxygen consumption. This incubation was necessary to allow the binding of the hormones to the tissues. The samples treated with catecholamines and Corticosteroids were incubated only for 15 minutes in the incubation chamber of the oxygen electrode at 25^{0} C before the measurement of oxygen consumption. Readings were taken at an interval of 5 minutes for half an hour when the oxygen consumption was found to be linear. All tissues were processed within 15 days of storage.

For <u>in vitro</u> experiments during active phase (July/August; minimum temperature: 12^{0} C; maximum temperature: 23^{0} C; Relative humidity: 85-90%), experimental procedures were the same as mentioned for <u>in vitro</u> experiments during Winter months.

Chemicals:

Hormones, agonists, antagonists and inhibitors used in the experiments were purchased from Sigma chemicals company, U.S.A . General chemicals were purchased from BDH. Cyproterone acetate was gifted by Prof.Dr. M.F. ElEtreby, Berlin.

Measurement of Tissue respiration:

The rate of oxygen consumption of each tissue (Liver, skeletal muscle and kidney) was measured with the help of an oxygen electrode (Digital Oxygen System, Model 10; Rank Brothers Ltd, England). The system is composed of an incubation chamber of plexi glass (with Ag as anode and Platinum as cathode , and a control panel (with knobs to regulate sensitivity, polarizing voltage and speed of the magnetic-bar). A thermostatically controlled water circulator is used for circulating water in the incubation chamber. For measuring the rate of respiration, polarizing voltage was kept at 0.6V. Frog Ringer-phosphate buffer (pH 7.4) was used as the polarizing medium.

For the measurement of tissue respiration, the tissues were blotted, weighed and homogenized in a loose fitting all-glass homogenizer (Remi homogenizer; Remi equipments, Bombay) in ice-cold Frog Ringer-phosphate buffer (9:1) solution (pH 7.4). Since the preferred body temperature for most of the amphibians ranges between 25^0 and 28^0 C (Duellman and Trueb, 1986), the rate of oxygen consumption of tissue homogenates was measured at 25^0 C by circulating water at 25^0 C in the water jacket of the incubation chamber using the thermostatic water circulator. For measuring the rate of respiration of tissues from <u>in vivo</u> experiments, 1ml of homogenate was added to 4 ml of frog Ringer-phosphate buffer solution and placed into the incubation chamber of the oxygen electrode. The homogenates were incubated in the chamber of the Oxygen electrode for 15-20 minutes before recording the readings. Readings were taken at an interval of 5 minutes for half an hour. The rate of tissue respiration was expressed as ul O_2/mg wet tissue /hr.

<u>Statistical analysis</u>:

All data were analyzed statistically with the help of student's "t" test (Snedecor, 1961). $P < 0.05$ was considered as significant.

The above mentioned procedures were followed in all the experiments discussed in the following chapters (Chapter 2 to Chapter 6).

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CHAPTER 2

Chapter 2

Annual Variations in the Oxidative Metabolism of Rana limnocharis and Rana cyanophlyctis

Introduction

In amphibians, knowledge of seasonal variations in the oxidative metabolism is very limited. Gilles-Ballien (1974) suggested that in the animals undergoing hibernation, seasonal variations in the metabolic rate reflect an annual metabolic cycle that is probably genetically determined and expresses physiological adaptation to the cold climate. Amphibians are ectothermic vertebrate and their body temperature is easily changed by ambient temperature. Seasonal variations in body temperature is greater among temperate amphibians than among tropical species. The species living at higher altitudes in the low latitudes experience dial temperature variations approximating the annual variations (Gills-Ballien, 1974).

Seasonal differences in the metabolic rate (MR) of salamanders, Plethodon cinereus and Eurycea bistineata has been reported (Vernberg, 1952). Seasonal variations in the rate of oxygen consumption might be related to seasonal differences in activity levels. Seasonal variations in the metabolic rate of Amphibians is supposed to be influenced by thyroid hormones and autonomic nervous system (Lagersptez, 1977).

There are few reports on the effects of photoperiod on the metabolic activity as measured by O_2 consumption. However, experimental findings and interpretations are inconsistent (Turney and Hutchinson,1974). It is also reported that amphibians have endogenous rhythms associated with daily changes in ambient atmospheric pressure (Brown *et al.*,1955) but Hutchinson and Kohl (1971) did not find any correlation in *Bufo marinus*. Jameson and Co-workers (1970) reported a great deal of variability in the metabolic rates between population of the frogs, *Hyla* from British Columbia to Baja California. A partial compensation for temperature in the metabolic rate has been reported for several species of Rana and for *Bufo boreus* (Stangenburg, 1955; Rieck, Belli and Blaskovics, 1960; Jankowsky, 1960; Bishop and Gordon, 1967). It has been reported that frogs acclimated at high temperature have higher metabolic rates when measured at an intermediate temperature than those acclimated at low temperatures (Dunlap, 1972).

In general, the long daylengths are associated with increased ambient temperature. Therefore, it is very difficult to dissociate the effects of photoperiod from that of temperature on the metabolic rate of vertebrates. There is scarcity of carefully designed experimental studies regarding the effects of photoperiod on the oxidative metabolism of amphibians.

Most of the above mentioned information are based on studies conducted on temperate ambphibians. Practically there is no information on tropical/subtropical amphibian species. Even in temperate zone amphibians, studies were conducted mainly on whole body oxygen consumption but not on tissue respiration. Further, there is scarcity of information on seasonal variation in the metabolic rate of temperate and tropical/sub-tropical amphibian species. The Indian streaked frog, Rana limnocharis is a hibernating species while the Indian skipper frog, Rana cyanophlyctis does not hibernate even at low temperature of winter months. Very little is known about the changes that take place in the metabolically important tissues like liver, muscle and kidney of amphibians during different seasons/months of the year. So far no attempt has been made to compare the annual/seasonal variations in the metabolic rate of hibernating and non-hibernating amphibians. Therefore, it was thought worthwhile to study annual variations in the oxidative metabolism of R. limnocharis and Rana cyanophlyctis with special reference to their breeding cycles, natural ambient temperature and the annual activity cycles.

Materials and Methods

The Indian streaked frog, Rana limnocharis is a small-sized amphibian which is found in all parts of India. This species is available in and around Shillong throughout the year. In nature, Rana *limnocharis* breeds during the months of April to August in temporary ponds/water p00ls and paddy fields. It hibernates in nature as well as under Laboratory condition during the months of November to February. During the hibernation phase, the frogs become lethargic and anorexic. The Indian skipper frog, *Rana cyanophlyctis* is a medium sized frog, found in plenty in and around Shillong throughout the year. In nature *Rana cyanophlyctic* does not hibernate and remains active throughout the year. This species is found to be active and feeding even during the winter months. Since, *Rana cyanophlyctis* remains active and its gut is found to be full of semi-digested materials (personal observation), this species is considered as a non-hibernating species.

For investigating monthly variations in the metabolic rate of tissues, adult male frogs of the two species were collected locally during the 3rd week (20th-22nd day) of each month (January to December). After collection, the frogs were brought to the laboratory where they were immediately decapitated, tissues (liver, skeletal muscle and kidney) removed, rinsed in ice-cold Frog Ringer's solution and stored in a refrigerator at -6^0C to -8^0C for the measurement of tissue respiration. The tissues were processed for the measurement of the rate of respiration within 15 days.

Measurement of Oxygen Consumption

The rate of O_2 consumption of tissues was measured with the help of an Oxygen electrode following the methodology discussed in "Chapter I : Materials and Methods."

The Data on temperature, rainfall and relative humidity at Shillong were obtained for every month from the Meteorological Department, Guwahati Airport, Guwahati (Fig. 1 and 2).

Results

The annual variations in the rate of respiration of liver, muscle and kidney tissues of *Rana limnocharis* and *Rana cyanophlyctis* are shown in Figs. 1 and 2. During the hibernation phase (November, December and January), the rate of respiration of all the three tissues (Liver, muscle and kidney) of both the species was found to be the lowest during the month of January. However, all the three tissues of both the species differed in their annual pattern of variations in the rate of oxygen consumption.

In *Rana limnocharis*, the rate of oxygen consumption of liver was found to be always higher than that of muscle and kidney

(Fig.1). The respiratory rate of liver was found to be minimum during the month of January, then it increased significantly during the month of February and remained at that level during the month of March. Then the metabolic rate of liver increased significantly during the month of April and remained high upto September and reached its highest level in the month of October. After October, the rate of liver respiration decreased significantly and continuously reaching to a minimum level during January.

The rate of muscle respiration in Rana limnocharis was also found to be minimum during January which increased significantly during the month of February. However, unlike the rate of liver respiration, the respiratory rate of muscle tissues declined significantly during the month of March. Then the muscle respiration increased gradually and significantly upto May followed by no significant alterations in its respiratory rate upto July. Thereafter, the metabolic rate of muscle tissue increased significantly during the month of August and remained high upto the month of October. After October, it decreased during the month of November and remained as such during the month of December. Further decrease in the rate of muscle respiration was observed leading to a minimum level during the month of January.

In Rana limnocharis, the rate of kidney tissue respiration was found to be higher than that of muscle and lower than that of liver (Fig.1). The annual variations in the rate of kidney oxygen consumption followed, more or less, a similar pattern of the liver tissue except that the highest kidney oxygen uptake was recorded during the month of August and September.

The annual variations in the rate of respiration of liver, muscle and kidney tissues of *Rana cyanophlyctis* has been presented in Fig.2. In *Rana cyanophlyctis*, as in *Rana limnocharis*, the rate of oxygen consumption of the liver tissue remained higher than that of kidney and muscle throughout the year (Fig. 2; Tab. 2). The rate of liver oxygen consumption was found to be low during winter months (November,December and January). Liver metabolic rate was found to be minimum during the month of January. Then it increased significantly during the month of February followed by a significant decrease during the month of March. Thereafter, it increased significantly during the month of April and was maintained at that level till August, reaching to the maximum level during September, October and November. Then the liver respiratory rate decreased gradually during the month of December and reached to the minimum level during the month of January.

The rate of muscle respiration was lowest in January and it increased significantly during the month of February followed by a sharp decrease during the month of March (Fig.2; Tab.2). Thereafter, it increased significantly during the month of April and remained at that level during the month of May to October, and reached the highest level during the month of August. The muscle tissue respiratory rate decreased gradually through the months of November and December and reached to the minimum level during January.

The rate of kidney tissue respiration of *Rana cyanophlyctis* was found to be higher than that of muscle and lower than liver (Fig. 2; Tab. 2). The pattern of annual variation in the rate of kidney respiration, more or less, followed the pattern of muscle tissue respiration. The kidney oxygen uptake increased during the months of August and remained at that level upto December. Then it declined significantly during the month of January.

When the average rate of tissue respiration in *Rana limnocharis* was calculated for the hibernation phase and the active phase (Fig. 2; Tab. 2), the average respiratory rates of all the three tissues were found to be significantly higher during active phase (February-October) as compared to hibernation phase (November-January). However, when the average respiratory rate was calculated for *Rana cyanophlyctis* during the winter months and rest of the months, only the average respiratory rate of muscle tissue was found to be significantly higher during summer/rainy months as compared to winter months (Nov-Jan). The average respiratory rate of kidney and liver did not change significantly during winter months as compared to other months (Fig.2B ;Tab.2B).

Discussion

The rate of energy metabolism of an animal is a critical indicator of its overall physiology.particulaly if the pattern of variation of the rate through time is known (Bartholomew.1977). The temporal pattern of energy metabolism provides a realistic appraisal of the adjustment that it makes to the availability and demand of energy both on a daily and or/seasonal basis (Bartholomew.1977). It is.however. dependent on a number of factors.viz.activity.body size. photoperiod. temperature. age. sex. seasons/month and the time of the day. (Prosser.1973).

Unlike in other vertebrates. there is scarcity of information on the circannual variations in the oxidative metabolism of amphibians. (Prosser.1973.1986). There are a few reports on seasonal variations in the rate of O_2 consumption in amphibians (Duellman and Trueb.1986). Seasonal variations in the whole body oxygen consumption are reported in only three temperate zone amphibians i.e.<u>Plethodon cinereus</u>. <u>Eurycea bislineata</u> (Vernberg.1952) and <u>Desmognathus ochrophaeus</u> (Fitzpatrick and Brown.1975). However.there is practically no information on the seasonal/circannual variations in the oxidative metabolism of any tropical or subtropical amphibians (Prosser, 1973; Duellman and Trueb, 1986). Different tissues are supposed to differ in their energy requirements during different months/seasons over the annual time-scale, but so far no attempt has been made to study seasonal variations in tissues respiration in any amphibian species. Further, there is also practically no information on the metabolic rate of non-hibernating amphibian species (which do not undergo cold torpor and remain active throughout the year).

In the present study, monthly variations in tissues (liver, muscle and kidney) respiration in adult males of a hibernating (Rana limnocharis) and a non-hibernating (Rana cyanophlyctis) tropical/sub-tropical species were recorded. It is important to mention that, to the best of our knowledge, this is the first comparative and comprehensive study on annual variations in the metabolic rate of tissues of a hibernating and a non-hibernating amphibian species under natural climatic-conditions (Prosser, 1973, 1986; Duellman and Trueb,1986).

Present observations indicate that the pattern of variations in metabolic rate in Rana limnocharis and Rana cyanophlyctis are similar. However, the metabolic rates of the two species are different during winter and summer/rainy months. The two species exhibit differences in their energy demand during different phases of the annual activity cycle. While the average rate of oxygen consumption of liver, muscle and kidney in Rana limnocharis was found to be significantly low during the hibernation (Winter) as compared to the active phase (Fig.2B,Tab.2B). However, in Rana cyanophlyctis the average metabolic rate of liver and kidney did not change significantly between winter and summer-rainy months (Fig. 2B, Tab. 2B). But the muscle O_2 uptake in Rana cyanophlyctis was significantly low during the winter months than during the summer-rainy months (Fig. 2B, Tab. 2B). Further, the average metabolic rate of all the tissues in Rana cyanophlyctis during the winter months was significantly higher than that of Rana cyanophlyctis. It seems that the significant difference during winter between the average metabolic rates of all tissues (liver, muscle and kidney) of Rana limnocharis and Rana cyanophlyctis might be due to differences in their activity during winter months. Since Rana limnocharis becomes lethargic and anorexic in nature during winter months, it needs minimum energy just enough to keep itself alive. Unlike Rana limnocharis,Rana cyanophlyctis remains active and takes food even during winter months. Further, it is found to swim in cold water (minimum water temperature 2^0-5^0C). Therefore, Rana cyanophlyctis requires more energy for maintaining its activities as well as to keep it alive while being exposed to the cold unfavorable climatic conditions prevalent during winter months. Increased rate of tissue respiration in active R. limnocharis reaching to the level of Rana cyanophlyctis, also seems to strongly suggest that the significant differences in the metabolic rates of tissues in the two species during winter months might be due to differences in the levels of their activity.

Significant decrease in the rate of tissues respiration in Rana limnocharis during winter months seems to be directly related to its decreased physical activity (lethargy). Since this species becomes anorexic during winter, prolonged fasting might also be responsible for the decreased metabolic rate of the tissues. In temperate amphibian species also the rate of oxygen consumption reportedly depends upon the activity level, i.e., increased activity is associated with increased rate of respiration (Bennett and Wake, 1974; Feder, 1978b). Further, great reductions in metabolic rate have been reported in dormant amphibians of temperate climate (Gehlbach et.al., 1973; Seymour, 1973b, Van Buerden, 1980). Slight but significant decrease in the average rate of muscle O_2 consumption in Rana cyanophlyctis . might be due to comparative decrease in its physical/muscular activity.

In general, ambient temperature influences physical and metabolic activities of amphibians (Whiteford, 1973; Weathers and

Snyder, 1977; Feder, 1978a, 1978b, 1982a). However, it does not seem to be true for all amphibian species. For example, the metabolic rate of two vital tissues (Liver and kidney) of Rana cyanophlyctis were not influenced by very low temperature and the frog remained active during the cold winter months. Even in Rana limnocharis, the metabolic rate of the tissues has not been found always to increase or decrease with ambient temperature (Fig. 1). For example, the metabolic rate of tissues increased significantly during the month of February and March without any significant increase in ambient temperature. Similarly, the rate of O_2 consumption by liver and kidney did not decrease with the sharp decline in the ambient temperature during the month of November. It is also important to mention that Rana limnocharis becomes fully active during the month of February when temperature remains low (Average temperature :10.5 ^{0}C). These observations strongly suggest that the metabolic rate of amphibians seems to be directly related to the level of activity and low temperature may not always decrease the metabolic rate of all tissues and induce inactivity in all amphibian species. Inhibitory effect of low temperature on amphibian metabolic rate seems to depend on the phases of annual activity cycle.

While the metabolic rate of liver was always found to be higher than kidney and muscle tissues, muscle tissue respiratory rate was found to be lower than liver and kidney tissues in both the species (Fig.1 and 2). The metabolic rate of muscle tissue seems to be associated with the activity level and/or temperature. However, the rate of oxygen consumption of liver and kidney seems to be associated with food intake. Since liver is involved in metabolism and kidney in excretion, food intake probably increases the metabolic activities of these tissues. As a result, their increased energy requirements lead to increase in their metabolic rate. A consistently high average metabolic rate of liver and kidney in Rana cyanophlyctis, which feeds during winter months, also suggests that the metabolic rate of these tissues might be associated with feeding status. Fasting leads to decrease in the metabolic rate of all the tissues in Rana cyanophlyctis during Summer months (Unpublished Data).

There are inconsistent reports regarding the role of photoperiod in the regulation of the oxidative metabolism in amphibians (Turney and Hutchinson, 1974; Weathers and Snyder, 1977). Under natural conditions, ambient temperature is associated with the day length. Therefore, it is difficult to dissociate the effects of temperature and photoperiod on the metabolic rate in the present study. In general, photoperiod activates the hypothalamo-hypophyseal-gonadal axis leading to increased level of testicular hormones (Church, 1960a; Werner, 1969; Ralph, 1983; Kupwade and Saidapur, 1986; Duellman and

Trueb, 1986; Saidapur,1989). Testicular hormones are reportedly involved in the regulation of the oxidative metabolism in reptiles (Gupta and Thapliyal, 1991). It, thus, seems that photoperiod may influence the metabolic rate of amphibian tissues indirectly by increasing androgen level. We have conducted experiments to elucidate the role of androgens in the oxidative metabolism of Rana limnocharis and Rana cyanophlyctis, and the data are presented in the chapter 4 . Further experimental studies are essential to establish the role of photoperiod in the energy metabolism of amphibians.

On the basis of the present findings, it can be concluded that the pattern of the annual variations in the metabolic rate of tissues is different in the hibernating species,Rana cyanophlyctis and non-hibernating Rana cyanophlyctis. The metabolic rate of liver and kidney tissues seems to be dependent on the feeding status in both the species. However, the metabolic rate of the skeletal muscle seems to depend upon the level of physical/muscular activity. Present findings also suggest that the effect of low temperature on the metabolic rate depends on the function of tissues, species and phase of the annual activity cycle. It is difficult to delineate the role of photoperiod from that of environmental temperature. It also remains to be investigated that what internal factors protect Rana cyanophlyctis from the ill-effect of low temperature and keep the frog physically and metabolically active during the cold winter months.

****

Table 1 : Annual variations in the rate of oxygen consumption of tissues (liver, muscle and kidney) of *Rana limnocharis*

Month	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
November	1.97 ± 0.06^{*,c}	0.87 ± 0.02^{c}	1.76 ± 0.05
December	1.65 ± 0.07^{a}	0.86 ± 0.06	1.42 ± 0.07^{b}
January	0.79 ± 0.02^{c}	0.51 ± 0.08^{a}	0.63 ± 0.10^{c}
February	1.51 ± 0.16^{b}	1.14 ± 0.10^{b}	1.32 ± 0.04^{c}
March	1.60 ± 0.08	0.55 ± 0.05^{b}	1.17 ± 0.20
April	2.15 ± 0.13^{b}	0.98 ± 0.18	1.86 ± 0.09^{a}
May	2.28 ± 0.20	1.59 ± 0.10^{a}	1.89 ± 0.06
June	2.19 ± 0.14	1.40 ± 0.10	1.80 ± 0.07
July	2.19 ± 0.14	1.22 ± 0.03	1.84 ± 0.10
August	1.98 ± 0.16	1.78 ± 0.05^{c}	2.02 ± 0.30
September	2.30 ± 0.25	1.65 ± 0.05	2.63 ± 0.07
October	2.48 ± 0.03	1.66 ± 0.02	1.90 ± 0.12

[*] Mean ± Standard error; n = 4.

a, b, and c Differ from the preceding month : $P < 0.05$, 0.01 and 0.001 respectively.

Table 2 : Annual variations in the rate of oxygen consumption of tissues (liver, muscle and kidney) of <u>Rana cyanophlyctis</u>

Month	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
November	$2.22 \pm 0.23^{*}$	1.32 ± 0.02	2.13 ± 0.15
December	2.05 ± 0.06	1.16 ± 0.05^{a}	1.89 ± 0.07
January	1.10 ± 0.07^{c}	0.73 ± 0.03^{c}	0.99 ± 0.04^{c}
February	1.58 ± 0.02^{c}	0.88 ± 0.03^{a}	1.29 ± 0.08^{a}
March	0.82 ± 0.04^{c}	0.72 ± 0.07	0.80 ± 0.13^{a}
April	1.89 ± 0.02^{c}	1.31 ± 0.03^{c}	1.67 ± 0.02^{c}
May	2.03 ± 0.33	1.45 ± 0.06	1.77 ± 0.15
June	2.08 ± 0.26	1.40 ± 0.04	1.69 ± 0.07
July	1.81 ± 0.09	1.50 ± 0.07	1.61 ± 0.05
August	1.85 ± 0.40	1.64 ± 0.05	2.13 ± 0.07^{c}
September	2.20 ± 0.30	1.47 ± 0.06	2.01 ± 0.14
October	2.29 ± 0.20	1.53 ± 0.09	1.87 ± 0.14

[*] Mean ± Standard error; n = 4.

a and c Differ from the preceding month : $P < 0.05$ and 0.001 respectively.

Table 2B : Average rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during hibernation/Winter and active phase/Summer-Rainy season.

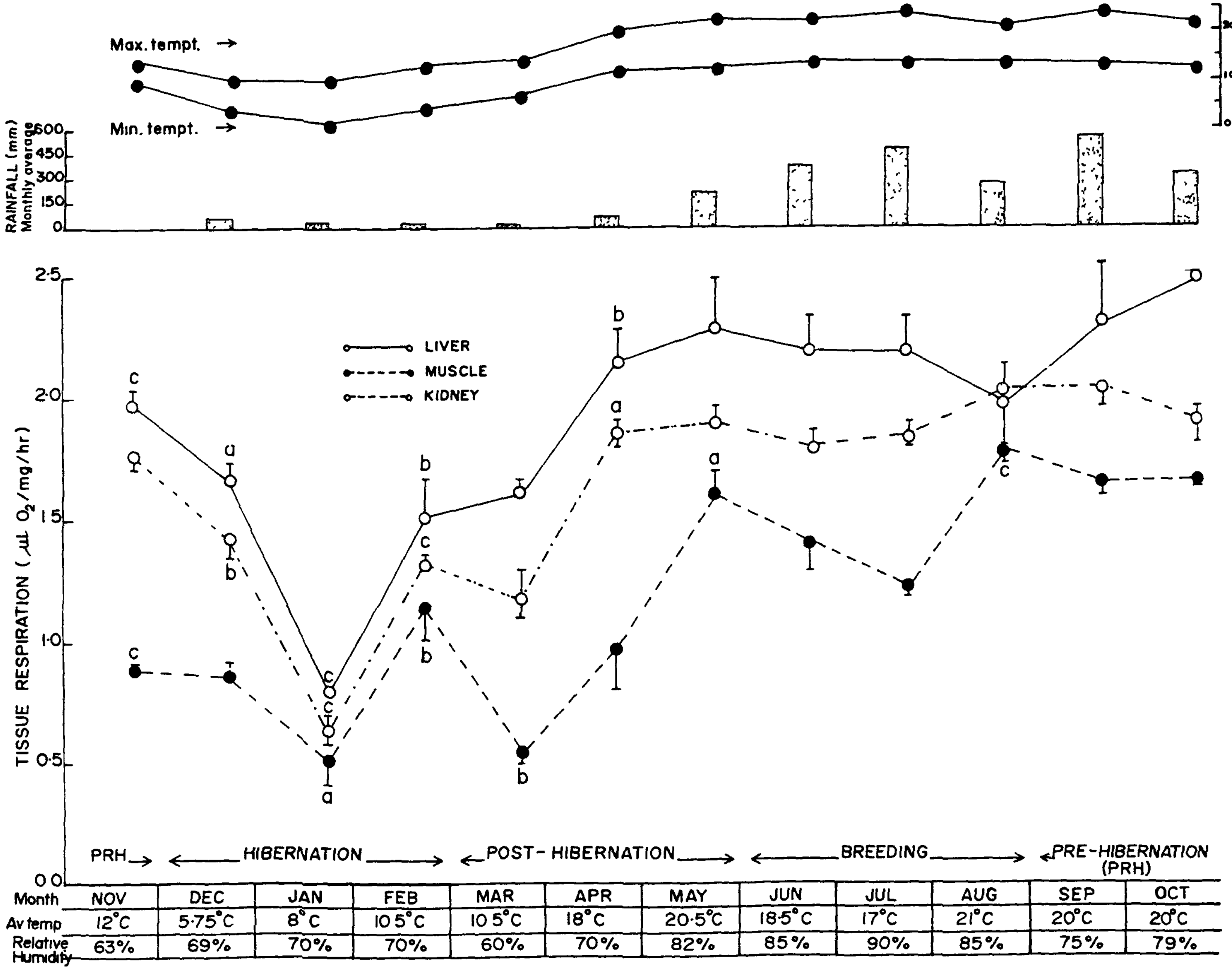
Tissues	average rate of oxygen consumption($\mu l\ O_2$/mg/h)			
	Rana limnocharis		*Rana cyanophlyctis*	
	Winter	Summer-Rain	Winter	Summer-Rain
Liver	$1.47 \pm 0.05^{*}$	2.08 ± 0.14^{b}	1.79 ± 0.12	1.84 ± 0.18
Muscle	0.75 ± 0.05	1.55 ± 0.10^{c}	1.07 ± 0.03	1.32 ± 0.07^{a}
Kidney	1.27 ± 0.07	1.75 ± 0.11^{b}	1.67 ± 0.09	1.65 ± 0.09

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the values during Winter : $P < 0.05$, 0.01 and 0.001 respectively.

Fig. 1 : Annual variations in the rate of oxygen consumption of tissues (liver, muscle and kidney) of *Rana limnocharis*

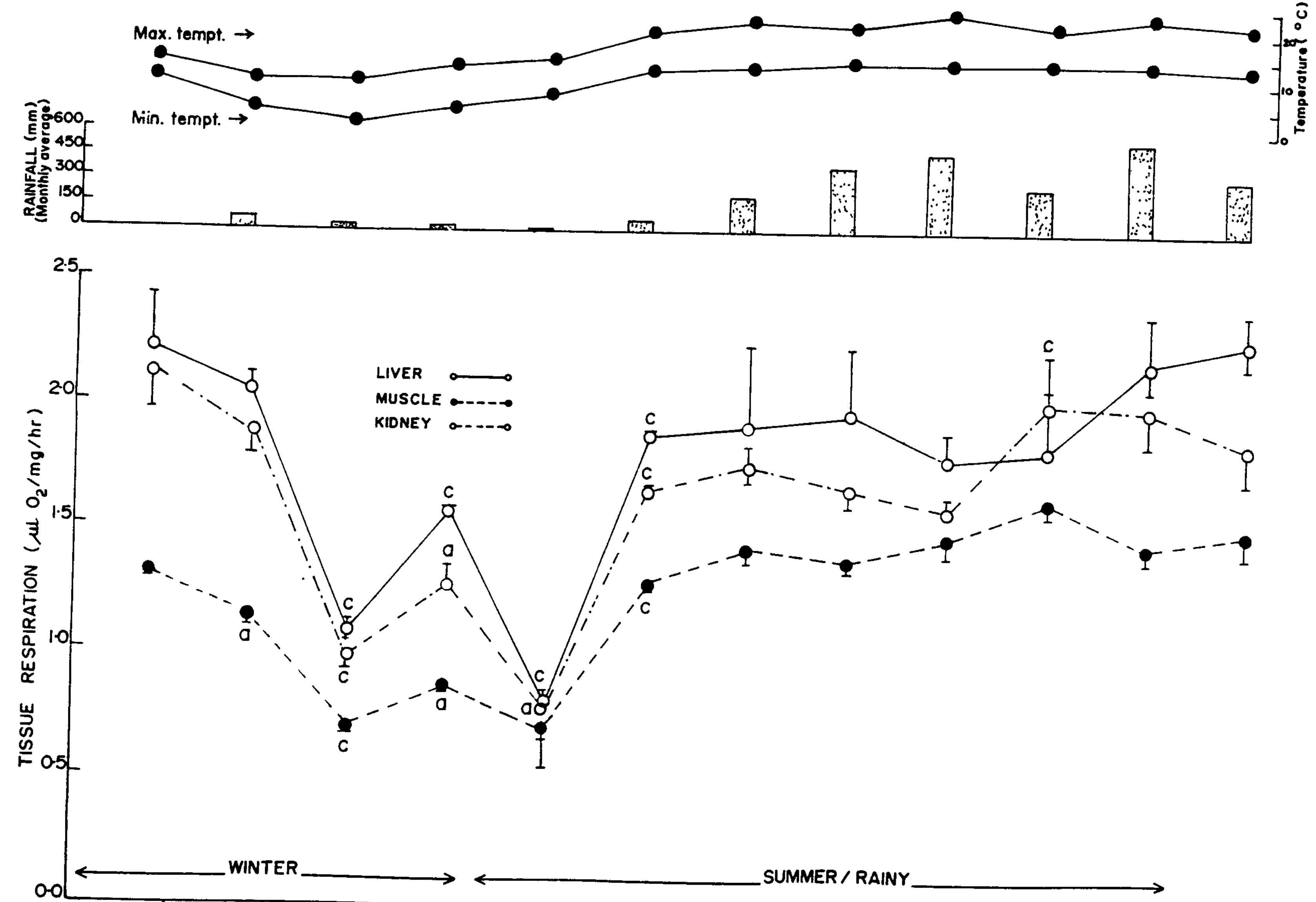
^{a,b,c}Differ from the preceding month : $P < 0.05$, 0.01 and 0.001 respectively.



Month	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Av temp	12°C	5·75°C	8°C	10·5°C	10·5°C	18°C	20·5°C	18·5°C	17°C	21°C	20°C	20°C
Relative Humidity	63%	69%	70%	70%	60%	70%	82%	85%	90%	85%	75%	79%

Fig. 2 : Annual variations in the rate of oxygen consumption of tissues (liver, muscle and kidney) of *Rana cyanophlyctis*

^{a,c}Differ from the preceding month : $P < 0.05$ and 0.001 respectively.



Month.	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Av.temp :	12°C	5·75°C	8°C	10·5°C	10·5°C	18°C	20·5°C	18·5°C	17°C	21°C	20°C	20°C
Relative Humidity	63%	69%	70%	70%	60%	70%	82%	85%	90%	85%	75%	79%

Fig. 2A : Comparison of annual variations in the rate of oxygen consumption of tissues in *Rana limnocharis* and *Rana cyanophlyctis*.

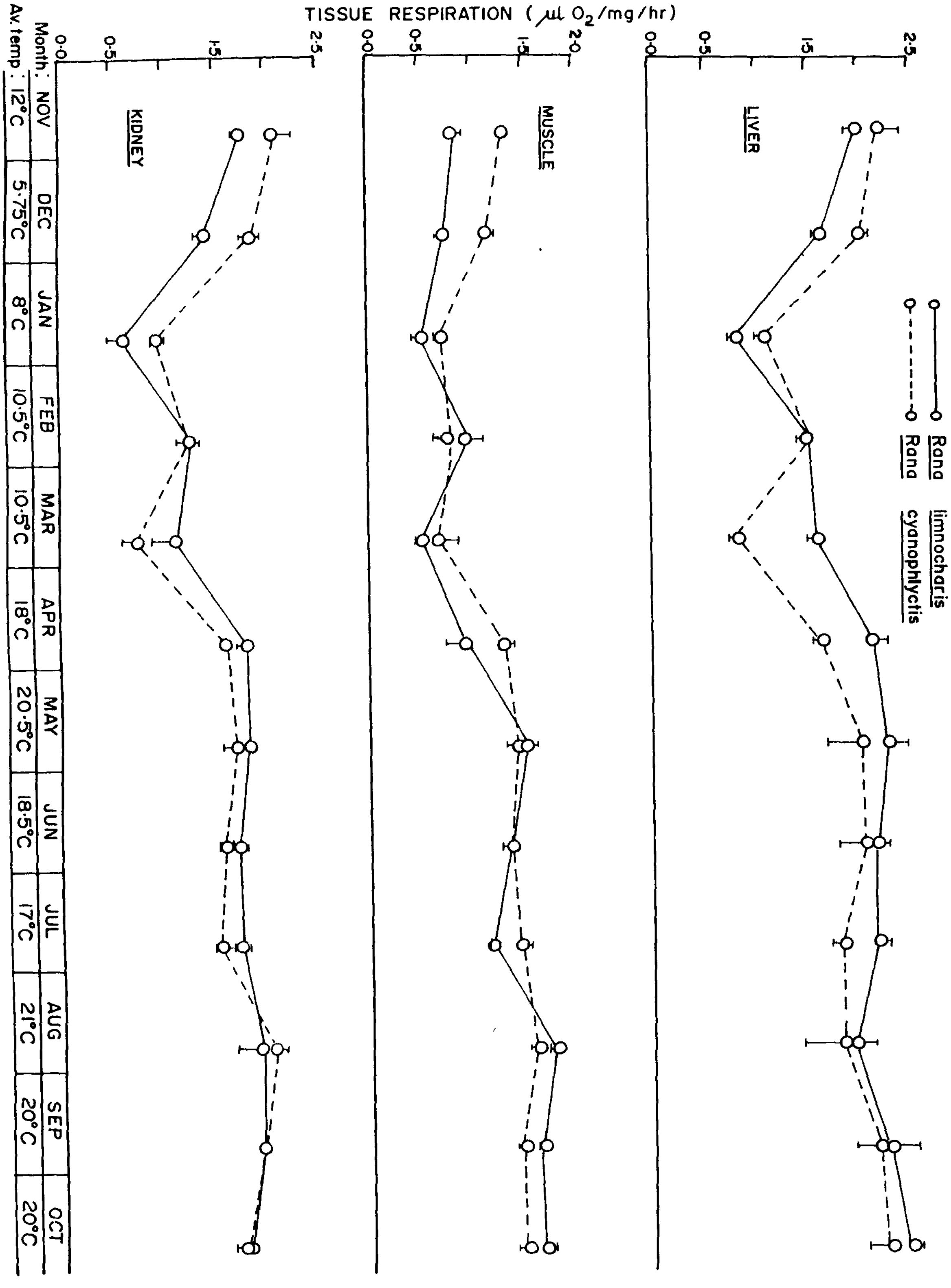
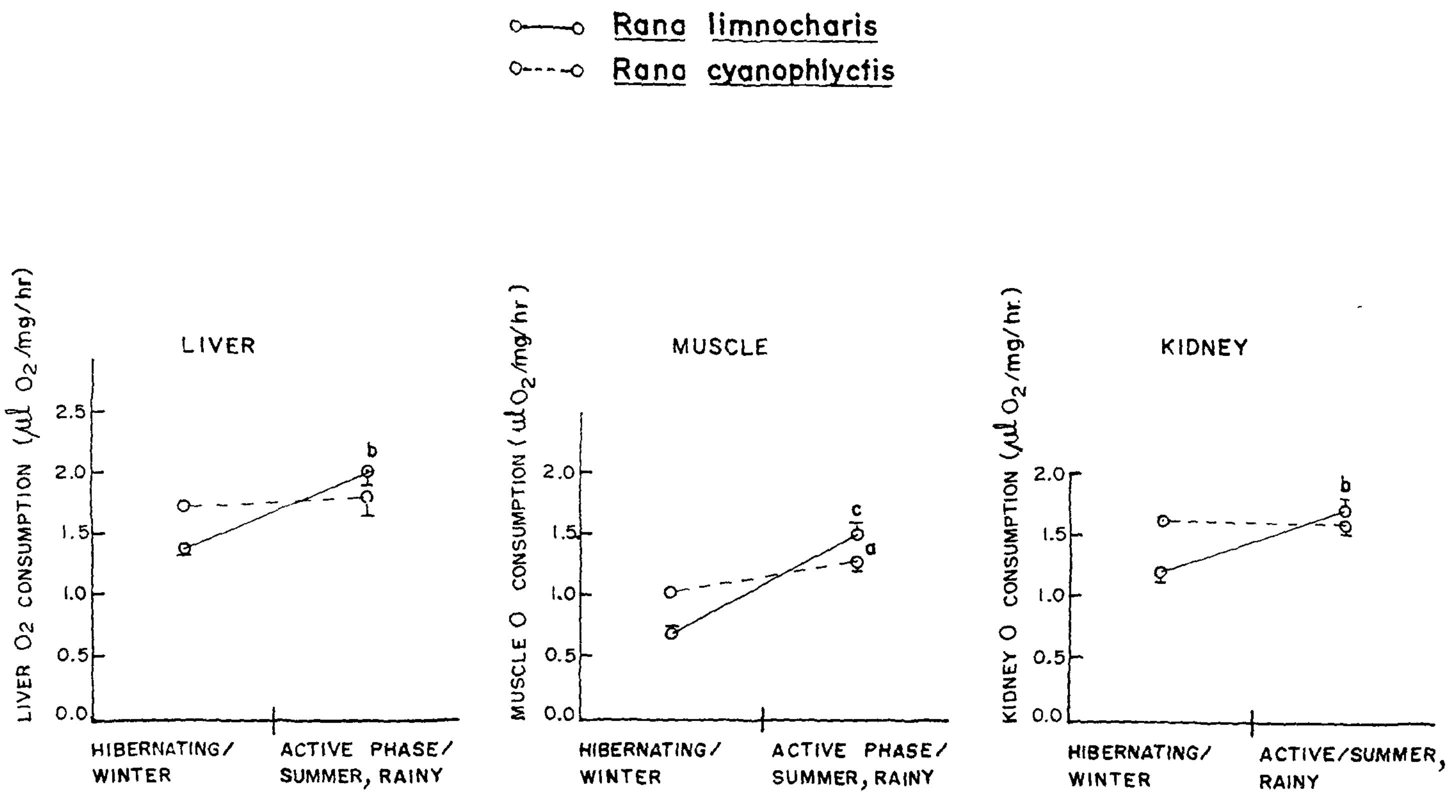

TISSUE RESPIRATION (µl O2/mg/hr)
0·0
0·5
1·5
2·5
2·0
LIVER
MUSCLE
KIDNEY
Rana limnocharis
Rana cyanophlyctis
Month:
Av. temp.:
NOV
12°C
DEC
5·75°C
JAN
8°C
FEB
10·5°C
MAR
10·5°C
APR
18°C
MAY
20·5°C
JUN
18·5°C
JUL
17°C
AUG
21°C
SEP
20°C
OCT
20°C

Fig. 2B : Average rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during hibernation/Winter and active phase/Summer-Rainy season.

^{a,b,c} Differ from the values during Winter : $P < 0.05$, 0.01 and 0.001 respectively.


Rana limnocharis
Rana cyanophlyctis
LIVER
LIVER O2 CONSUMPTION (µl O2/mg/hr)
0.0
0.5
1.0
1.5
2.0
2.5
b
HIBERNATING/ WINTER
ACTIVE PHASE/ SUMMER, RAINY
MUSCLE
MUSCLE O CONSUMPTION (µl O2/mg/hr)
0.0
0.5
1.0
1.5
2.0
c
a
HIBERNATING/ WINTER
ACTIVE PHASE/ SUMMER, RAINY
KIDNEY
KIDNEY O CONSUMPTION (µl O2/mg/hr.)
0.0
0.5
1.0
1.5
2.0
b
HIBERNATING/ WINTER
ACTIVE/SUMMER, RAINY

CHAPTER 3

Chapter 3

Role of Thyroid Hormones in Regulation of the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*

Introduction

The oxidative metabolism in homeotherms is controlled mainly by thyroid hormones. Thyroid hormones invariably increase and thyroidectomy decreases the metabolic rate of birds and mammals (Pitt-Rivers and Tata, 1959; Barrington, 1964; DeGroot and Stanbury, 1975; Bentley, 1976; Oppenheimer, 1979; Ingbar, 1985). However, there are contradictory reports regarding the calorigenic function of thyroid hormones in Poikilothermic vertebrates (see reviews by Eales, 1979, 1990; Rosenkilde, 1979; Thapliyal and Gupta, 1983; Gupta and Thapliyal, 1991). Recent studies indicate that thyroid hormones are calorigenic in reptiles only at high (at or above 25°C) ambient temperature (Gupta and Thapliyal, 1985a, 1991). Unlike in Reptiles, there are only a few studies on the calorigenic role of thyroid hormones in temperate amphibians. Some investigators have suggested calorigenic role of thyroid hormones in temperate amphibian species (Euler, 1933; Taylor, 1939; Warren, 1940; Donoso, 1960; Jankowsky, 1960; Matty and Green, 1963; Maher, 1967; McNabb, 1969; Packard, *et al.*, 1974; Packard and Packard, 1975; Packard, 1976). However, a number of investigators have reported that thyroid hormones are not involved in the regulation of energy metabolism of amphibians (Gayda, 1922; Henschel and Stauber, 1935; Galton and Ingbar, 1962; Taylor and Barker, 1967; Gupta and Chakrabarty, 1990). Thyroid hormones are reported to be actively involved in amphibian metamorphosis, but the endogenous thyroid hormones were not found to be involved in the oxidative metabolism of metamorphosing anurans (Etkin, 1934; Fletcher and Mayant, 1959; Lewis and Frieden, 1959; Marussic,*et al*., 1966; Funkhouser and Mills, 1969). It seems that the conflicting reports are based on the studies in which ambient temperature, dose(s) of hormones and months of year/phases of the annual activity cycle were not given their due importance. Further, so far no attempts have been made to study the calorigenic role of thyroid hormones in tropical/sub-tropical hibernating and non-hibernating (which do not exhibit cold torpor/lethargy) amphibian species. Keeping in view the scarcity of information and phylogenetic importance of amphibians, it was thought worth while to investigate the role of thyroid hormones in the regulation of the oxidative metabolism in a hibernating and a non-hibernating amphibian species. In the present study,*in vivo* and *in vitro* experiments were executed to investigate the effects of thyroid hormones and a goitrogenic (propyl thiouracil) on the metabolic rate of tissues in *Rana limnocharis* and *Rana cyanophlyctis* exposed to natural climatic conditions during winter and summer/rainy seasons at Shillong.

Materials and Methods

All the experiments were conducted on adult male *Rana limnocharis* (Body weight : 8-10 g) and *Rana cyanophlyctis* (Body weight: 10-12 g) which were captured locally. For *in vivo* experiments, frogs were maintained under natural climatic conditions in plastic cages and acclimatized for 10 days before the treatments were started (for details, please see "Chapter 1"). For *in vitro* experiments, frogs were captured from the nature and killed immediately. All the experiments were conducted during winter and summer/rainy months on both the species until/unless specified. Details of experimental protocol is given below:

Experimental protocol

Expt No.	Treatments	In vivo/In vitro	Months (Temperature)	Dose	Duration
1	Saline(Control)				
	L-T3	In vivo	December (7^{0}-15^{0} C)	2µg/frog/d	4 days
	L-T_4	-do-	-do-	-do-	-do-
2	Control				
	L-T3	In vitro	December (6^{0}-14^{0} C)	0.61µM	
	L-T_4	-do-	-do-	0.51µM	

3	Saline(Control)				
	L-T3	In vivo	July (12°-21° C)	2ug/frog/day	4 days
	L-T_4	-do-	-do-	-do-	-do-
4	Saline(Control)				
	L-T3	In vitro	July (14°-21° C)	0.61 µM	
	L-T_4	-do-		0.51 µM	
5	Control				
	L-T3	In vitro	August (13°-19° C)		1 µM
	L-T_4	-do-	-do-	1 µM	
6	Control (Tadpoles of *Rana cyanophlyctis*)				
	L-T3	In vitro	August (13°-19° C)	0.61 µM	
	L-T_4	-do-	-do-	0.51 µM	
	Corticosterone	-do-	-do-	1.15 µM	
	Corticosterone + L-T_4	-do-	-do-	1.15 µM	
7	Control				
	PTU(Propyl thiouracil)	In vivo	December (8.5°-16° C)	1µg/g	4 Days
8	Control				
	PTU	In vivo	August (14°-24° C)	-do-	-do-
9	Single injection of L-T_3		January (5°-10° C)	2 µg/frog	
10	Single injection of L-T_3		August (17-24 degree C)	-do-	

Twenty-four hours after the last injection, control and treated frogs were decapitated and tissues (liver, skeletal muscle and kidney) wererapidly removed, rinsed in ice-cold frog Ringer's solution and stored in a refrigerator. The rate of tissues respiration was measured with the help of an oxygen electrode (For details, please see "Chapter 1". In the case of experiment No.9 and 10, the control and the treated animals were decapitated at the time-intervals mentioned in the Tables. The data were analyzed statistically with the help of Student's "t" test.

Results

In vivo effects of L-T_3 and L-T_4:

In vivo administration of L-T_3 and L-T_4 in Rana limnocharis did not produce any significant effect on the metabolic rate of tissues during both winter and summer/rainy months (Tabs. 3 & 5; Figs. 3 & 5). As in Rana limnocharis, thyroxine administration did not alter the metabolic rate of any tissues in Rana cyanophlyctis during winter (Tab. 3; Fig. 3). However, L-T_3 administration induced significant increase in the rate of O_2 consumption of muscle and kidney during winter (Tab.3;Fig.3). During summer/rainy months administration of both L-T_3 and L-T_4 (separately) significantly increased the O_2 uptake of kidney only (Tab. 5; Fig. 5).

In vitro effects of L-T_3 and L-T_4 :

In vitro treatments with L-T_3 or L-T_4 during winter and summer/rainy months did not produce any effect on the rate of tissues respiration in both *Rana limnocharis* and *Rana cyanophlyctis* (Tabs. 4, 6 & 7; Figs. 4, 6 & 7).

In vitro effects of L-T_3 and L-T_4 in tadpoles:

When the tissues from the tadpoles of *Rana cyanophlyctis* were treated with L-T_3 during summer/rainy months, no significant changes were observed in the rate of tissues respiration (Tab. 8; Fig.8). However, L-T_4 administration induced significant increase in the rate of O_2 consumption of liver and tail-fin tissues (Tab. 8; Fig.8). But L-T_4 had no significant effect on corticosterone-induced increase in tissues respiratory rate (Tab. 8; Fig. 8).

In vivo effects of propyl thiouracil (PTU):

Administration of propyl thio-uracil (PTU) significantly decreased the respiratory rate of liver, muscle and kidney in both the species during winter and summer/rainy months except of liver in *Rana cyanophlyctis* during summer/rainy months (Tabs. 9 & 10; Figs. 9 & 10).

In vivo effects of a single dose of L-T_3 :

When a single dose of L-T_3 was administered in *Rana limnocharis* and *Rana cyanophlyctis* during winter and summer/rainy months, it had no significant effect on tissues (liver and muscle) oxygen consumption except during summer/rainy seasons in *Rana cyanophlyctis*, where it significantly increased liver respiratory rate at 6 and 12 hours (Tabs 11-14; Figs. 11-14).

Discussion

A critical analysis of earlier reports regarding the calorigenic action of thyroid hormones in amphibians gives a general impression that probably L-T_3 and L-T_4 might be calorigenic at high ambient temperature and ineffective at low temperature (Maher, 1967; McNabb, 1969; Packard*et al*. 1974; Packard and Packard, 1975; Rosenkilde, 1981; Gupta and Chakrabarty, 1988). However, temperature alone can not be considered as the factor which determines the stimulatory effects of these hormones on the oxidative metabolism.

In the present study, L-T_3 and L-T_4 never stimulated the respiratory rate of tissues in *Rana limnocharis* irrespective of tissues, ambient temperature and mode of treatments (Tabs. 3 & 5 ; Figs. 3 & 5). In *Rana cyanophlyctis*, *in vivo* administration of only L-T_3 significantly increased the respiratory rate of muscle and kidney during winter, while both L-T_3 and L-T_4 stimulated only kidney respiratory rate during summer/rainy season (Tabs. 3 & 5; Figs. 3 & 5). But *in vitro* treatments with L-T_3 and L-T_4 did not influence the tissue respiratory rate in any of the species irrespective of the season/temperature (Tabs. 4, 6 & 7; Figs. 4, 6 & 7). These findings seem to suggest that (i) thyroid hormones can stimulate tissues respiration even at low temperature, however, the stimulatory effect might be dependent on species, tissues and season and/or temperature, and (ii) calorigenic effect will depend on the mode of treatment (*in vivo*/*in vitro*). Since *in vitro* treatments with $L-T_3$ and $L-T_4$ did not stimulate tissues respiration in *Rana cyanophlyctis* irrespective of tissues and season/temperature (Tabs. 4, 6 & 7; Figs. 4, 6 & 7), the stimulatory effects of *in vivo* treatments with $L-T_3$ and $L-T_4$ in the frogs (Tabs. 3 & 5; Figs. 3 & 5) during winter and summer/rainy seasons might be indirect via other hormones. It has been reported that thyroid hormones are not calorigenic in mammmals exposed to very low temperature, but they potentiate the calorigenic action of the catecholamines (Himms-Hagen, 1983). Further, thyroid hormones are reported to attenuate the activity of the sympathetic nervous system (Axelrod, 1975; Gibson, 1981) and increase the concentration of adrenergic receptors in a number of tissues (Giudicelli, 1978; Malbon *et al*., 1978; Ciaraldi and Marinetti, 1978; Scarpace and Abrass, 1981 a; Saudin, 1981b). Similarly, it might be possible that thyroid hormone(s) potentiate the effects of catecholamines on the oxidative metabolism of *Rana cyanophlyctis* which does not hibernate during cold winter months. Experiments conducted to reveal the role of catecholamines in Rana limnocharis and Rana cyanophlyctis are presented in the "Chapter 5".

Unlike in adults, in vitro treatments with L-T_4 (but not L-T_3) significantly increased the respiratory rate of liver and tail-fin tissues of tadpoles (Two limbs-stage) of Rana cyanophlyctis. These findings strongly suggest that L-T_4 might be directly involved in the oxidative metabolism of tadpoles to meet the increased energy demand associated with the complex process of metamorphosis. The metabolic rate of tadpole tissues was not influenced by in vitro treatment with L-T_3. It seems that the oxidative machinary of tadpole tissues acquire responsiveness for thyroxine which is lost in adults (Rosenkilde, 1985). In vitro effectiveness of L-T_4 in tissues of tadpoles and ineffectiveness in stimulating the respiratory rate of tissues from adult seems to suggest that amphibian tissues acquire responsiveness to thyroid hormones during metamorphosis and become selectively insensitive in adults. The changes in sensitivity to thyroid hormones associated with growth and development might be of great adaptive and evolutionary significance. Further experimental studies are essential to know the mechanism and importance of changes in tissues sensitivity associated with growth, development and aging in amphibians.

Propyl-thiouracil (PTU), when administered in vivo, significantly decreased the respiratory rate of tissues (except liver of Rana cyanophlyctis during summer) in both the species during both winter and summer months (Tabs. 9 & 10; Figs. 9 & 10). Derivatives of thio-uria are reported to decrease iodine uptake by thyroid, thyroxi-nogenesis and peripheral conversion of L-T_4 to L-T_3 (Ingbar, 1985; Norman and Litwack, 1987). As a result, these compounds significantly reduce the circulating level of thyroid hormones. Thus, PTU-induced decrease in the endogenous thyroid hormones might be responsible for significant decline in the respiratory rate of tissues. As mentioned earlier, in vitro treatment with L-T_3 and L-T_4 did not alter the rate of tissues respiration, therefore, the decline in the metabolic rate of tissues might be due to decreased calorigenic potency of catecholamines and/or the activity of the sympathetic nervous system and/or concentrations of adrenergic receptors associated with decreased levels of thyroid hormones as reported in mammals (Giudicelli, 1978; Malbon et al., 1978; Ciaraldi and Marinetti, 1978; Scarpace and Abrass, 1981a; Saudin, 1981b). In vitro ineffectiveness of L-T_3 and L-T_4- and PTU-induced decline in the respiration of tissues in both the species seem to suggest that thyroid hormones are indirectly involved in the regulation of the oxidative metabolism of amphibians.

Thyroid hormones were found to be ineffective under in vitro condition. This ineffectiveness might be due to alteration(s) in their receptors, and/or very low concentrations of receptors. The probability of modifications in receptors seems to be rare, if possible, it might be species-dependent. There is a greater possibility of very low thyroid hormone receptors in amphibian tissues as compared to homeotherms (Oppenheimer, 1979; Galton, 1980a, 1988a,b; Eales, 1990). It seems that the concentrations of thyroid hormones receptors are so low in adults that binding level of the hormone molecules to receptors is not enough to influence the oxidative metabolism. Similar suggestions have been made for insensitivity of reptilian tissues to thyroid hormones (Gupta and Thapliyal, 1991).

Multiple injections of L-T_3 and L-T_4 increased only the respiratory rate of kidney in *Rana cyanophlyctis* during summer. However, a single injection of L-T_3 induced significant increase in the rate of oxygen consumption of liver tissues in *Rana cyanophlyctis* after 6 hours and the rate remained stimulated upto 12 hours. The stimulatory effect was not found 24 hours after the single injection (Tab. 14; Fig. 14). In *Rana limnocharis*, single injection of L-T_3 had no effect at any time-intervals irrespective of seasons and tissues. It, thus, seems that before drawing any firm conclusions, one must investigate the time-dependent calorigenic action of thyroid hormones in amphibians. It remains to be investigated whether the observed increase in liver respiration was due to direct action of L-T_3 or due to potentiation of calorigenic action of catecholamine(s).

On the basis of the present findings it can be concluded that the calorigenic effect of thyroid hormones in amphibians (frogs) depends upon the species, tissues, stage of life cycle, mode of treatment (*in vivo*/*in vitro*; single/multiple injection), intervals at which observations are made, and the phase (month/temperature) of the annual activity cycle. Further, at low temperature the thyroid hormones seem to increase the metabolic rate of tissues indirectly by potentiating the calorigenic actions of the catecholamine hormones.

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Table 3 : In vivo effects of T_3 and T_4 on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Winter (Temperature : 7 - 15 ^{o}C)

Treatments	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	1.04 ± 0.05[*]	0.79 ± 0.03	0.92 ± 0.05
L-T_3	1.08 ± 0.07	0.80 ± 0.01	0.98 ± 0.04
L-T_4	1.05 ± 0.04	0.80 ± 0.05	0.96 ± 0.03
	Rana cyanophlyctis		
Saline(Control)	1.44 ± 0.05	1.12 ± 0.02	1.30 ± 0.02
L-T_3	1.46 ± 0.01	1.20 ± 0.01[a]	1.48 ± 0.04[b]
L-T_4	1.44 ± 0.005	1.19 ± 0.03	1.45 ± 0.08

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated control group : P < 0.05 and 0.01 respectively.

Table 4 : In vitro effects of L-T_3 and L-T_4 on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature 6 - 14 °C)

Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline(Control)	0.88 ± 0.07*	0.61 ± 0.03
L-T_3	0.92 ± 0.09	0.71 ± 0.09
L-T_4	0.86 ± 0.02	0.60 ± 0.09
	Rana cyanophlyctis	
Saline(Control)	0.80 ± 0.07	0.40 ± 0.02
L-T_3	0.90 ± 0.06	0.45 ± 0.04
L-T_4	0.81 ± 0.05	0.40 ± 0.05

* Mean ± Standard error; n = 4.

Table 5 : In vivo effects of T_3 and T_4 on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer(Temperature : 12 - 21 ^{o}C)

Treatments	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	2.05 ± 0.07[*]	1.44 ± 0.09	1.82 ± 0.03
L-T_3	2.10 ± 0.09	1.52 ± 0.03	1.93 ± 0.05
L-T_4	2.04 ± 0.02	1.45 ± 0.05	1.84 ± 0.06
	Rana cyanophlyctis		
Saline(Control)	2.01 ± 0.08	1.36 ± 0.06	1.72 ± 0.02
L-T_3	2.17 ± 0.03	1.44 ± 0.06	2.02 ± 0.06[b]
L-T_4	2.12 ± 0.04	1.42 ± 0.08	1.96 ± 0.05[a]

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated group : $P < 0.05$ and 0.01 respectively.

Table 6 : In vitro effects of L-T_3 and L-T_4 on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature 14 - 21 °C)

Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline(Control)	1.81 ± 0.02*	1.65 ± 0.07
L-T_3	1.98 ± 0.08	1.76 ± 0.06
L-T_4	1.82 ± 0.04	1.62 ± 0.06
	Rana cyanophlyctis	
Saline(Control)	1.69 ± 0.005	1.32 ± 0.04
L-T_3	1.86 ± 0.09	1.44 ± 0.07
L-T_4	1.80 ± 0.10	1.34 ± 0.04

* Mean ± Standard error: n = 4.

Table 7 : In vitro effects of equimolar concentration (10^{-6} M) of L-T_3 and L-T_4 on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature : 13 - 19 °C)

Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline(Control)	1.79 ± 0.005[*]	1.25 ± 0.01
L-T_3	1.81 ± 0.10	1.30 ± 0.09
L-T_4	1.89 ± 0.08	1.30 ± 0.07
	Rana cyanophlyctis	
Saline(Control)	1.67 ± 0.03	1.08 ± 0.06
L-T_3	1.74 ± 0.05	1.09 ± 0.04
L-T_4	1,79 ± 0.04	1.20 ± 0.05

[*] Mean ± Standard error; n = 4.

Table 8 : In vitro effects of L-T_3 , L-T_4 and corticosterone on the rate of tissues respiration of <u>Rana cyanophlyctis</u> (tadpoles) during Summer (Temperature 13 - 19 °C)

Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
	Liver	Tailfin
Saline(Control)	1.51 ± 0.05[*]	1.35 ± 0.03
L-T_3	1.49 ± 0.07	1.32 ± 0.03
L-T_4	1.96 ± 0.12[a]	1.59 ± 0.04[b]
Corticosterone	1.74 ± 0.07[a]	1.65 ± 0.05[b]
Corticosterone + L-T_4	1.87 ± 0.06[b]	1.76 ± 0.09[b]

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 9 : In vivo effects of Propyl thiouracil (PTU) on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 8.5 - 16 ^{o}C)

Treatments	Tissue oxygen consumption (μl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
		Rana limnocharis	
Saline(Control)	1.16 ± 0.05[*]	0.94 ± 0.03	1.04 ± 0.03
PTU	0.91 ± 0.03^{b}	0.67 ± 0.07^{a}	0.79 ± 0.05^{b}
		Rana cyanophlyctis	
Saline(Control)	1.54 ± 0.05	1.20 ± 0.07	1.29 ± 0.01
PTU	1.30 ± 0.06^{a}	0.76 ± 0.12^{a}	0.96 ± 0.09^{a}

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 10 : In vivo effects of Propyl thiouracil (PTU) on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 14 - 24 °C)

Treatments	Tissue oxygen consumption ($\mu l\ O_2$/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	$2.05 \pm 0.04^{*}$	1.72 ± 0.04	1.99 ± 0.05
PTU	1.67 ± 0.03^{c}	1.60 ± 0.01^{a}	1.80 ± 0.03^{a}
	Rana cyanophlyctis		
Saline(Control)	2.20 ± 0.07	1.75 ± 0.005	1.93 ± 0.05
PTU	2.05 ± 0.03	1.50 ± 0.07^{a}	1.70 ± 0.05^{a}

[*] Mean ± Standard error; n = 4.

[a,c] Differ from the saline treated control group : $P < 0.05$ and 0.001 respectively.

Table 11 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in _Rana limnocharis_ during Winter (Temperature : 5 - 10 °C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h) Liver	Muscle
10 Minutes	Saline(Control)	1.50 ± 0.05*	1.29 ± 0.07
	L-T_3	1.50 ± 0.08	1.30 ± 0.05
30 Minutes	Saline(Control)	1.54 ± 0.03	1.30 ± 0.08
	L-T_3	1.58 ± 0.09	1.30 ± 0.03
1 Hour	Saline(Control)	1.55 ± 0.07	1.33 ± 0.03
	L-T_3	1.53 ± 0.09	1.36 ± 0.09
6 Hour	Saline(Control)	1.81 ± 0.12	1.66 ± 0.03
	L-T_3	1.73 ± 0.03	1.59 ± 0.09
24 Hour	Saline(Control)	1.55 ± 0.07	1.33 ± 0.05
	L-T_3	1.48 ± 0.05	1.24 ± 0.11

* Mean ± Standard error; n = 4.

Table 12 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in <u>Rana</u> <u>cyanophlyctis</u> during Winter (Temperature : 5 - 10 °C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes	Saline(Control)	1.58 ± 0.03[*]	1.35 ± 0.05
	L-T_3	1.58 ± 0.05	1.32 ± 0.07
30 Minutes	Saline(Control)	1.60 ± 0.07	1.35 ± 0.07
	L-T_3	1.59 ± 0.05	1.36 ± 0.02
1 Hour	Saline(Control)	1.60 ± 0.09	1.41 ± 0.03
	L-T_3	1.65 ± 0.07	1.36 ± 0.07
24 Hour	Saline(Control)	1.75 ± 0.10	1.53 ± 0.10
	L-T_3	1.70 ± 0.05	1.40 ± 0.09

[*] Mean ± Standard error; n = 4.

Table 13 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in _Rana limnocharis_ during Summer (Temperature : 17 - 24 °C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	2.42 ± 0.05[r*]	1.30 ± 0.09
	L-T_3	2.55 ± 0.11	1.47 ± 0.18
30 Minutes :	Saline(Control)	2.30 ± 0.10	1.42 ± 0.08
	L-T_3	2.25 ± 0.09	1.40 ± 0.05
1 Hour :	Saline(Control)	2.30 ± 0.07	1.42 ± 0.09
	L-T_3	2.26 ± 0.07	1.42 ± 0.06
6 Hour :	Saline(Control)	2.25 ± 0.03	1.47 ± 0.03
	L-T_3	2.39 ± 0.07	1.42 ± 0.03
12 Hour :	Saline(Control)	2.26 ± 0.12	1.45 ± 0.10
	L-T_3	2.35 ± 0.03	1.47 ± 0.07
24 Hour :	Saline(Control)	2.24 ± 0.04	1.31 ± 0.11
	L-T_3	2.26 ± 0.08	1.35 ± 0.09

[*] Mean ± Standard error; n = 4.

Table 14 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in *Rana cyanophlyctis* during Summer (Temperature : 16 - 25 °C)

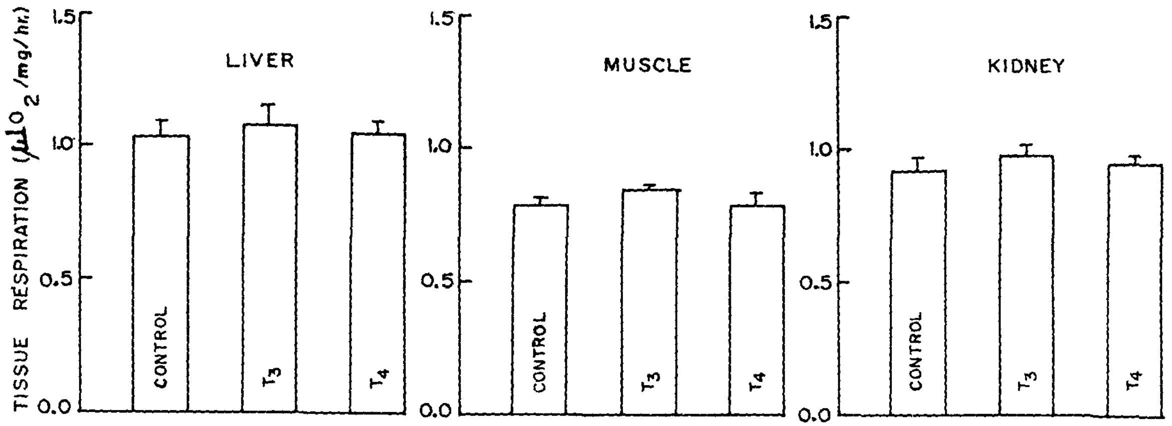
Time	Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h) Liver	Muscle
10 Minutes	Saline(Control)	2.50 ± 0.03[*]	1.80 ± 0.07
	L-T_3	2.59 ± 0.04	1.88 ± 0.03
30 Minutes	Saline(Control)	2.51 ± 0.05	1.80 ± 0.05
	L-T_3	2.59 ± 0.02	1.87 ± 0.06
1 Hour	Saline(Control)	2.50 ± 0.03	1.95 ± 0.03
	L-T_3	2.65 ± 0.07	2.09 ± 0.07
6 Hour	Saline(Control)	2.48 ± 0.07	1.90 ± 0.06
	L-T_3	2.70 ± 0.03[a]	2.01 ± 0.05
12 Hour	Saline(Control)	2.52 ± 0.04	1.90 ± 0.03
	L-T_3	2.70 ± 0.05[a]	1.95 ± 0.02
24 Hour	Saline(Control)	2.52 ± 0.03	1.87 ± 0.04
	L-T_3	2.55 ± 0.04	1.85 ± 0.09

[*] Mean ± Standard error; n = 4.

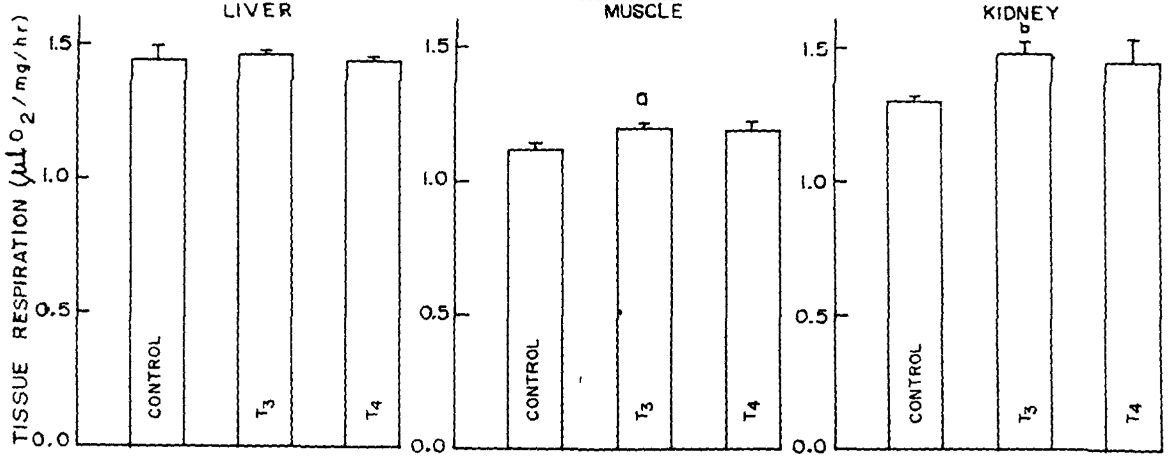
[a] Differs from the respective saline treated control group : $P < 0.05$.

Fig. 3 : In vivo effects of T_3 and T_4 on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Winter (Temperature : 7 - 15 °C)

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

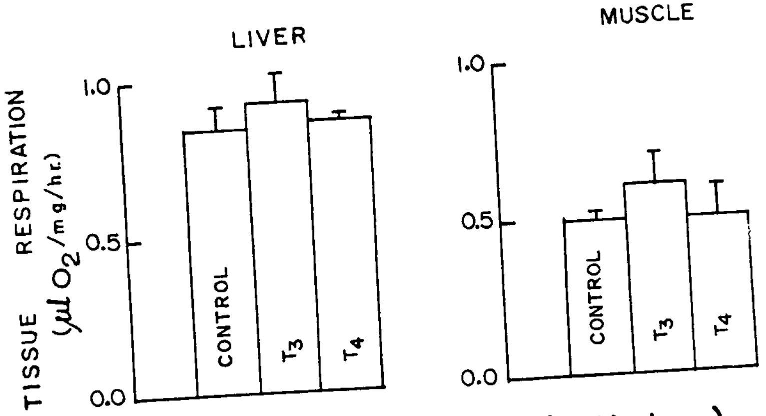


Rana limnocharis (Winter)

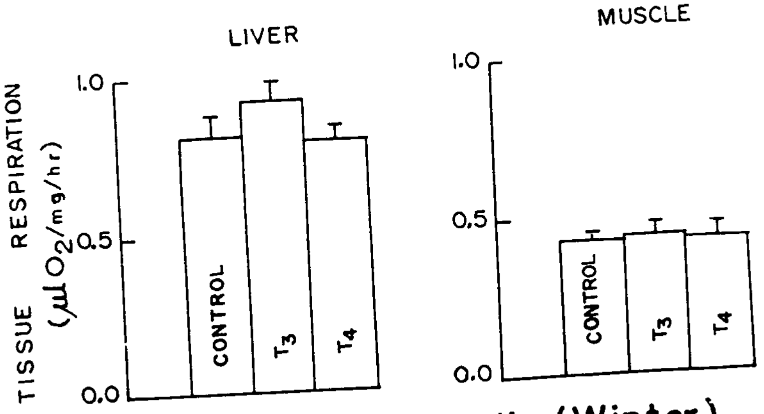


Rana cyanophlyctis (Winter)

Fig. 4 : In vitro effects of L-T_3 and L-T_4 on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Winter (Temperature 6 - 14 °C)



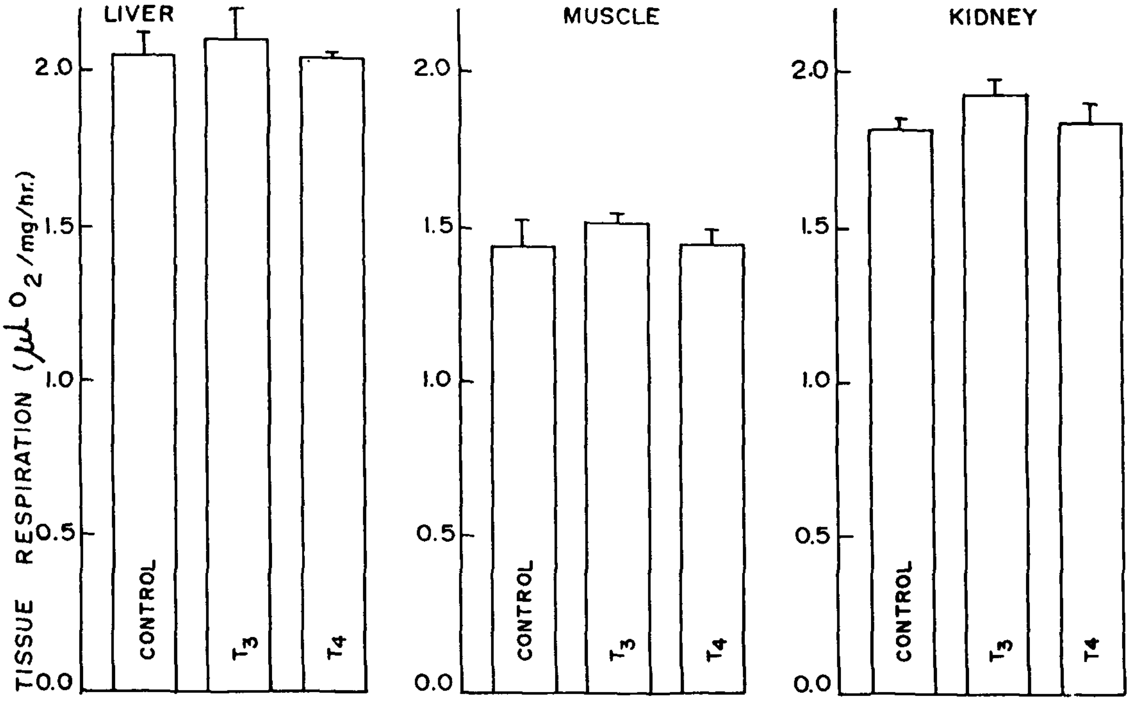
Rana limnocharis (Winter)



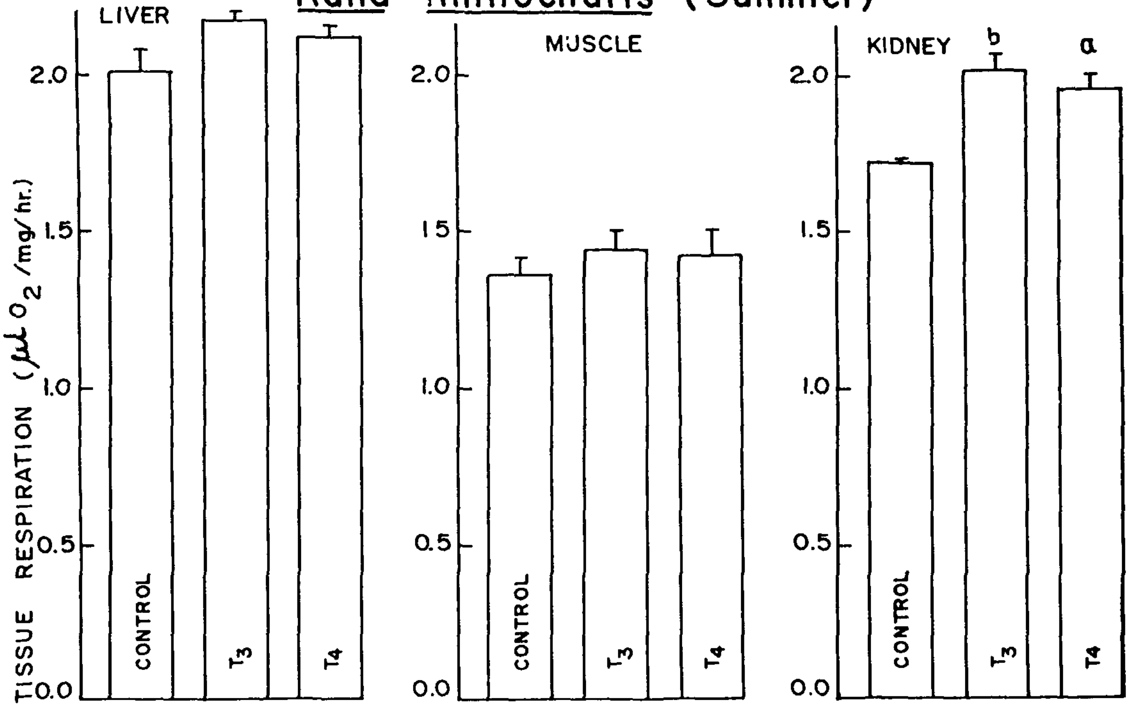
Rana cyanophlyctis (Winter)

Fig. 5 : In vivo effects of T_3 and T_4 on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer(Temperature : $12 - 21\,^{o}C$)

[a,b] Differ from the saline treated group : $P < 0.05$ and 0.01 respectively.

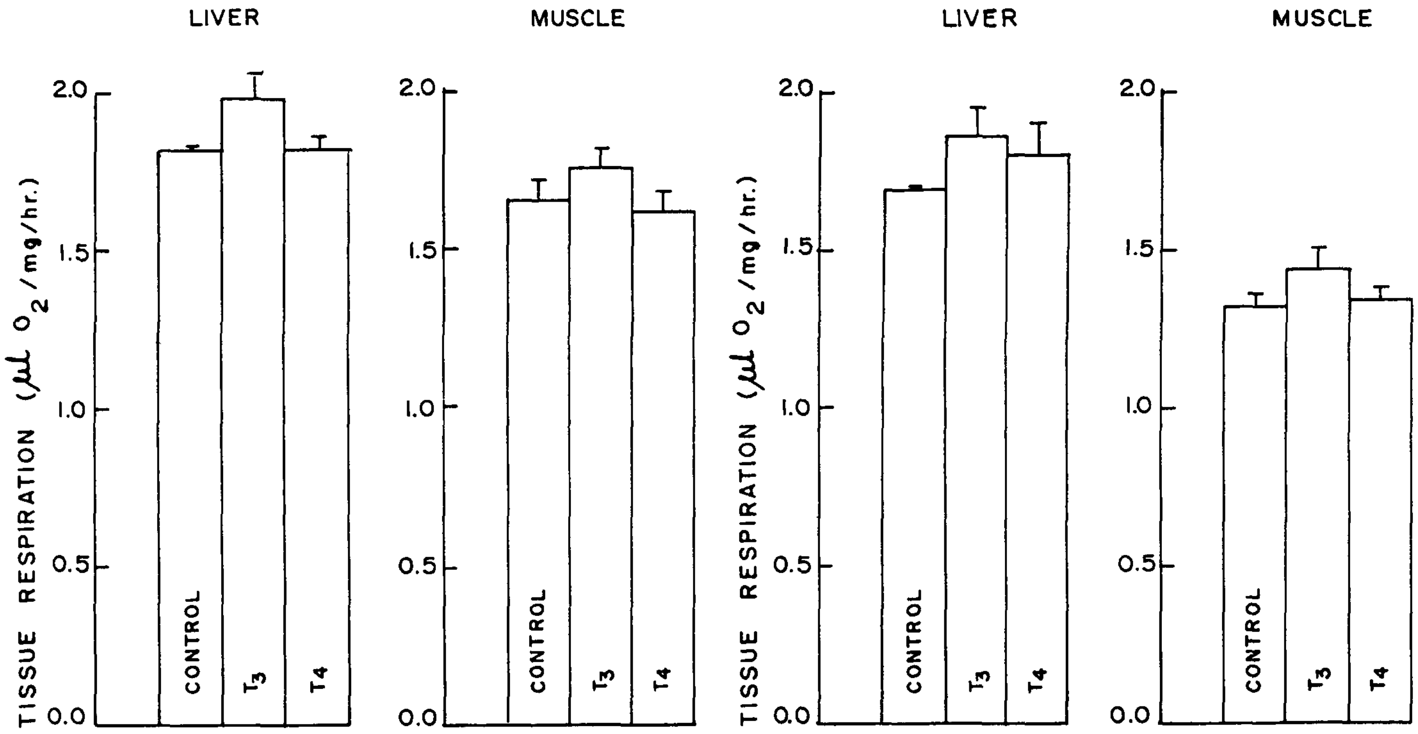


Rana limnocharis (Summer)



Rana cyanophlyctis (Summer)

Fig. 6 : In vitro effects of L-T_3 and L-T_4 on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature 14 - 21 °C)



Rana limnocharis (Summer)

Rana cyanophlyctis (Summer)

Fig. 7 : In vitro effects of equimolar concentration (10^{-6} M) of $L-T_3$ and $L-T_4$ on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature 13 - 19 ^{o}C)

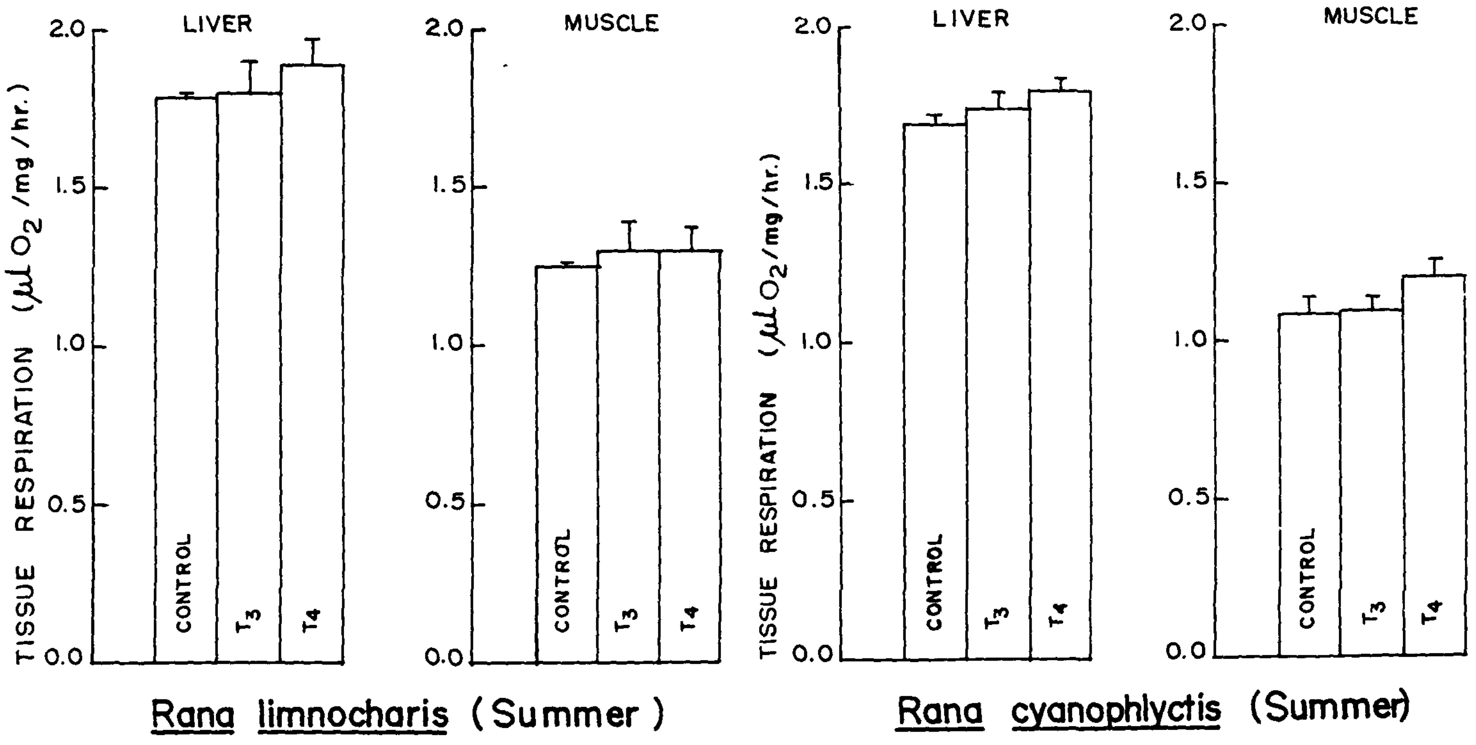
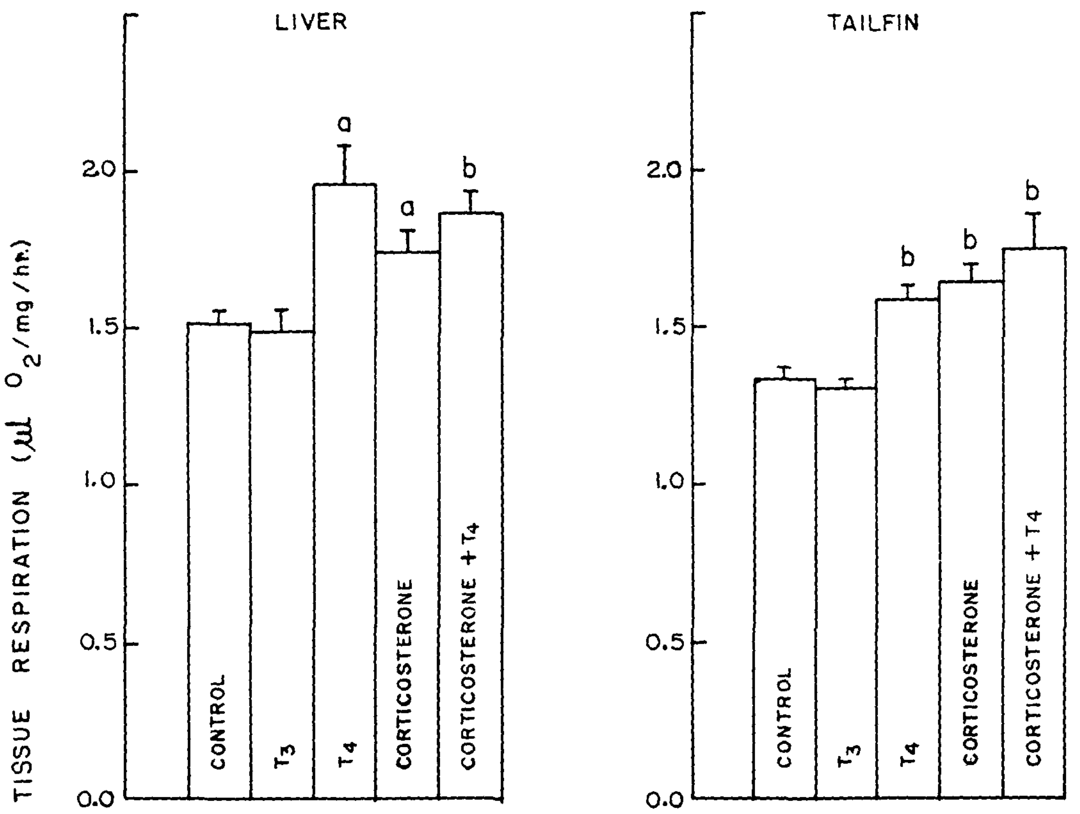

TISSUE RESPIRATION ($\mu l\ O_2$/mg/hr.)
LIVER
MUSCLE
2.0
1.5
1.0
0.5
0.0
CONTROL
T_3
T_4
Rana limnocharis (Summer)
Rana cyanophlyctis (Summer)

Fig. 8 : In vitro effects of L-T_3, L-T_4 and corticosterone on the rate of tissues respiration of <u>Rana</u> <u>cyanophlyctis</u> (tadpoles) during Summer (Temperature 13 - 19 °C)

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

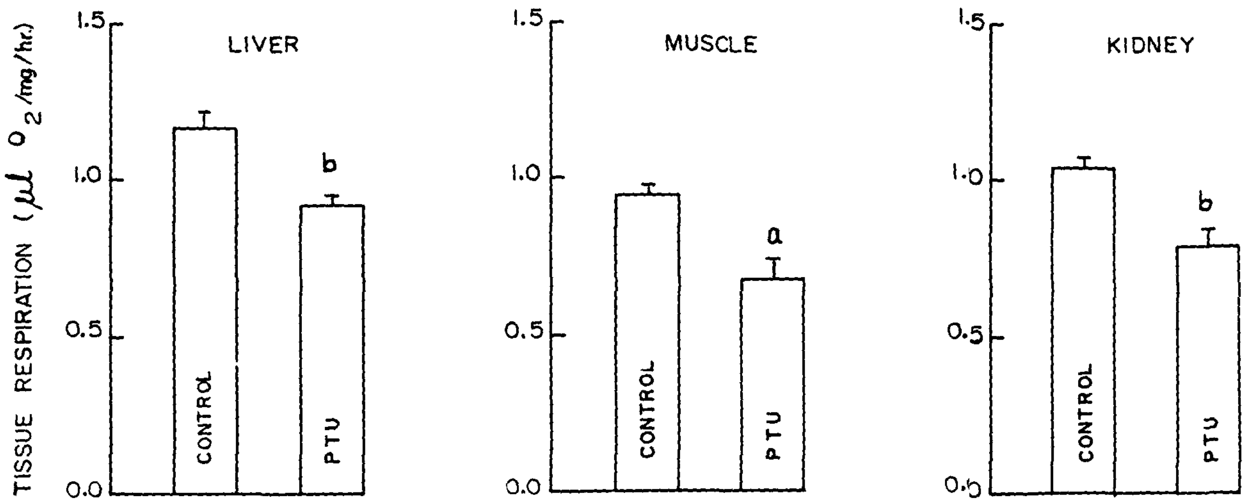
TADPOLES



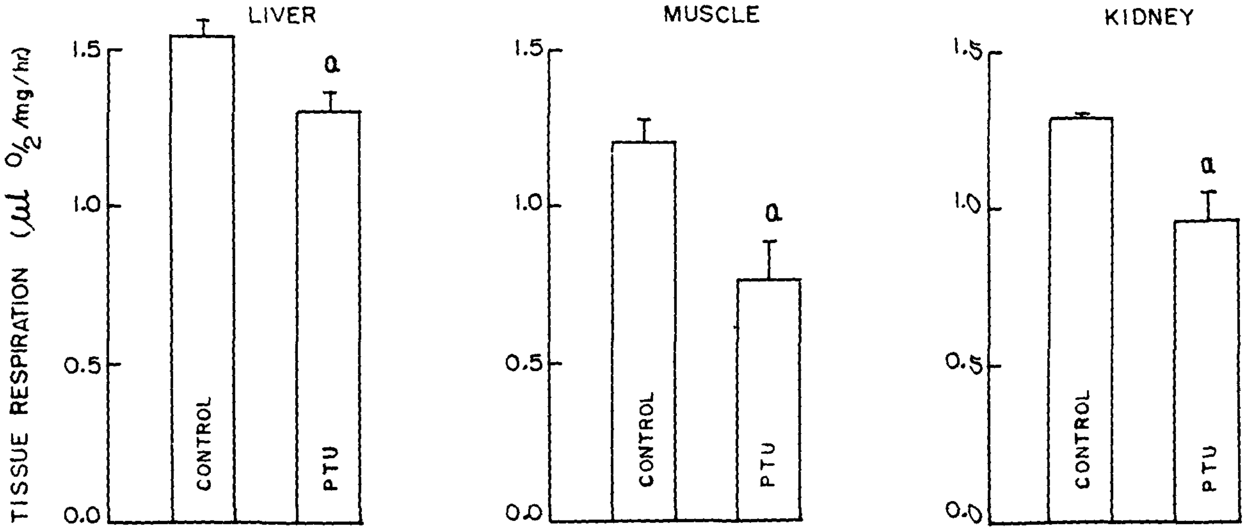
Rana cyanophlyctis (Summer)

Fig. 9 : In vivo effects of Propyl thiouracil (PTU) on the rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during Winter (Temperature : 8.5 - 16 °C)

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.



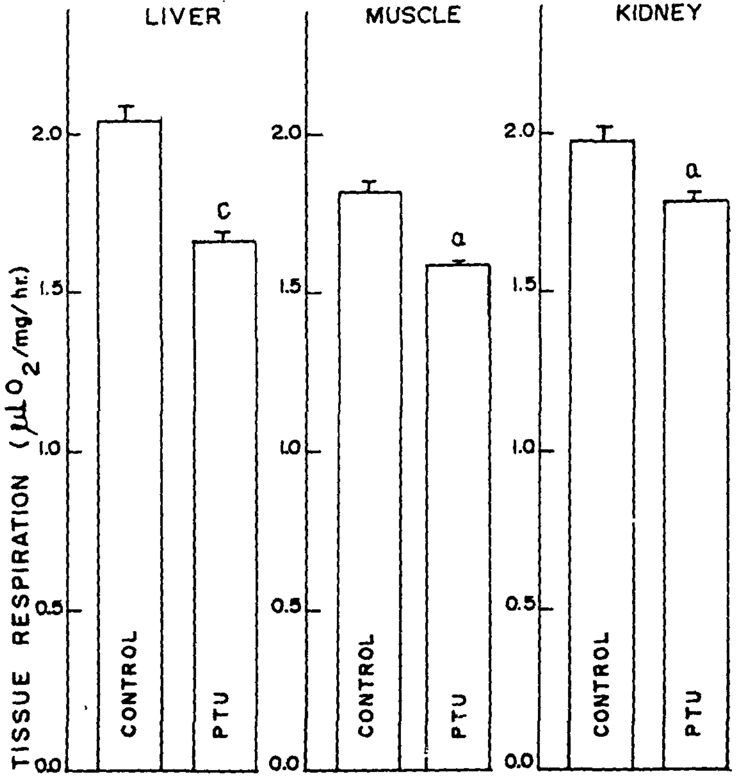
Rana limnocharis (Winter)



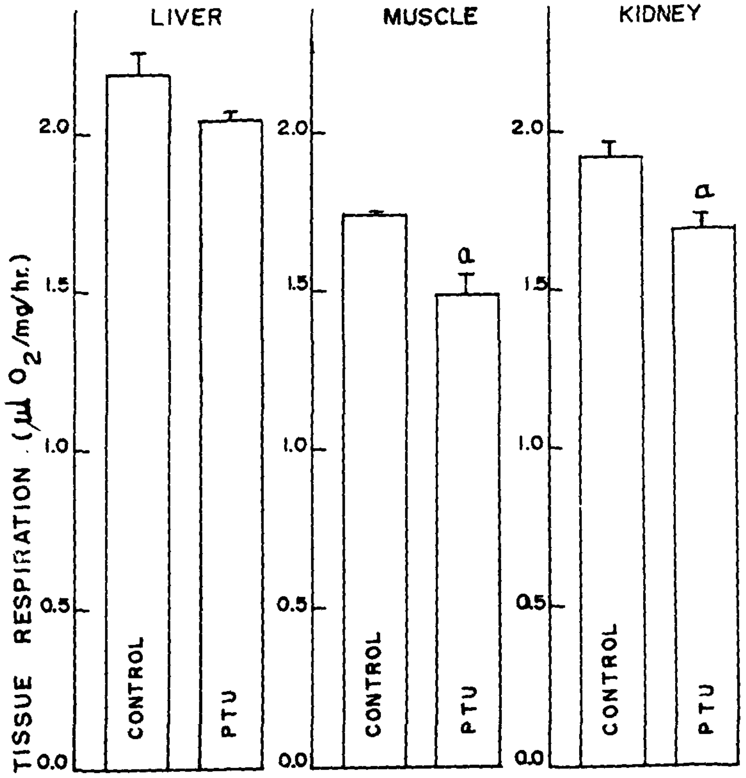
Rana cyanophlyctis (Winter)

Fig. 10 : In vivo effects of Propyl thiouracil (PTU) on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 14 - 24 ^{o}C)

[a,c] Differ from the saline treated control group : $P < 0.05$ and 0.001 respectively.

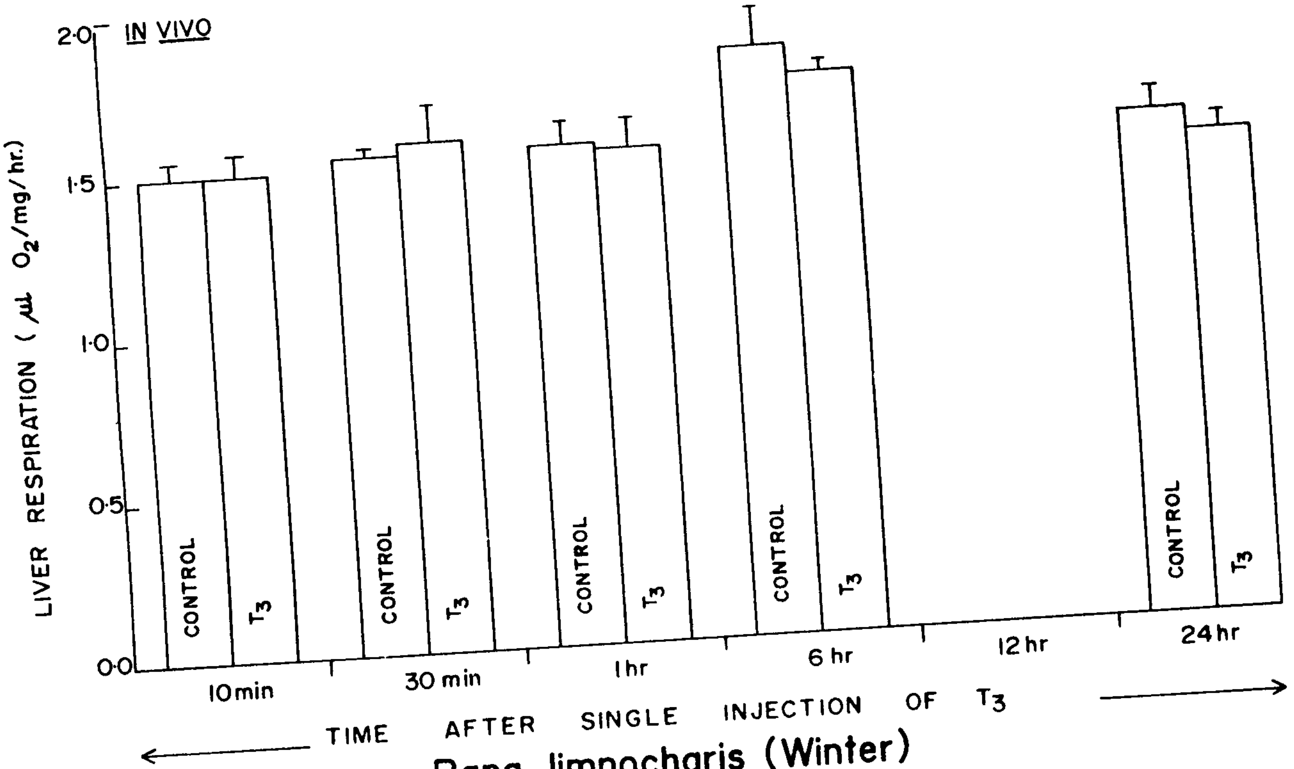


Rana limnocharis (Summer)

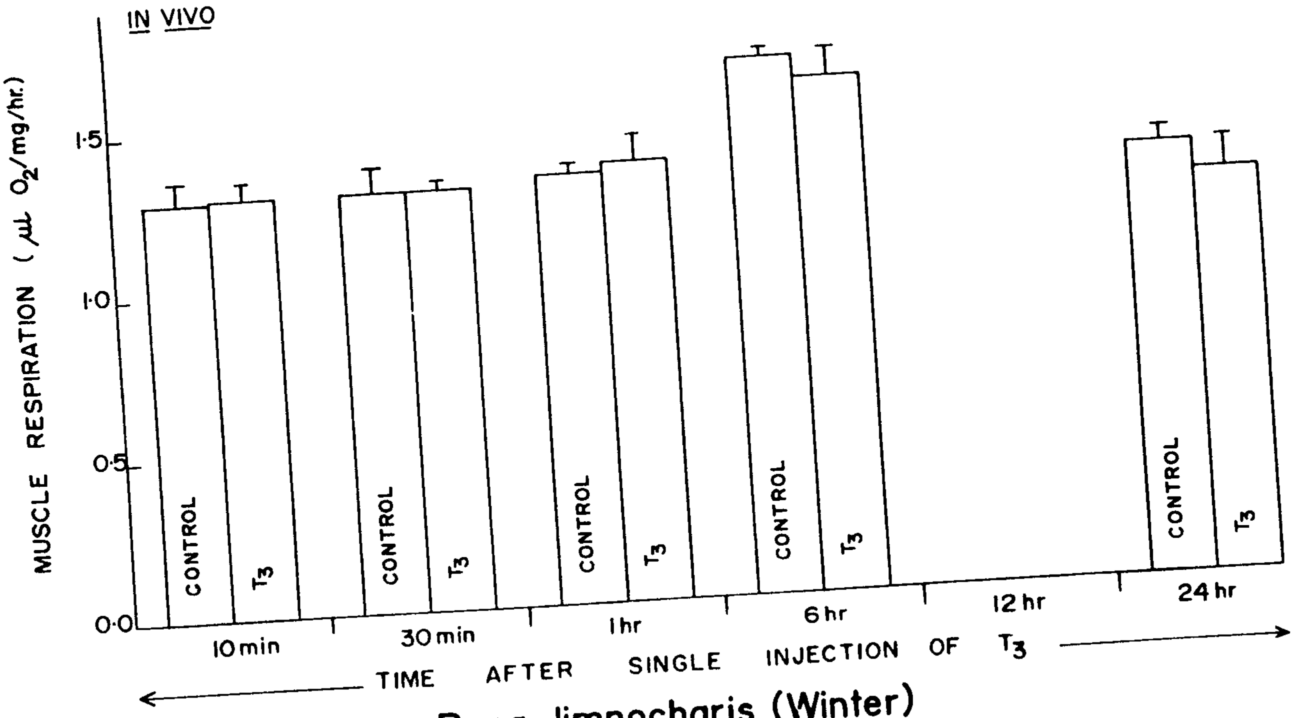


Rana cyanophlyctis (Summer)

Fig. 11 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in <u>Rana limnocharis</u> during Winter (Temperature : 5 - 10°C)

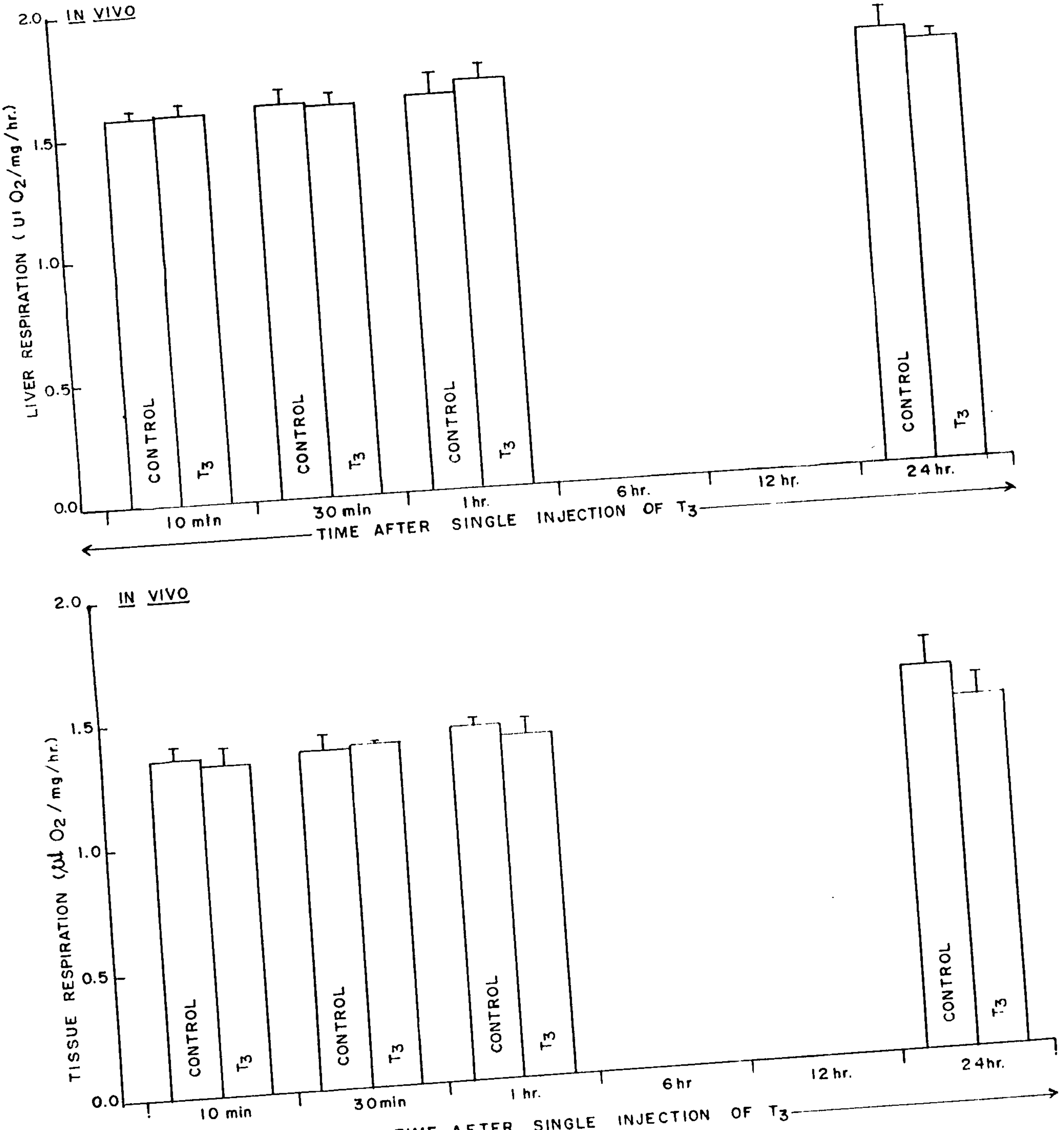


Rana limnocharis (Winter)



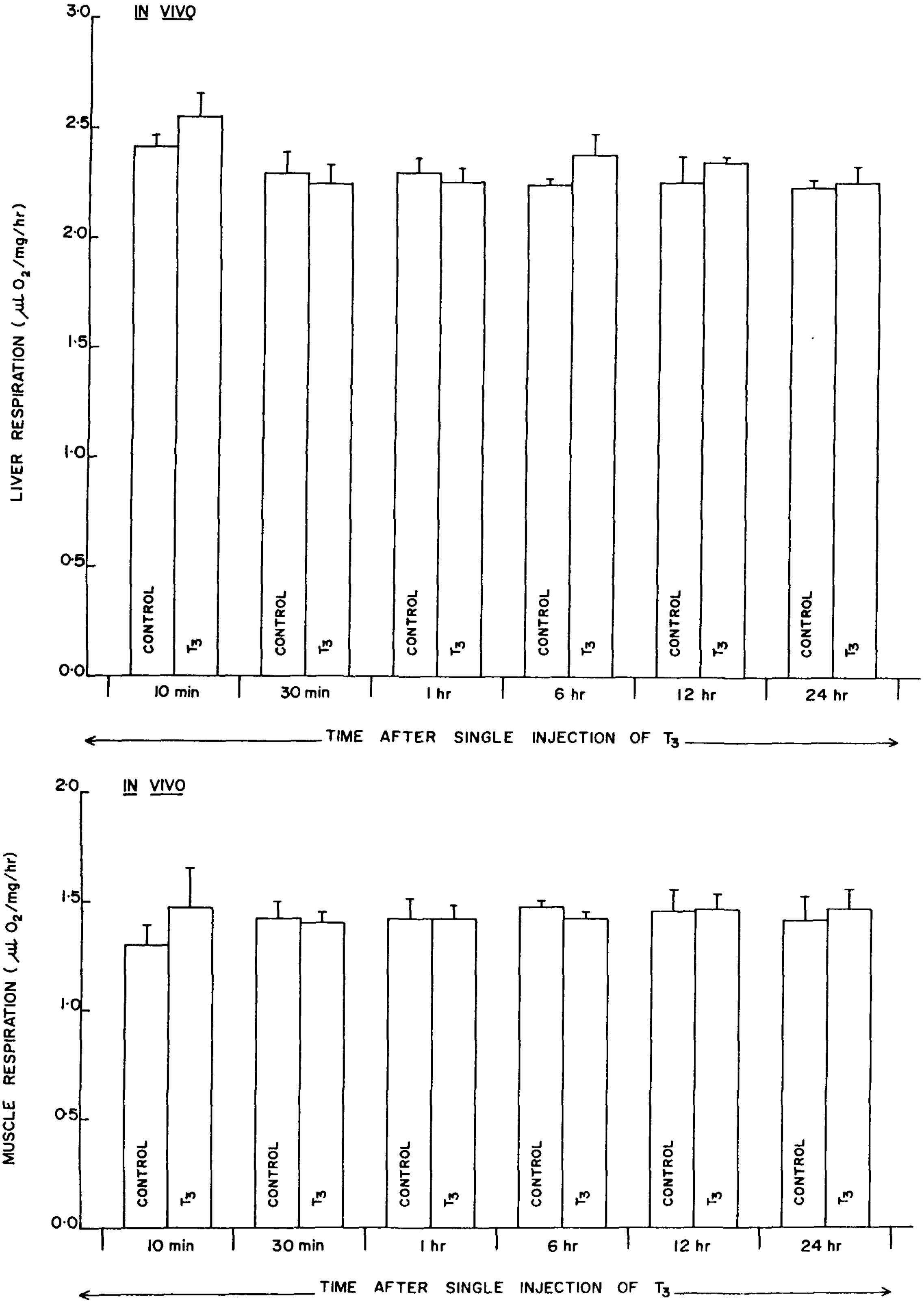
Rana limnocharis (Winter)

Fig. 12 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in <u>Rana</u> <u>cyanophlyctis</u> during Winter (Temperature : 5 - 10 °C)



Rana cyanophlyctis (Winter)

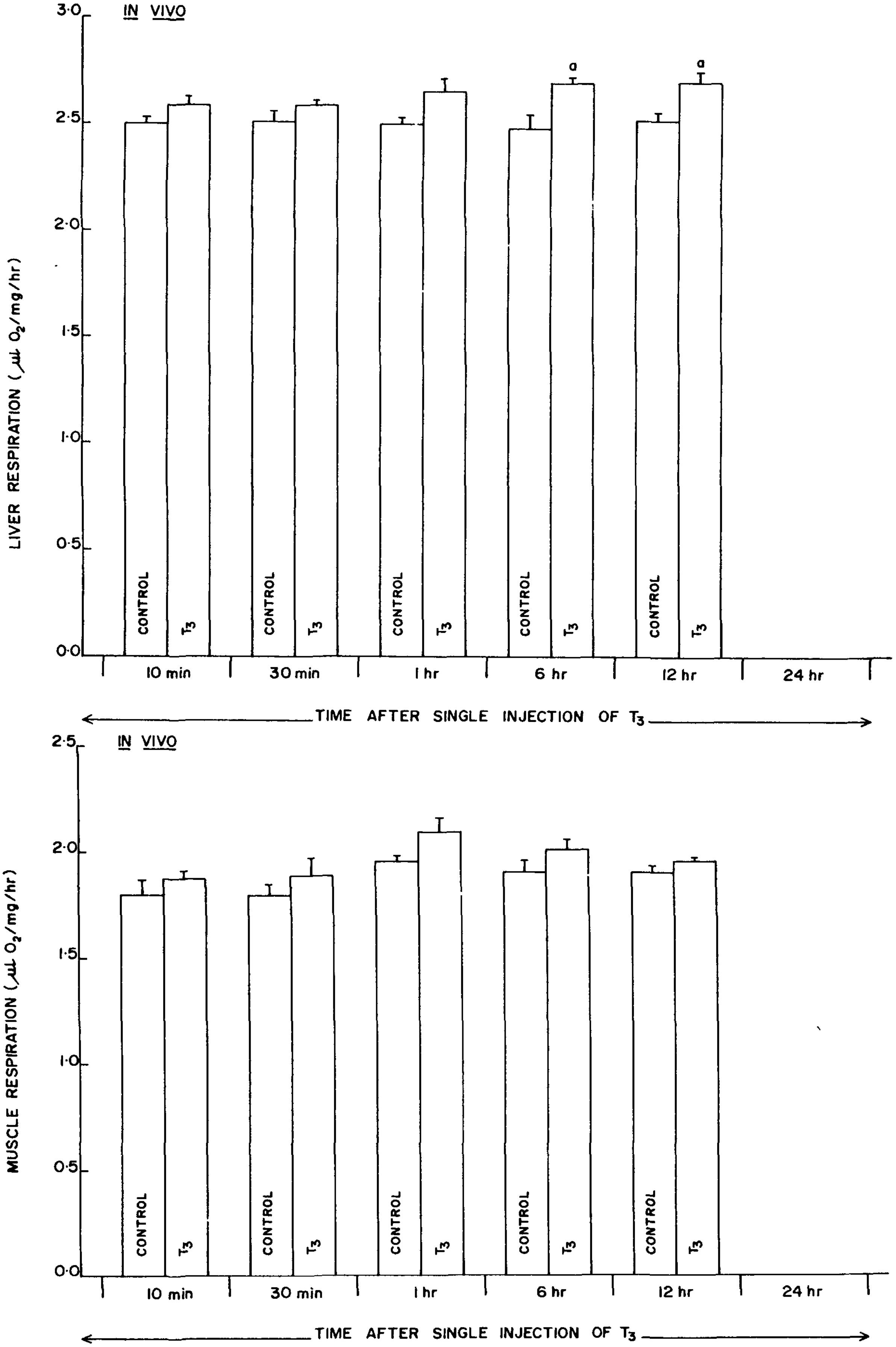
Fig. 13 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in *Rana limnocharis* during Summer (Temperature : 17 - 24 °C)



<u>Rana limnocharis</u> (Summer)

Fig. 14 : Time-dependent in vivo effects of L-triiodothyronine (T_3) on the rate of tissues respiration in _Rana_ _cyanophlyctis_ during Summer (Temperature : 16 - 25 °C)

[a] Differs from the respective saline treated control group : $P < 0.05$.



Rana cyanophlyctis (Summer)

CHAPTER 4

Chapter 4

Role of Testicular Hormones in Regulation of the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

Introduction

Unlike in mammals and birds.thyroid hormones are not directly involved in the oxidative metabolism of poikilotherms (Eales,1979.1985,1990:Rosenkilde,1979.1981:Gupta and Thapliyal.1991). Since ,the calorigenic effects of thyroid hormones were prominent in reptiles only at high temperature.attempts were made to investigate the involvement of gonadal steroids at low and high natural ambient temperature. Now.it is well established that gonadal steroids are directly involved in the oxidative metabolism of reptiles.especially at low ambient temperature (Thapliyal and Gupta.1983:Gupta and Thapliyal,1985a;1991). As it is evident from the findings reported in Chapter 3,thyroid hormones are not very important and/or dependable for the energy metabolism of amphibians.especially in species exposed to mederate/low temperature. Sex steroids have been reported to induce metabolic altertions other than reproductive changes in Amphibian (Follet and Red Shaw .1968). But.so far no attempt has been made to investigate the involvement of gonadal steroids in the energy metabolism of amphibian species at low temperature. Even in reptiles.the mechanism(s) by which gonadal hormones stimulate the oxidative metabolism is not known. Since amphibians occupy a crucial phylogenetic position, we decided to investigate in detail the calorigenic role of testicular hormones (androgens) in two amphibian species, *Rana limnocharis* (hibernating species) and *Rana cyanophlyctis* (non-hibernating species) involving both *in vivo* and *in vitro* experiments conducted during winter and summer/rainy months under natural ambient temperature and daylength. The findings of the present study strongly suggest that testicular hormones are directly involved in the oxidative metabolism of these two species.

Materials and Methods

All Experiments were conducted on male *Rana limnocharis* (Body weight 8-10 g) and *Rana cyanophlyctis* (Body weight 10-12 g) which were captured locally. *In vivo* and *in vitro* experiments were conducted during winter and summer/rainy months on both the species until/unless specified. For *in vivo* experiments, frogs were maintained in plastic cages exposed to natural climatic conditions and acclimatized for 10 days before the treatments were started. For *in vitro* experiments, frogs were killed immediately after collection from the nature (for details, please see "Chapter 1"). Details of experimental protocol is given on the next page.

Experimental Protocol

Exp No.	Treatments	In vivo/vitro	Months (Temperature)	Dose	Duration
1.	Control	In vivo	January		
	Testosterone		(6.1-14 ^{0}C)	2µg/frog/Day	4 days
2.	Control	In vitro	January (6.1-14 ^{0}C)		
	Testosterone			1.38 µM	
3.	Control	In vivo	July		
	Testosterone		(12-20 ^{0}C)	2µg/frog/Day	4 days
4.	Control	In vitro	August (13-19 ^{0}C)		
	Testosterone			1.38 µM	
5.	Control Cyproterone acetate (CA)	In vivo	January (6-14 ^{0}C)	1µg/g Body wt.	4 days
6.	Control	-do-	August (14-23 ^{0}C)	-do-	-do-
	CA			-do-	-do-
7.	Single injection of Testosterone	In vivo	January (7-15 degree C)	2µg/frog	10 Min 30 Min 1 hr 6 hr 24 hr
8.	Single injection of Testosterone	-do-	September (16-23 ^{0}C)	-do-	-do-
9.	Control	in vitro	July (12-19 ^{0}C)		
	CA			1 µM	
	Testosterone			1.38 µM	
	CA + T			1 µM + 1.38 µM	

10. Control	in vitro	August (13-19 ^{0}C)	
Testosterone (T)			1.38 µM
Actinomycin D			1 µM
Cyclohexamide			1 µM
Ouabain			1 µM
Actinomycin-D + T			1 µM + 1.38 µM
Cyclohexamide + T			1 µM + 1.38 µM
Ouabain + T			1 µM + 1.38 µM
(<u>Rana cyanophlyctis</u>)			

Twenty-four hours after the last <u>in vivo</u> injections, control and treated frogs were decapitated and tissues (Liver, skeletal muscle and kidney) were removed, rapidly rinsed in ice-cold frog Ringer's solution and stored in a refrigerator. The rate of tissues respiration was measured with the help of an oxygen electrode (For details, please see "Chapter 1"). In the case of experiment Nos. 7 and 8, control and treated animals were decapitated at the time-intervals mentioned in the experimental protocol. The data were analyzed statistically with the help of student's "t" test.

Results:

In vivo effects of testosterone and cyproterone acetate (CA):

In vivo administration of testosterone significantly stimulated the rate of oxygen consumption of liver, muscle and kidney tissues in both *Rana limnocharis* and *Rana cyanophlyctis* during winter as well as during summer/rainy months (Tabs. 15 & 17; Figs. 15 & 17). *In vivo* injections of cyproterone acetate (androgen receptor blocker) significantly decreased the respiratory rate of all the tissues during winter and summer/rainy months (Tabs. 19 & 20; Figs. 19 & 20).

In vitro effects of testosterone:

In vitro treatments with testosterone invariably stimulated the rate of oxygen consumption of tissues (liver and skeletal muscle) of both *Rana limnocharis* and *Rana cyanophlyctis* during winter and summer/rainy months (Tabs. 16 & 18; Figs. 16 & 18).

Effects of a single in vivo injection of Testosterone:

A single dose of testosterone evoked different responses at different time-intervals in *Rana limnocharis* and *Rana cyanophlyctis* during different months/seasons. In *Rana limnocharis*, a single dose of testosterone stimulated the respiratory rate of both liver and muscle after 6 hours during winter (Tab. 21; Fig. 21). During summer, liver O_2 uptake was stimulated after 30 minutes and remained significantly high up to one hour, while muscle respiratory rate was stimulated after 10 minutes and remained significantly high upto 6 hours (Tab. 23; Fig. 23). Unlike in *Rana limnocharis*, a single dose of testosterone in *Rana cyanophlyctis* significantly stimulated only liver respiratory rate after 6 hours during winter (Tab. 22; Fig. 22). However, the respiratory rate of both liver and skeletal muscle were stimulated significantly after 30 minutes and remained significantly high upto 6 hours after the injection during summer/rainy months (Tab. 24; Fig. 24).

In vitro effects of testosterone in the presence of inhibitors:

When liver and muscle homogenates were first incubated with cyproterone acetate (androgen receptor blocker), Actinomycin-D (inhibitor of transcription), cyclohexamide (inhibitor of Na^+-K^+-ATPase) separately, testosterone (when added *in vitro*) could not stimulate the respiratory rate of the tissues (Tabs. 25 & 26; Figs. 25 & 26).

Discussion

In vivo and In VITRO treatments with testosterone invariably stimulated the rate of tissues respiration in both Rana limnocharis and Rana cyanophlyctis irrespective of seasons and Temperature (Tabs. 15-18; Figs. 15 - 18). Further, cyproterone acetate (CA) always inhibited the respiratory rate of tissues (Tabs. 19 & 20; Figs. 19 & 20). These findings strongly suggest that testicular hormones are calorigenic in frogs, and the calorigenic effect seems to be direct and independent of seasons/ambient temperature. The temperature-independent stimulatory effect of testosterone in frogs at low temperature might be of great adaptational significance. In Rana limnocharis and Rana cyanophlyctis, Leydig cells are found to be active throughout the year, except during winter when there is marginal decline in their activity (Saidapur, 1989). In Rana limnocharis, Leydig cell activity has been found to be comparatively low during winter (our unpublished data). Since continuous hyperactivity of Leydig cells in the testis of Rana cyanophlyctis represents increased production of steroid throughout the year, the difference between the activity level of Rana cyanophlyctis and Rana limnocharis during winter months might be due to differences in the circulating levels of androgens (testosterone). However, this suggestion remains to be confirmed by measuring the circulating level of testosterone in both the species.

In vitro stimulation of tissues respiration by testosterone seems to suggest that testosterone acts directly on the oxidative machinery of the cells without involving any other hormone(s). Similar suggestion has been made for reptiles (Gupta and Thapliyal, 1991). The direct involvement of testosterone in amphibian and reptiles might be of evolutionary significance. It seems that the lower vertebrates (Poikilotherms) first involved gonadal hormones in the regulation of energy metabolism to ensure efficient energy production to meet the increased energy demand associated with reproductive activities. The development of sensitivity of the oxidative metabolism in poikilotherms to gonadal hormones was probably essential to ensure successful breeding and survival at low temperature. It seems that, with the evolution of homeothermy, thyroid hormones assumed the calorigenic function and gonadal steroids became involved exclusively in the process of reproduction.

Though the calorigenic effect of a single dose of testosterone was found to depend on the species, tissues and seasons, testosterone could stimulate respiratory rate of tissues within 30 minutes during summer (breeding phase) and within 6 hours during winter in both the species (Tabs. 21-24; Figs. 21-24). It, thus, seems that testosterone can stimulate the oxidative metabolism in amphibians within a short span of time.

When tissue homogenates were pre-incubated with cyproterone acetate, Actinomycin-D, Cyclohexamide and Oubain, testosterone was found to be ineffective in stimulating the respiratory rate of tissues *in vitro* (Tabs. 25 & 26; Figs. 25 & 26). Since cyproterone acetate binds to androgen receptors with high affinity, testosterone could not produce its calorigenic action due to unavailability of unoccupied receptors. Ineffectiveness of testosterone in stimulating respiratory rate of tissues in the presence of Actinomycin-D and cyclohexamide (inhibitor of transcription), suggest that the hormone produces its stimulatory effect on the oxidative metabolism by stimulating the process of transcription. However, at present it is difficult to explain the mechanism by which Ouabain (inhibitor of the enzyme Na^+-K^+-ATPase) interferes with the calorigenic action of testosterone. Whether testosterone stimulates oxidative phosphorylation by stimulating Na^+-K^+-ATPase activity also remains to be unknown.

These findings strongly suggest that testicular hormones play an important role in the regulation of the oxidative metabolism in amphibians. The calorigenic action of testosterone seems to be direct and independent of ambient temperature. Further, testosterone acts via androgen receptors and involves the process of transcription while stimulating the respiratory role of tissues. The role of Na^+-K^+-ATPase in testosterone-induced increase in respiratory rates deserves further exploration.

****

Table 15 : In vivo effects of Testosterone on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Winter (Temperature : 6.1 - 14 °C)

Treatments	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	0.86 ± 0.05[*]	0.48 ± 0.04	0.67 ± 0.03
Testosterone	1.09 ± 0.07[a]	0.59 ± 0.01[a]	0.84 ± 0.02[b]
	Rana cyanophlyctis		
Saline(Control)	0.74 ± 0.03	0.48 ± 0.02	0.76 ± 0.05
Testosterone	1.21 ± 0.05[c]	0.82 ± 0.03[c]	1.01 ± 0.09[a]

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 16 : In vitro effects of testosterone on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 - 14 °C)

Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline(Control)	0.88 ± 0.07[*]	0.59 ± 0.03
Testosterone	1.26 ± 0.10[a]	0.80 ± 0.03[b]
	Rana cyanophlyctis	
Saline (Control)	0.80 ± 0.07	0.40 ± 0.02
Testosterone	1.24 ± 0.08[b]	0.51 ± 0.03[a]

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 17 : In vivo effects of Testosterone on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

Treatments	Tissue oxygen consumption ($\mu l\ O_2$/mg wet tissue/h)		
	Liver	Muscle	Kidney
Rana limnocharis			
Saline (Control)	$2.20 \pm 0.07^{*}$	1.36 ± 0.03	1.76 ± 0.07
Testosterone	2.24 ± 0.03^{a}	1.50 ± 0.02^{b}	1.92 ± 0.05^{b}
Rana cyanophlyctis			
Saline (Control)	1.98 ± 0.07	1.36 ± 0.05	1.72 ± 0.04
Testosterone	2.39 ± 0.11^{a}	1.60 ± 0.7^{a}	2.04 ± 0.07^{b}

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 18 : In vitro effects of testosterone on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature : 13 - 19 ^{o}C)

Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline(Control)	$1.79 \pm 0.005^{*}$	1.25 ± 0.01
Testosterone	1.99 ± 0.05^{b}	1.49 ± 0.08^{a}
	Rana cyanophlyctis	
Saline (Control)	1.67 ± 0.03	1.08 ± 0.06
Testosterone	1.86 ± 0.06^{a}	1.31 ± 0.04^{a}

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 19 : In vivo effects of cyproterone acetate (CA) on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 - 14 °C)

Treatments	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	1.16 ± 0.05[*]	0.94 ± 0.03	1.04 ± 0.03
CA	0.74 ± 0.07[b]	0.84 ± 0.05	0.90 ± 0.03[a]
	Rana cyanophlyctis		
Saline(Control)	1.54 ± 0.05	1.20 ± 0.07	1.29 ± 0.01
CA	1.28 ± 0.07[a]	0.96 ± 0.03[a]	0.96 ± 0.04[c]

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 20 : In vivo effects of cyproterone acetate on the rate of tissues respiration in <u>Rana limnocharis</u> and <u>Rana cyanophlyctis</u> during Summer (Temperature : 14 - 23 ^{o}C)

Treatments	Tissue oxygen consumption ($\mu l\ O_2$/mg wet tissue/h)		
	Liver	Muscle	Kidney
		Rana limnocharis	
Saline (Control)	2.05 ± 0.04[*]	1.72 ± 0.04	1.99 ± 0.05
CA	1.85 ± 0.02[b]	1.55 ± 0.07	1.77 ± 0.03[a]
		Rana cyanophlyctis	
Saline (Control)	2.20 ± 0.07	1.75 ± 0.005	1.93 ± 0.05
CA	1.90 ± 0.05[a]	1.46 ± 0.09[a]	1.72 ± 0.04[a]

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the saline treated group : $P < 0.05$ and 0.01 respectively.

Table 21 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana limnocharis* during Winter (Temperature : 7 - 15 ^{o}C)

Time	Treatments	Tissues oxygen consumption (µl O_2 /mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	1.50 ± 0.02[*]	1.26 ± 0.04
	Testosterone	1.51 ± 0.01	1.28 ± 0.03
30 Minutes :	Saline(Control)	1.50 ± 0.03	1.28 ± 0.07
	Testosterone	1.51 ± 0.07	1.28 ± 0.05
1 Hour :	Saline(Control)	1.50 ± 0.07	1.28 ± 0.03
	Testosterone	1.50 ± 0.09	1.30 ± 0.03
6 Hour :	Saline(Control)	1.92 ± 0.06	1.49 ± 0.05
	Testosterone	3.15 ± 0.07[a]	1.63 ± 0.02[a]
24 Hour :	Saline(Control)	1.45 ± 0.09	1.30 ± 0.01
	Testosterone	1.33 ± 0.01	1.20 ± 0.03[a]

[*] Mean ± Standard error; n = 4.

[a] Differs from the respective saline treated control group : $P < 0.05$.

Table 22 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana cyanophlyctis* during Winter (Temperature : 7 - 15 ^{o}C)

		Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
Time	Treatments	Liver	Muscle
10 Minutes	: Saline(Control)	1.54 ± 0.09[*]	1.36 ± 0.07
	Testosterone	1.55 ± 0.07	1.32 ± 0.02
30 Minutes	: Saline(Control)	1.56 ± 0.02	1.37 ± 0.03
	Testosterone	1.55 ± 0.07	1.38 ± 0.01
1 Hour	: Saline(Control)	1.59 ± 0.07	1.38 ± 0.07
	Testosterone	1.59 ± 0.02	1.39 ± 0.01
6 Hour	: Saline(Control)	1.80 ± 0.02	1.60 ± 0.07
	Testosterone	2.01 ± 0.05[b]	1.79 ± 0.05
24 Hour	: Saline(Control)	1.50 ± 0.06	1.32 ± 0.05
	Testosterone	1.39 ± 0.04	1.20 ± 0.03

[*] Mean ± Standard error; n = 4.

[b] Differs from the respective saline treated control group : $P < 0.05$.

Table 23 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of _Rana limnocharis_ during Summer (Temperature : 16 - 23 ^{o}C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h) Liver	Muscle
10 Minutes :	Saline(Control)	2.30 ± 0.01[*]	1.55 ± 0.02
	Testosterone	2.44 ± 0.03[b]	1.62 ± 0.09
30 Minutes :	Saline(Control)	2.33 ± 0.05	1.50 ± 0.03
	Testosterone	2.50 ± 0.02[a]	1.75 ± 0.09[a]
1 Hour :	Saline(Control)	2.32 ± 0.04	1.55 ± 0.01
	Testosterone	2.55 ± 0.07[a]	1.75 ± 0.05[b]
6 Hour :	Saline(Control)	2.44 ± 0.03	1.55 ± 0.07
	Testosterone	2.32 ± 0.07	1.80 ± 0.03[a]
12 Hour :	Saline(Control)	2.30 ± 0.04	1.65 ± 0.09
	Testosterone	2.32 ± 0.07	1.80 ± 0.07
24 Hour :	Saline(Control)	2.30 ± 0.05	1.55 ± 0.03
	Testosterone	2.32 ± 0.07	1.59 ± 0.09

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the respective saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 24 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature 15 : 23 °C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	$2.32 \pm 0.06^{*}$	1.59 ± 0.03
	Testosterone	2.55 ± 0.08	1.62 ± 0.05
30 Minutes :	Saline(Control)	2.40 ± 0.03	1.62 ± 0.04
	Testosterone	2.60 ± 0.04^{b}	1.78 ± 0.05^{a}
1 Hour :	Saline(Control)	2.40 ± 0.05	1.67 ± 0.03
	Testosterone	2.61 ± 0.01^{b}	1.80 ± 0.04^{a}
6 Hour :	Saline(Control)	2.45 ± 0.03	1.67 ± 0.04
	Testosterone	2.61 ± 0.02^{b}	1.82 ± 0.05^{a}
12 Hour :	Saline(Control)	2.40 ± 0.06	1.60 ± 0.03
	Testosterone	2.60 ± 0.07	1.80 ± 0.09
24 Hour :	Saline(Control)	2.40 ± 0.03	1.60 ± 0.07
	Testosterone	2.59 ± 0.09	1.62 ± 0.05

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the respective saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 25 : In vitro effects of cyproterone acetate and testosterone on the rate of tissues respiration of <u>Rana cyanophlyctis</u> during Summer (Temperature : 12 - 19 °C)

Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
	Liver	Muscle
Saline(Control)	1.20 ± 0.02*	0.76 ± 0.08
Cyproterone acetate(CA)	0.87 ± 0.04[c]	0.59 ± 0.05
Testosterone	1.41 ± 0.06[a]	0.99 ± 0.05[a]
CA + Testosterone	1.34 ± 0.09	0.85 ± 0.09

* Mean ± Standard error; n = 4.

[a,c] Differ from the saline treated control group : $P < 0.05$ and 0.001 respectively.

Table 26 : In vitro effects of testosterone, ouabain, cyclohexamide, and Actinomycin-D on the rate of tissues respiration of male *Rana cyanophlyctis* during summer (Temperature : 13 - 19 °C)

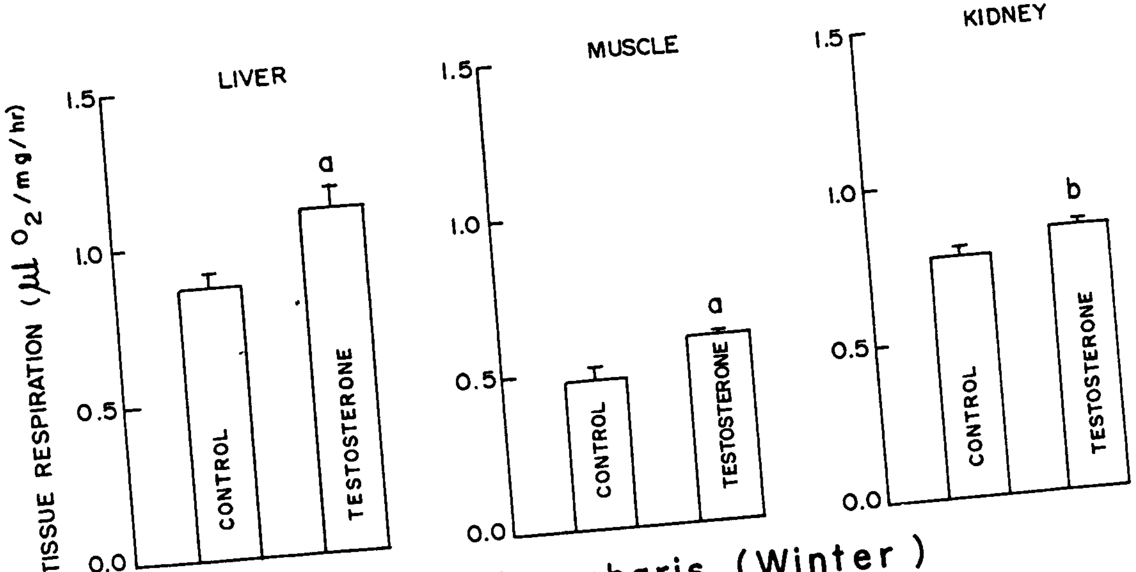
Treatments	Rate of oxygen consumption (ul O_2/mg wet tissue/h)	
	Liver	Muscle
Saline (Control)	1.67 ± 0.03[*]	1.08 ± 0.06
Testosterone (T)	1.86 ± 0.06[a]	1.31 ± 0.04[a]
Actinomycin-D	1.60 ± 0.08	0.90 ± 0.10
Actinomycin-D + T	1.70 + 0.06	1.08 ± 0.12
Cyclohexamide	1.64 ± 0.08	1.10 ± 0.08
Cyclohexamide + T	1.68 ± 0.05	1.14 ± 0.05
Ouabain	1.63 ± 0.07	1.00 ± 0.10
Ouabain + T	1.70 ± 0.05	1.02 ± 0.10

[*] Mean ± Standard Error; n = 4.

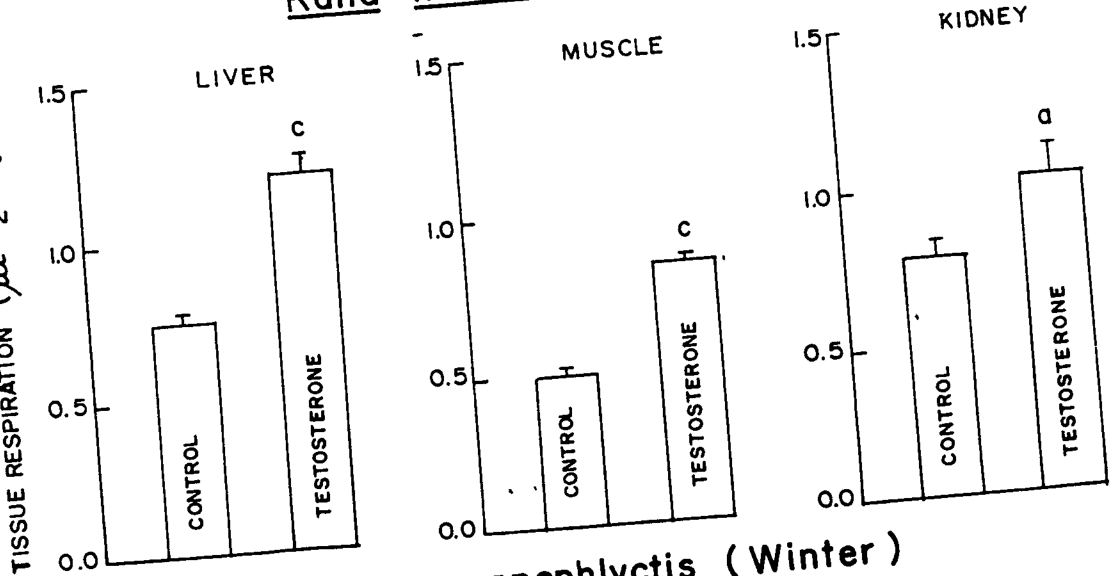
[a] Differs from the saline treated control group : $P < 0.05$.

Fig. 15 : In vivo effects of Testosterone on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Winter (Temperature : 6.1 - 14 °C)

^{a,b,c} Differ from the saline treated control : $P < 0.05$, 0.01 and 0.001 respectively.



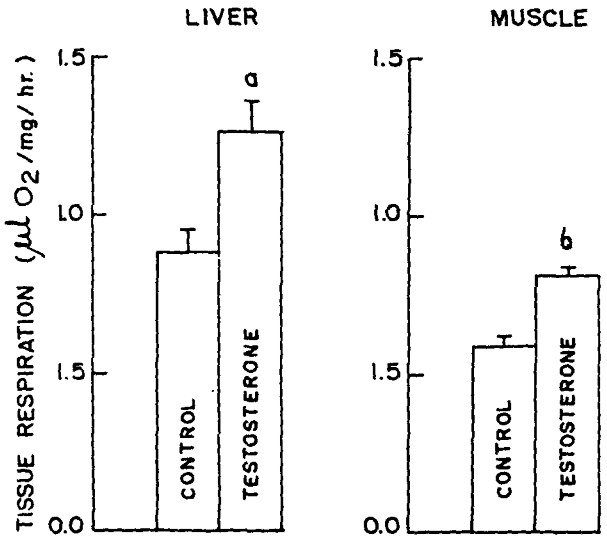
Rana limnocharis (Winter)



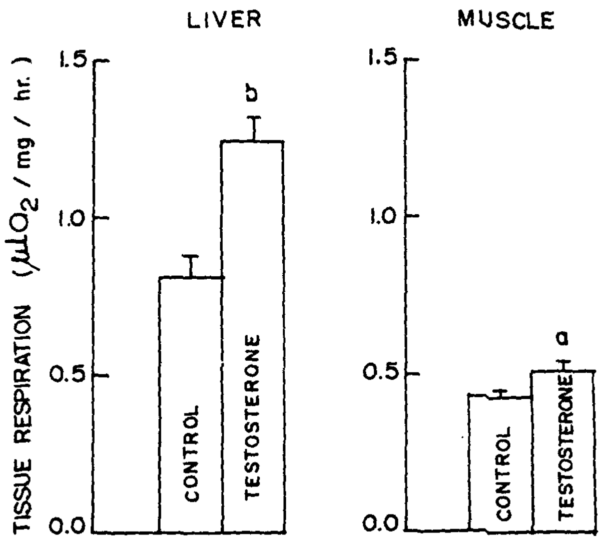
Rana cyanophlyctis (Winter)

Fig. 16 : In vitro effects of testosterone on the rate of tissues respiration of Rana limnocharis and Rana cyanophlyctis during Winter (Temperature : 6 - 14 °C)

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.



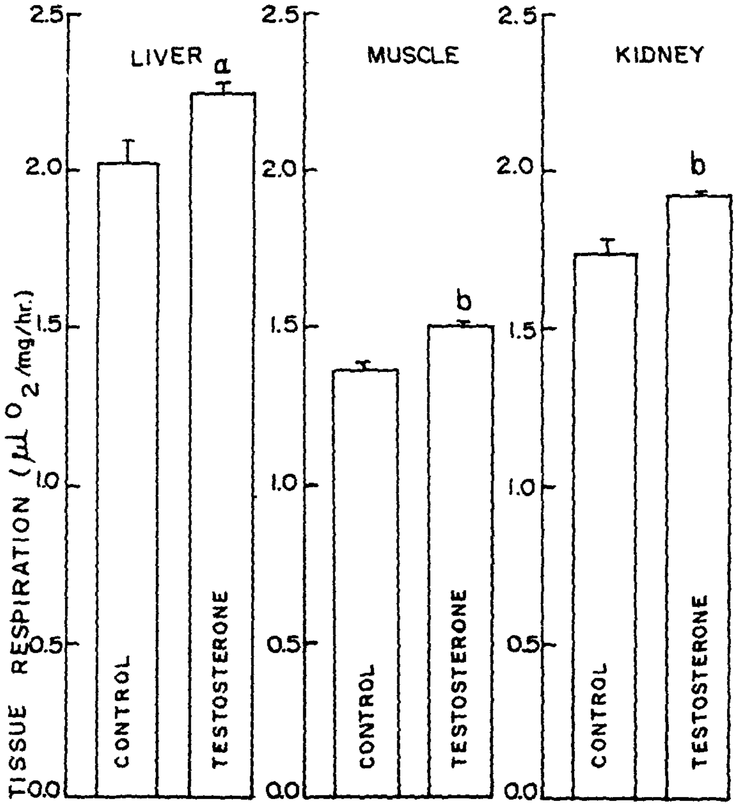
Rana limnocharis (Winter)



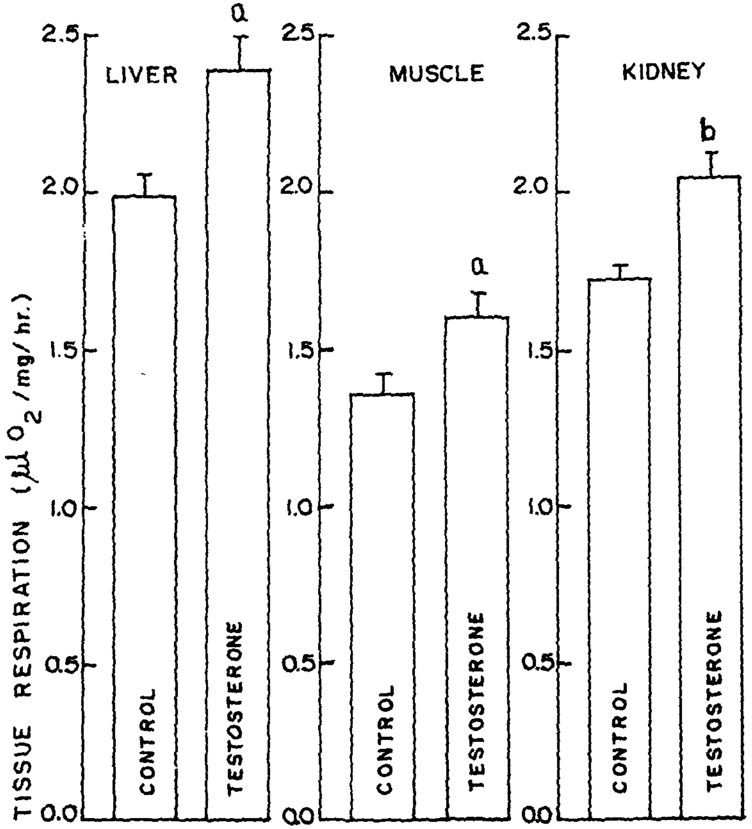
Rana cyanophlyctis (Winter)

Fig. 17 : In vivo effects of Testosterone on the rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during Summer (Temperature : 12 - 20 °C)

a,b Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.



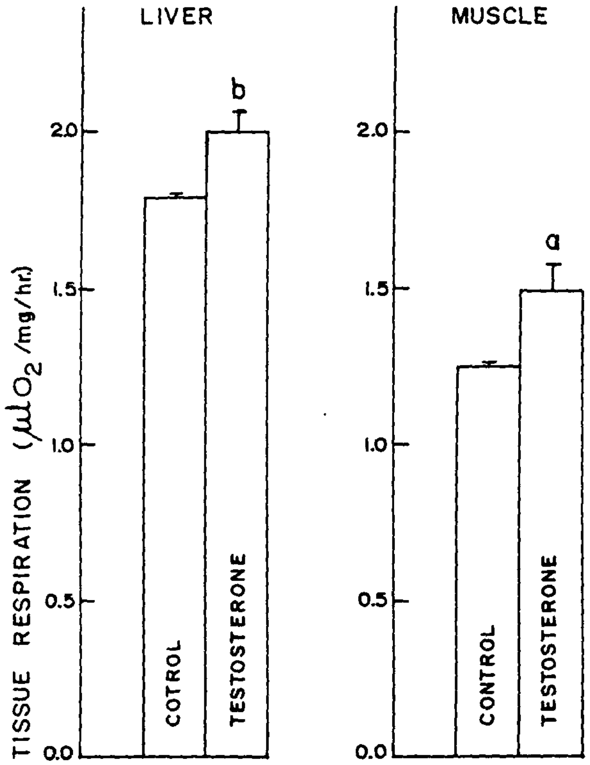
Rana limnocharis (Summer)



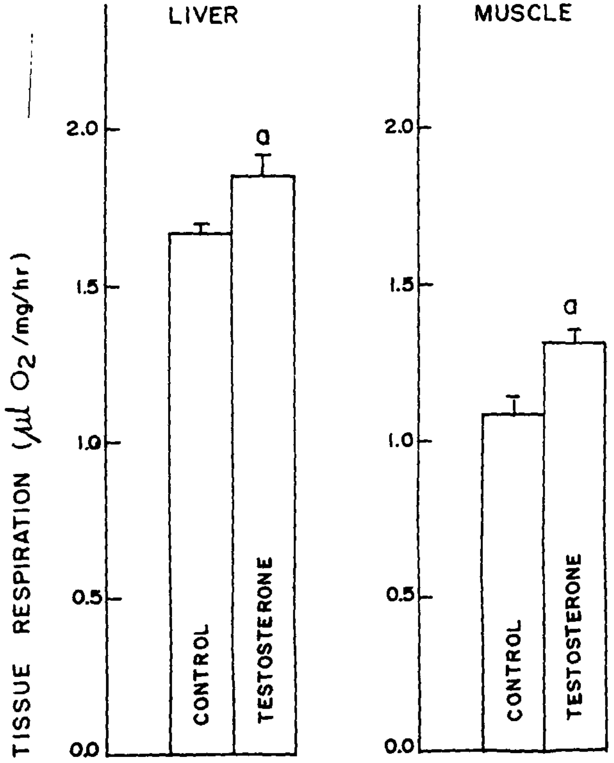
Rana cyanophlyctis (Summer)

Fig. 18 : In vitro effects of testosterone on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature : 13 - 19 °C)

[a,b] Differ from the saline treated control group : $P < 0.05$ and 0.01 respectively.



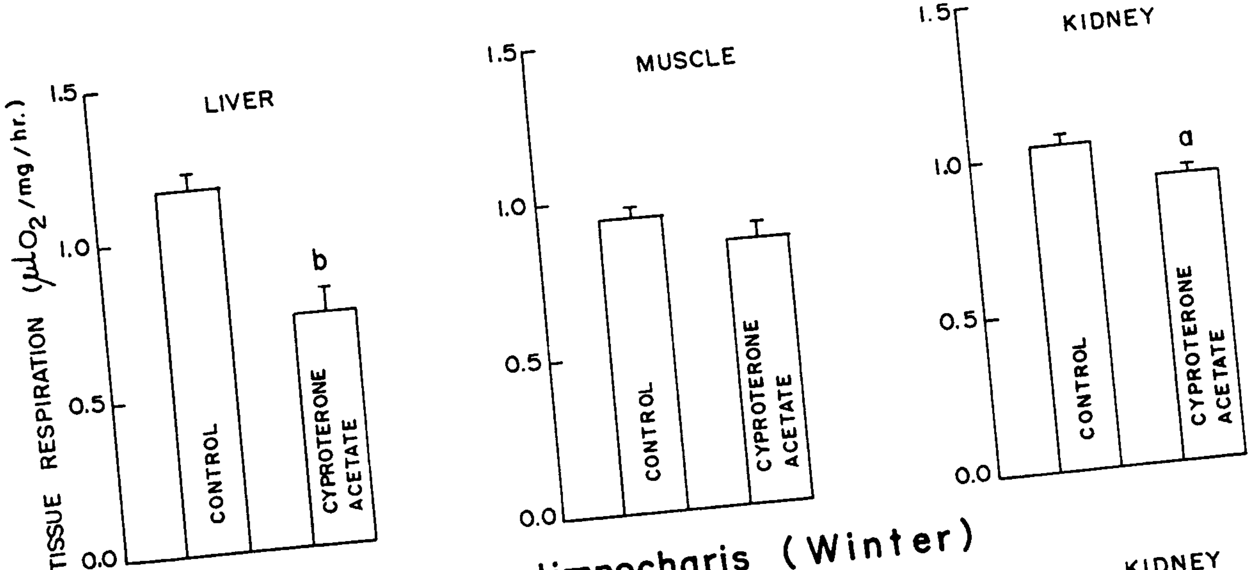
Rana limnocharis (Summer)



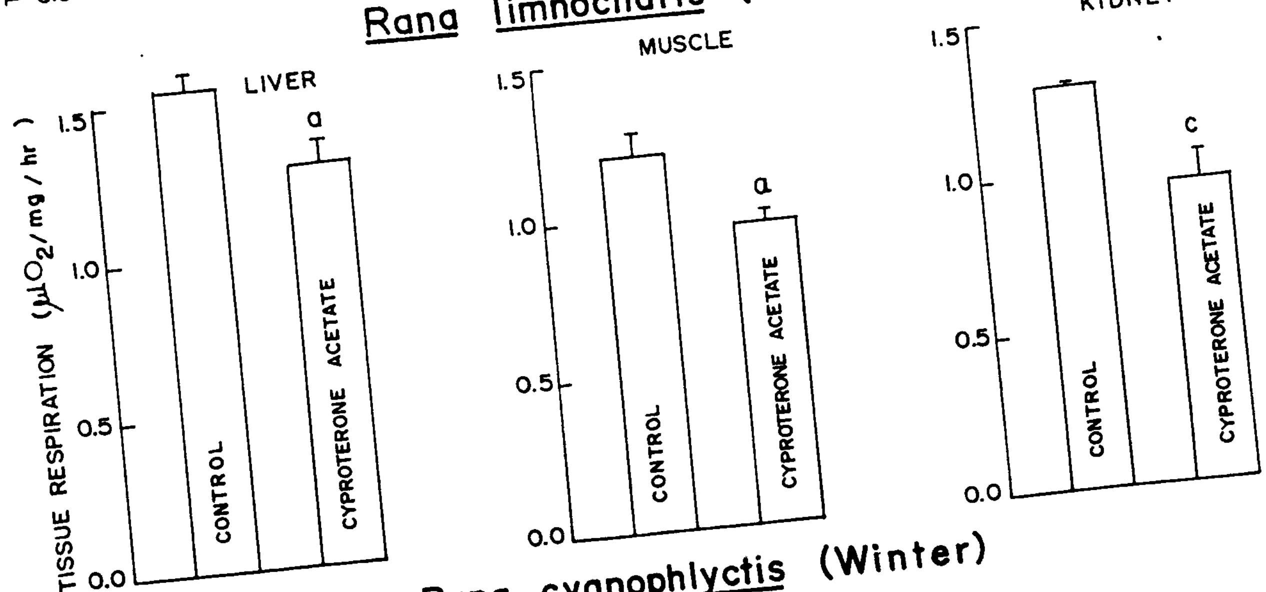
Rana cyanophlyctis (Summer)

Fig. 19 : In vivo effects of cyproterone acetate (CA) on the rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during Winter (Temperature : 6 - 14^{o})

^{a,b,c} Differ from the saline treated control group P < 0.05, 0.01 and 0.001 respectively.



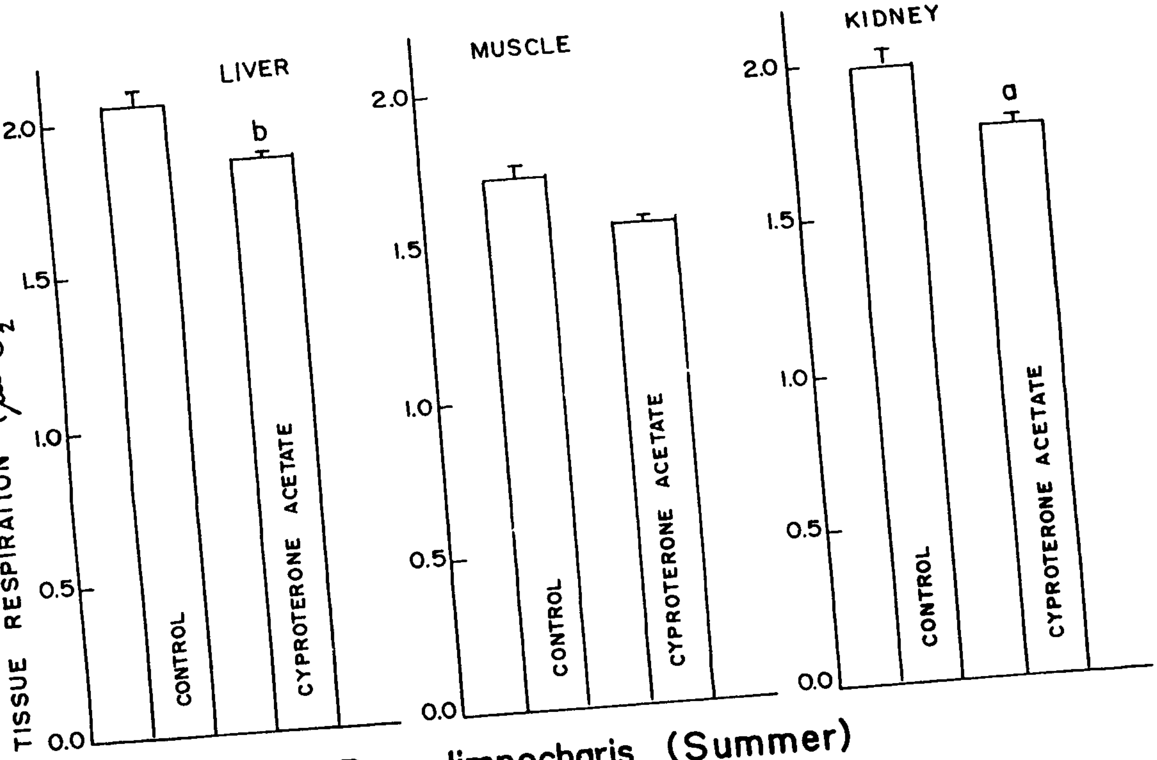
Rana limnocharis (Winter)



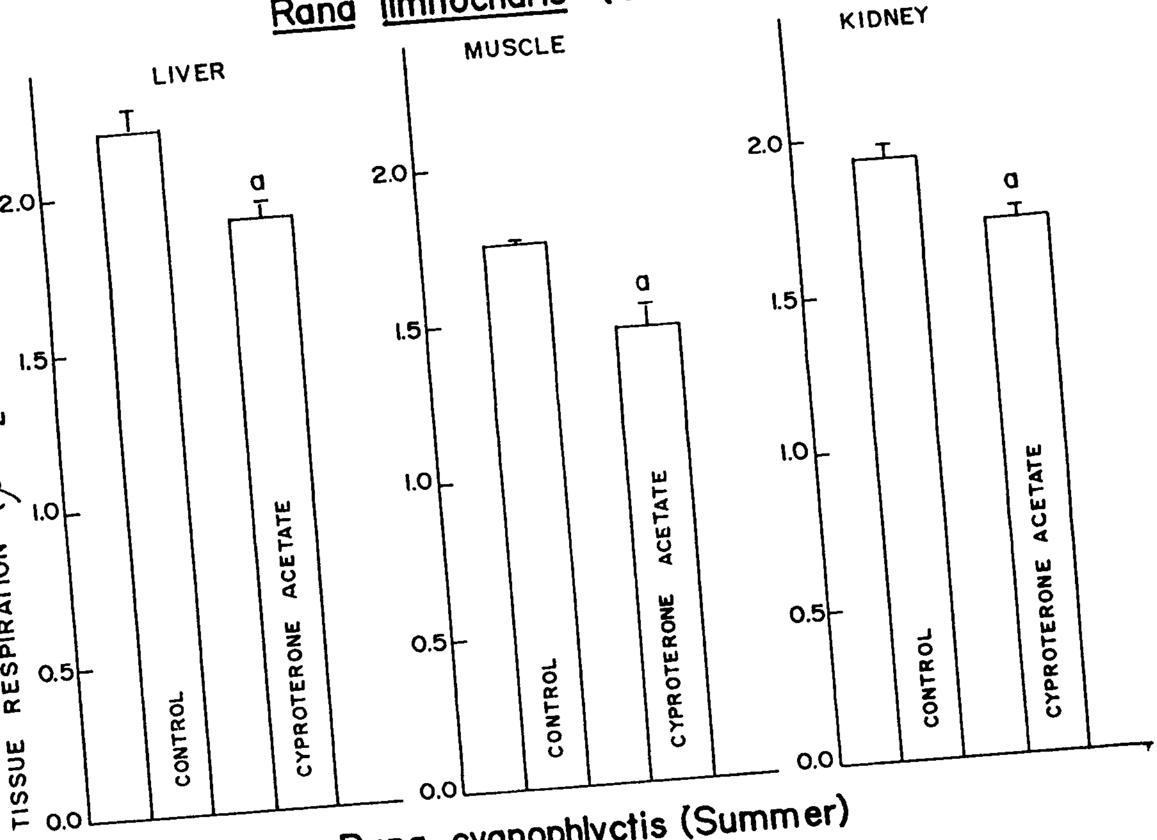
Rana cyanophlyctis (Winter)

Fig. 20 : In vivo effects of cyproterone acetate on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 14 - 23°C)

^{a,b}Differ from the saline treated group : $P < 0.05$ and 0.01 respectively.



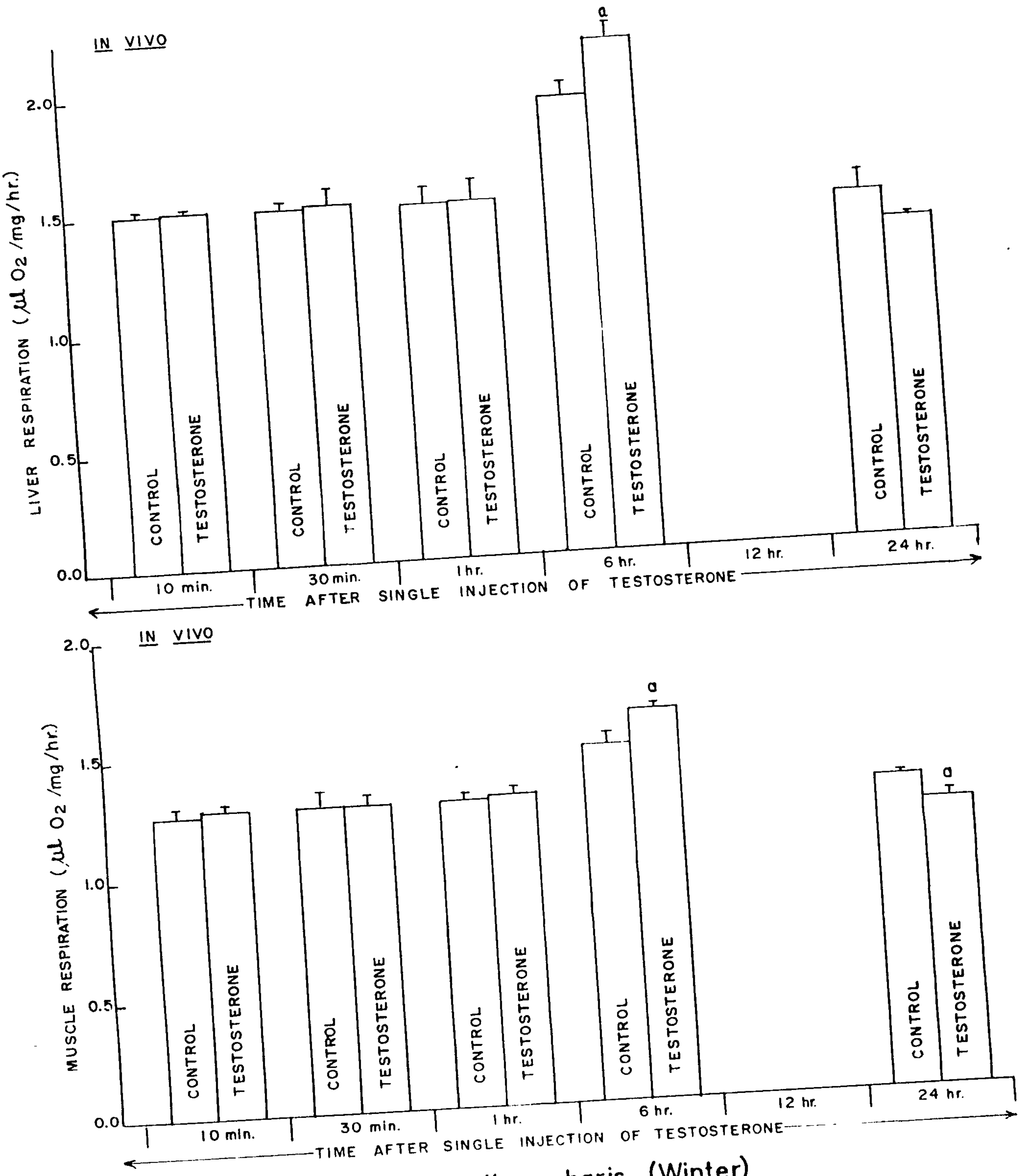
Rana limnocharis (Summer)



Rana cyanophlyctis (Summer)

Fig. 21 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana limnocharis* during Winter (Temperature : 7 - 15 °C)

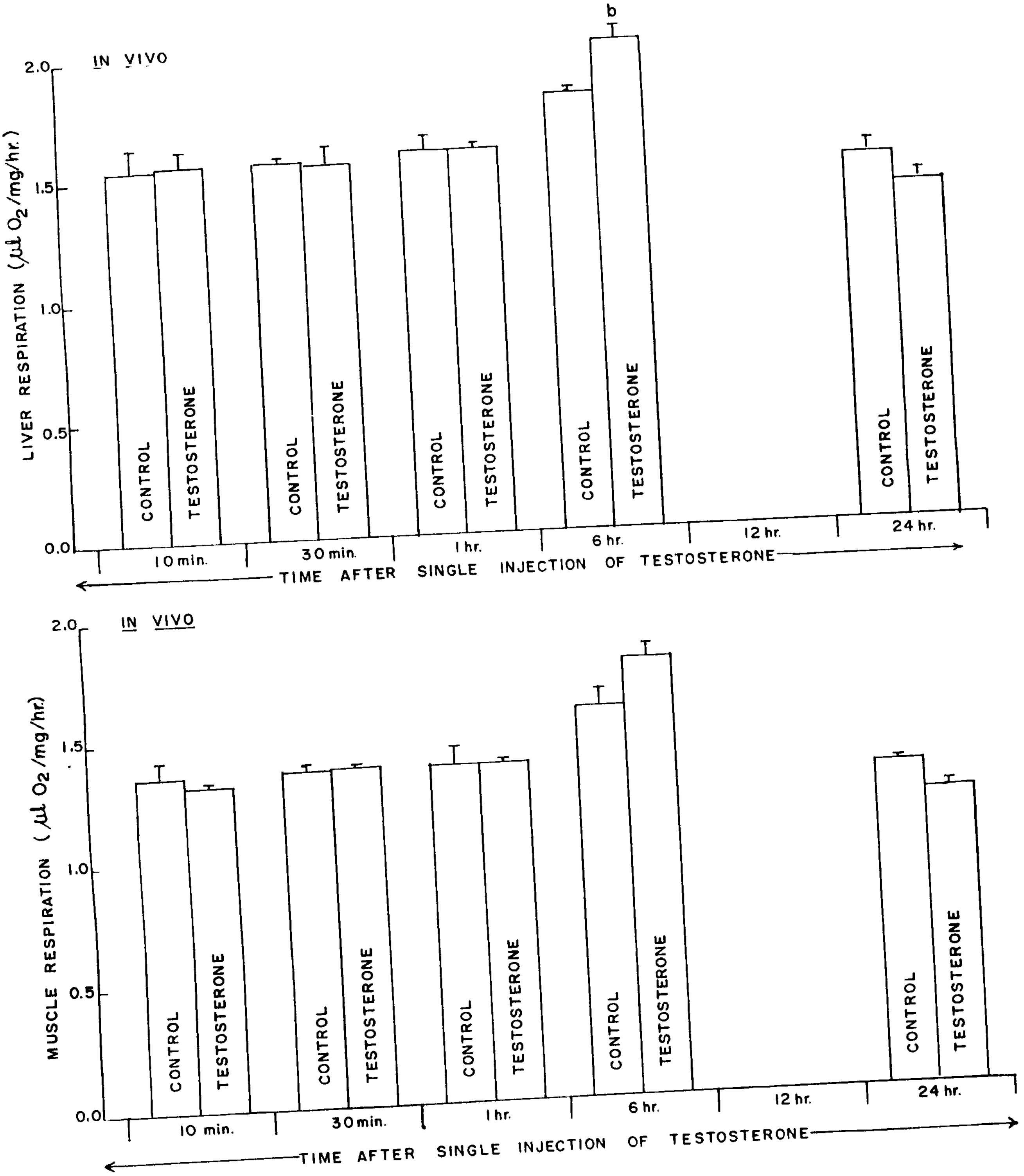
[a] Differs from the respective saline treated control group : $P < 0.05$.



Rana limnocharis (Winter)

Fig. 22 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana cyanophlyctis* during Winter (Temperature : 7 - 15^{o}C)

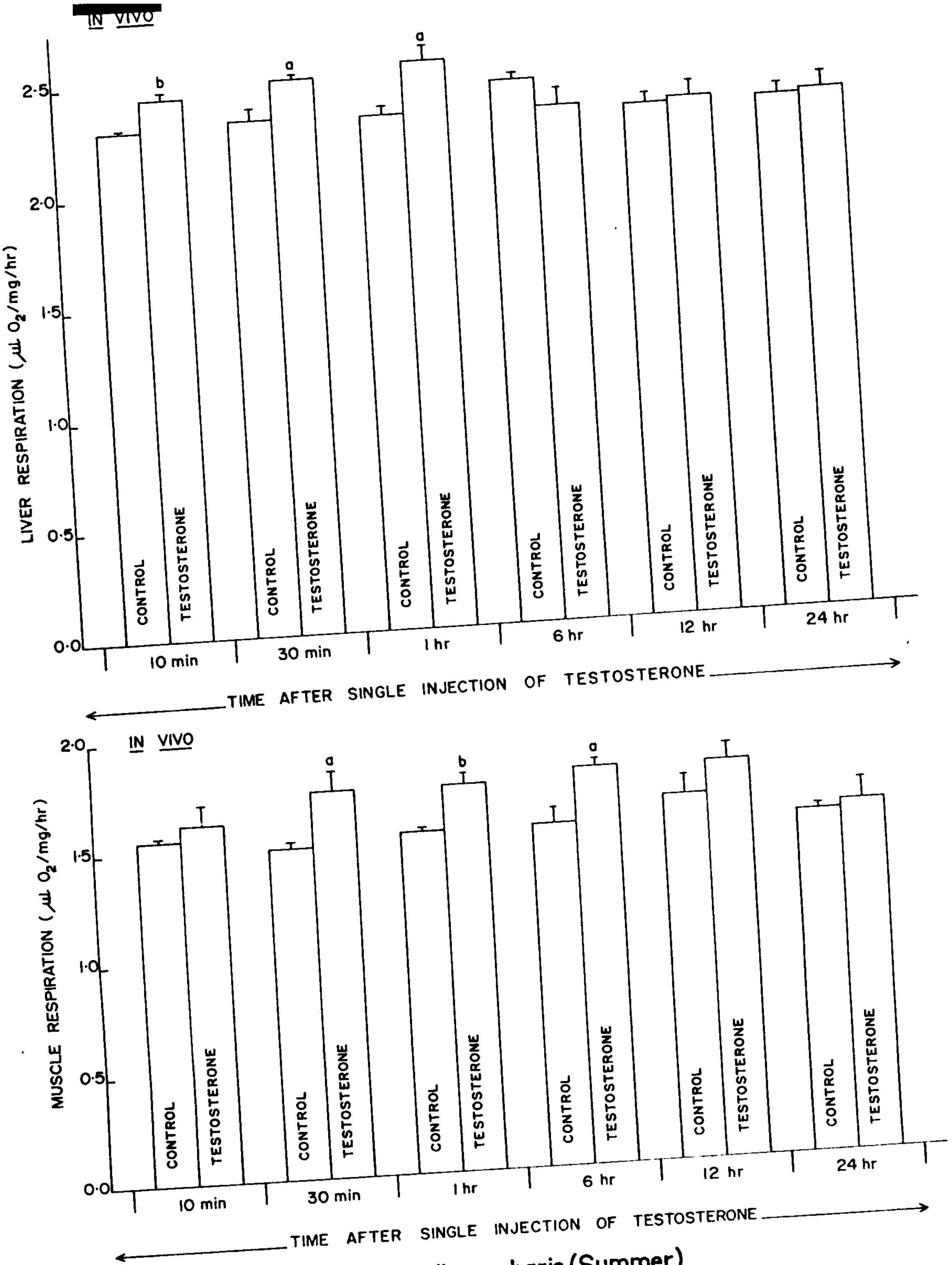
[b] Differs from the respective saline treated control group : $P < 0.05$.



Rana cyanophlyctis (Winter)

Fig. 23 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana limnocharis* during Summer (Temperature : 16 - 23 °C)

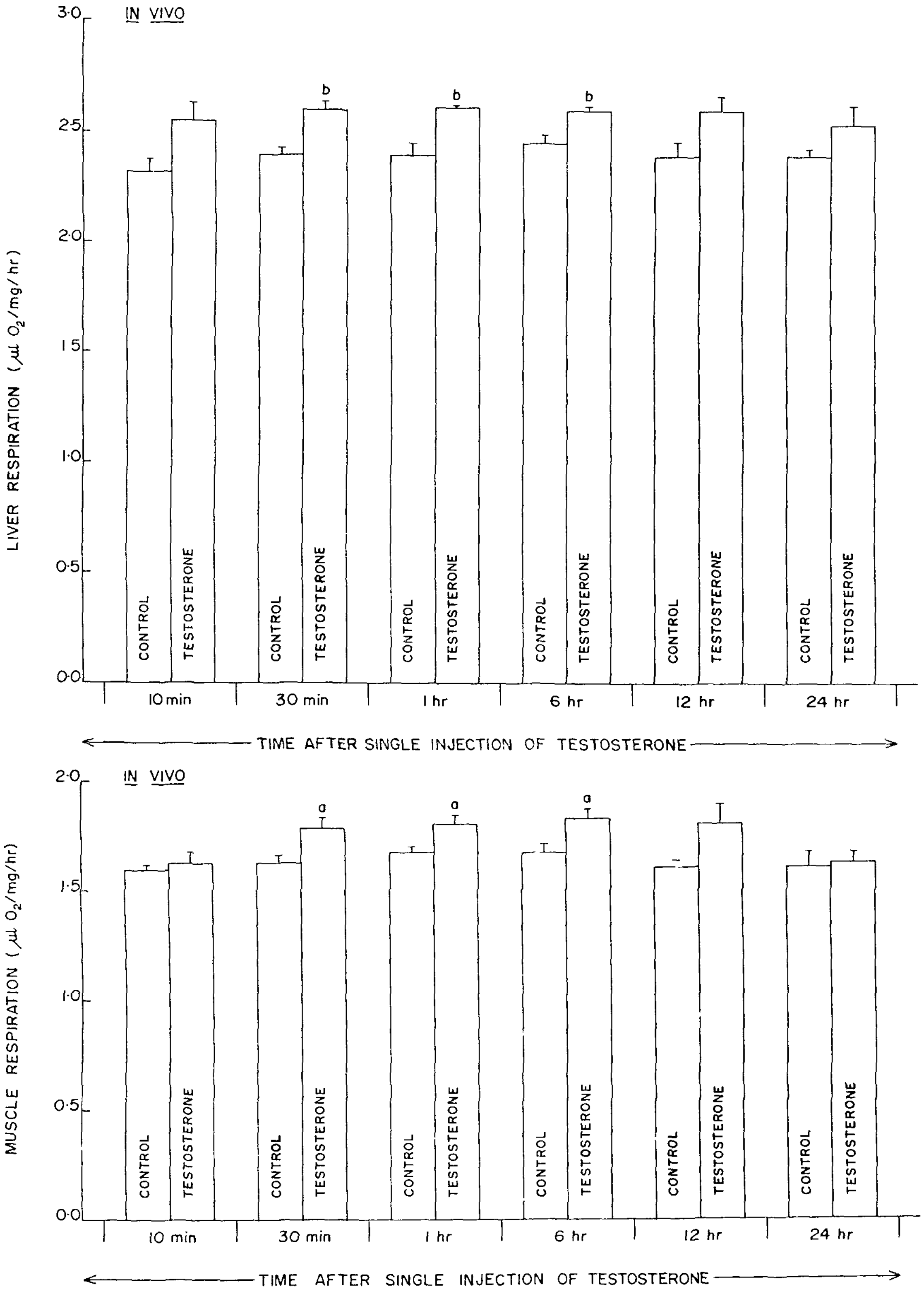
[a,b] Differ from the respective saline treated control group : $P < 0.05$ and 0.01 respectively.



Rana limnocharis (Summer)

Fig. 24 : Time-dependent in vivo effects of testosterone on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature 15 : 23 ^{o}C)

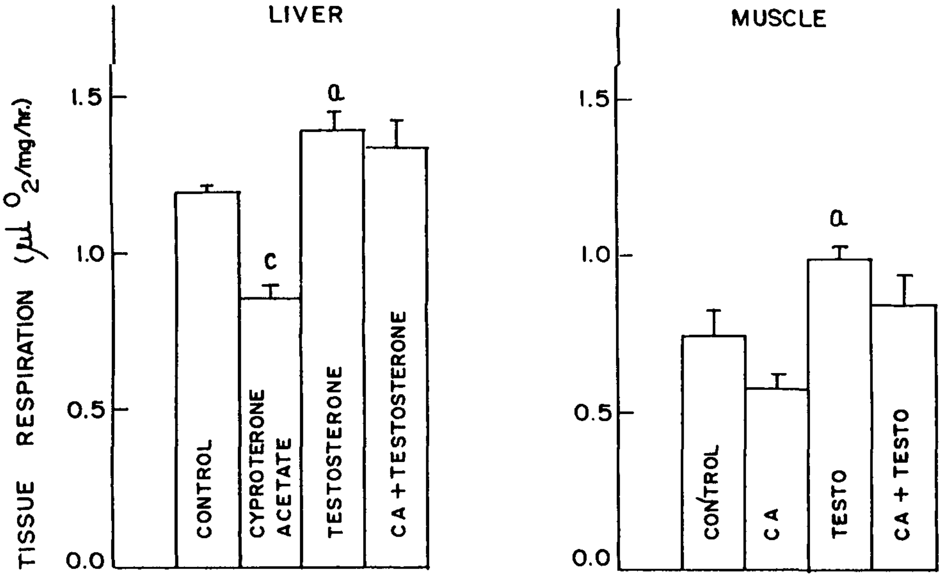
^{a,b} Differ from the respective saline treated control group : $P < 0.05$ and 0.01 respectively.



Rana Cyanophlyctis (Summer)

Fig. 25 : In vitro effects of cyproterone acetate and testosterone on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature : 12 - 19 °C)

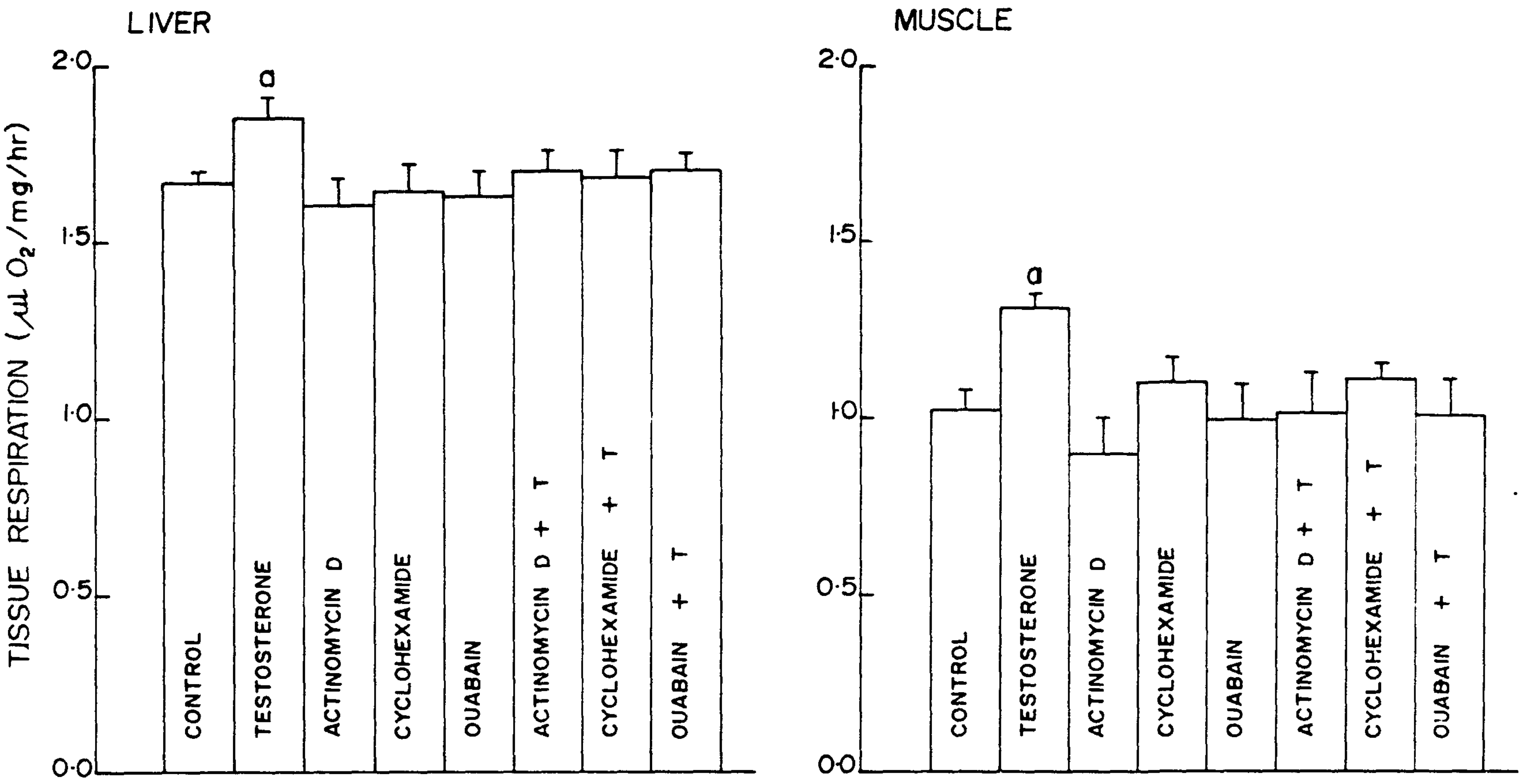
^{a,c} Differ from the saline treated control group : $P < 0.05$ and 0.001 respectively.



Rana cyanophlyctis

Fig. 26 : In vitro effects of testosterone, ouabain, cyclohexamide, and Actinomycin-D on the rate of tissues respiration of male <u>Rana</u> <u>cyanophlyctis</u> during summer (Temperature : 13 - 19 ^{o}C)

^{a}Differs from the saline treated control group : $P < 0.05$.



Rana cyanophlyctis (Summer)

CHAPTER 5

Chapter 5

Role of Adrenal Hormones in Regulation of the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*

Introduction

Adrenal hormones (Corticosteroids and Catecholamines) regulate a number of metabolic pathways in vertebrates (Gorbman *et al.*, 1983b). These hormones play an important role in adaptations of vertebrates against changes in environment and stressful conditions. Adrenal hormones regulate intermediary metabolism, electrolyte metabolism and oxidative metabolism (Gorbman, *et al.*, 1983b; Landsberg and Young, 1985).

Catecholamines (epinephrine and norepinephrine) are released from the adrenal medulla as well as from the sympathetic nervous system (Himms-Hagen, 1975, 1983). These hormones are reported to be indispensable for the oxidative metabolism (non-shivering thermogenesis) in mammals exposed to low temperature (Carlson, 1960; Jansky and Hart, 1963; Heroux, 1969; Himms-Hagen, 1970). Norepinephrine and epinephrine play a major role in the process of cold acclimation in mammals (Hsieh and Carlson, 1957; Depocas, 1960; Leduc, 1961; Himms-Hagen, 1975). Attempts were made to investigate the calorigenic role of catecholamines in poikilothermic vertebrates (Gupta and Thapliyal.1991). Catecholamines are reported to act as emergency hormones for the regulation of the oxidative metabolism in reptiles (Gupta and Thapliyal.1982.1983). Preliminary studies suggest that catecholamines might be involved in the oxidative metabolism of amphibians (Harri and Hadenstam.1972: Farrar and Frye.1977: Janssens.*et al.*.1983: Deka-Borah.1989. Gupta and Chakravartty.1990). However. so far no comprehensive attempt has been made to study in detail the role of catecholamines in the oxidative metabolism of amphibians with special reference to low temperature and hibernation (cold torpor). Further.there is practically no information on the mechanism of calorigenic action of catecholamines in the poikilothermic vertebrates. Similarly. it is also not known whether corticosteroid hormones (which play a major role in electrolyte and carbohydrate metabolism) are somehow involved in the oxidative metabolism of amphibians (Hanke.1990).

Cold exposure reportedly increases blood level of catecholamines in lower vertebrates (Gorbman *et al*.. 1983b). Further. catecholamines and adrenergic receptors are speculated to be evolved early during the course of evolution. Therefore.there is a possibility that. in the absence of the direct calorigenic action of thyroid hormones in lower vertebrates, catecholamines might be actively involved in the energy metabolism in cold-blooded animals at low temperature. Therefore, keeping in view the lack of information, crucial phylogenetic position of amphibians and the vital role of catecholamines in the calorigenesis of mammals at low temperature, it was decided to investigate in detail the role of adrenal hormones in the oxidative metabolism of a hibernating and a non-hibernating species of frogs at low temperature. Findings of the present study strongly suggest that catecholamines play a major role in the energy metabolism of frogs. Further,both alpha- and beta-adrenergic receptors seem to be involved in the calorigenic action of the hormones.

Materials and Methods

All experiments were conducted on adult male Rana limnocharis (Body weight : 8-10 g) and Rana cyanophlyctis (Body weight : 10-12 g) which were captured locally. In vivo and in vitro experiments were conducted during winter and summer/rainy months on both the species until/unless specified. For in vivo experiments, frogs were maintained under natural climatic conditions in plastic cages and acclimatized for 10 days before the treatments were started. For in vitro treatments, frogs were killed immediately after collection from the nature (for details, please see "Chapter 1"). *In vivo* and *in vitro* experiments were conducted to study the effects of adrenal hormones, adrenergic agonists and antagonists on the rate of tissues respiration. Details of experimental protocol is given below.

Experimental Protocol

Exp No.	Treatment(s)	In vivo/vitro	Months (Temperature)	Dose	Duration
1.	Control	In vivo	January (6-14 ^{0}C)		
	Epinephrine (EP)			2 µg/frog/d	4 days
	Norepinephrine (NE)			-do-	-do-
	Control		July (12-20 ^{0}C)		
	EP			2 and 5 µg/ frog/Day	-do-
	NE		-do-	-do-	-do-
2.	Control	In vitro	January (6-14 ^{0}C)		
	EP			2.2 µM	
	NE			2.2 µM	
	Control	In vitro	August (14-20 ^{0}C)		
	EP			2.2 µM	
	NE			2.2 µM	

3.	Single injection of Epinephrine	in vivo	January (4.6-14 degree C)	2 µg/frog	10 Min 30 Min 1 h 6 h 12 h 24 h
	-dO-		September (15^0 -24^{0}C)	-do-	-do-
4.	Single injection of Norepinephrine	In vivo	January (4.6-14^0 C)	-do-	-do-
	-do-		September (15-24^0 C)	-do-	-do-
5	Control	In vivo	July (12-21^{0}C)		
	Epinephrine			2 µg/f/day	4 days
	Norepinephrine			-do-	-do-
	L-T$_3$			-do-	-do-
	PTU			1 µg/g body weight	-do-
	Epinephrine+T$_3$			2 µg + 2 µg/f/day	-do-
	Norepinephrine+T$_3$			-do-	-do-
	PTU + Epinephrine			1 µg/g + 2 µg/f/day	-do-
	PTU + Norepinephrine			-do-	-do-

6. Control In vitro April (11-23 °C)

Phenylephrine	1 µM
Isoproterenol	1 µM
Phenylephrine + Isoproterenol	1 µM + 1 µM
Prazosin	1 µM
Propranolol	1 µM
Prazosin + Propranolol	1 µM + 1 µM
Phenylephrine + Prazosin	-do-
Isoproterenol + Propranolol	-do-
Isoproterenol+ Propranolol + Prazosin	1 µM + 1 µM + 1 µM
Phenylephrine + Propranolol + Prazosin	-do-

7. Control In vitro July (12-22 °C)

Epinephrine	2.2 µM
Norepinephrine	2.2 µM
Propranolol	1 µM
Prazosin	1 µM
Epinephrine + Propranolol	2.2 µM + 1 µM
Epinephrine + Propranolol + Prazosin	2.2 µM + 1 µM + 1 µM
Norepinephrine + Propranolol	2.2 µM + 1 µM
Norepinephrine + Prazosin	-do-
Norepinephrine + Propranolol + Prazosin	2.2 µM + 1 µM + 1 µM

8	Control	In vivo	January (6-14 °C)		
	Corticosterone			2 µg/f/day	4 days
	Cortisol			-do-	-do-
	-do-	-dO-	July (12-20 °C)	-do-	-do-
9.	Control	In vitro	January (6-14 °C)		
	Corticosterone			1.15 µM	
	Cortisol			2.7 µM	
	-do-	-do-	July (12-20 °)	-do-	
10.	Control	In vivo	December (7-15 °C)		
	Metapyrone			1 µg/g body wt/d	4 days
	-do-	-do-	August (14-24 °C)	-do-	-do-

Twenty-four hours after the last <u>in vivo</u> injections, control and treated frogs were decapitated and tissues (liver, skeletal muscle and kidney) were rapidly removed, rinsed in ice-cold frog Ringer's solution and stored in a refrigerator. The rate of tissues respiration was measured with the help of an oxygen electrode (for details, please see "Chapter 1"). In the case of experiment Nos. 3 and

4, control and treated animals were decapitated at the time-intervals mentioned in the experimental protocol. The data were analyzed with the help of students "t" test (Snedecor, 1961).

Results

In Vivo effects of Catecholamine hormones:

Results are presented in Tabs. 27, 28 & 29; Figs. 27, 28 & 29. *In vivo* administration of norepinephrine (NE) and epinephrine (EP) significantly increased the respiratory rate of liver, muscle and kidney tissues in both *Rana limnocharis* and *Rana cyanophlyctis* during winter and summer/rainy months (Tabs. 27, 28 & 29; Figs. 27, 28 & 29; Figs. 27, 28 & 29). NE was always found to be comparatively more potent than EP in stimulating the metabolic rate of tissues in both the species. Further, during summer/rainy season both NE and EP increased the metabolic rate of tissues in both the species in a dose-dependent manner (Tabs. 28 & 29; Figs. 28 & 29).

In vitro effects of catecholamine hormones:

Results are presented in Tabs. 30 & 31; Figs. 30 & 31. *In vitro* treatments with NE and EP also significantly increased the rate of oxygen uptake by liver and muscle tissues of both Rana limnocharis and Rana cyanophlyctis during winter as well as during summer/rainy seasons.

In vivo effects of a single dose of Norepinephrine:

Results are presented in Tabs. 32-35; Figs. 32-35. During winter, single dose of NE induced significant increase in the respiratory rate of liver and muscle tissues after 30 minutes and maintained a significantly higher metabolic rate of tissues upto 6 hours in both Rana limnocharis and Rana cyanophlyctis. No significant alteration in the metabolic rate of tissues was found after 10 min, 12 h. and 24 hours (Tabs. 32 & 33; Figs. 32 & 33).

During summer/rainy season, a single dose of NE significantly increased the rate of tissues respiration in Rana limnocharis after 10 minutes. Thereafter, the liver respiratory rate remained stimulated upto 12 hours, while the muscle oxygen uptake remained significantly high only upto 1 hr (Tab. 34; Fig. 34). In Rana cyanophlyctis during summer/rainy season, a single injection of NE significantly stimulated liver and muscle respiratory rate after 30 minutes and the respiratory rate of both the tissues remained stimulated upto 12 hours (Tab. 35; Fig. 35).

In vivo effect of a single dose of epinephrine:

Results are presented in Tabs. 36-39; Figs. 36-39. During winter, administration of a single dose of EP significantly increased the respiratory rate of liver and muscle tissues after 30 minutes, and the respiratory rate remained stimulated upto 6 hours in both *Rana limnocharis* and *Rana cyanophlyctis* (Tabs. 36 & 37; Figs. 36 & 37).

During summer/rainy season, a single dose of EP significantly increased the respiratory rate of liver in *Rana limnocharis* after 10 minutes, and it was found to be significantly high after 1h, 6h and 24 hours (Tab. 38; Fig. 38). However, the muscle respiratory rate was stimulated after 30 minutes and remained stimulated upto 1h only (Tab. 38; Fig. 38).

In *Rana cyanophlyctis* during summer/rainy season, a single injection of EP induced significant increase in the respiratory rate of liver after 30 minutes which remained increased upto 12 hours. However, the muscle respiratory rate was found to be stimulated only after 6 hours (Tab. 39; Fig. 39).

in vivo effects of Catecholamine hormones in Propyl thiouracil- treated frogs:

Results are presented in Tab. 40; Fig. 40. During summer, administration of propyl thiouracil (PTU) significantly inhibited/blunted the stimulatory effect of NE and EP on the metabolic rate of tissues (liver, muscle and kidney) in both Rana limnocharis and Rana cyanophlyctis (Tab. 40; Fig. 40). As compared to NE and EP treatment, PTU-induced inhibition of the metabolic action of NE and EP ranged between 10% to 17% in liver, 15% to 22% in muscle, and 6% to 13% in kidney tissue (Tab. 40; Fig. 40).

In vitro effects of adrenergic agonists and antagonists:

Results are presented in Tab. 41; Fig.41. Both phenylephrine (alpha-adrenergic agonist) and Isoproterenol (Beta-adrenergic agonist), when added separately in vitro, significantly increased the respiratory rate of tissues (liver and muscle) of both the species during summer/rainy season. When phenylephrine (PHE) and Isoproterenol (ISO) were added together in vitro, potentiated the stimulatory effect of each other in liver and muscle tissues of both Rana limnocharis and Rana cyanophlyctis (Tab. 41; Fig. 41).

Prazosin (alpha-adrenergic antagonist) significantly inhibited the PHE-induced increase in the respiratory rate of only muscle in both the species. Propranolol (Beta-adrenergic antagonist) significantly reduced the stimulatory effect of ISO only in the liver tissue of Rana limnocharis and in muscle of Rana cyanophlyctis. Prazosin (PRA) and Propranolol (PRO), when added together, significantly blocked the stimulatory effects of both PHE and ISO on the rate of oxygen consumption by liver and muscle tissues in both Rana limnocharis and Rana cyanophlyctis (Tab. 41; Fig. 41). In general, the inhibitory effect of PRA and PRO was more prominent when the two antagonists were added together as compared to their individual inhibitory effects.

In vitro effects of NE and EP in the presence of adrenergic antagonists:

Results are presented in Tab. 42; Fig. 42. In Rana cyanophlyctis during summer/rainy season, while PRA and PRO significantly inhibited the stimulatory effect of EP only in liver, PRA had no effect on EP-induced increase in the rate of tissues respiration. However, PRO significantly reduced the stimulatory effect of NE on both liver and muscle, PRA significantly inhibited the NE-induced increase in the respiratory rate of muscle only. PRO and PRA, when added together, invariably inhibited the NE and EP-induced increase in the respiratory rate of liver and muscle tissue, and the inhibitory effect was greater than their individual inhibitory effect on stimulatory effect of NE and EP.

In vivo and in vitro effects of corticosteroid hormones:

Results are presented in Tabs. 43-46; Figs. 43-45. *In vivo* administration of corticosterone invariably increased the respiratory rate of tissues (liver, muscle and kidney) irrespective of seasons in both *Rana limnocharis* *Rana cyanophlyctis* (Tabs. 43 & 44; Figs. 43 & 44). Similarly, *in vitro* administration of corticosterone always stimulated the rate of oxygen consumption by liver and muscle tissues of both the species (Tabs. 45 & 46; Figs. 45 & 46). However, both *in vivo* and *in vitro* treatments with cortisol did not alter the metabolic rate of tissues significantly in both the species during winter or summer/rainy seasons (Tabs. 45 & 46; Figs. 45 & 46).

In vivo effects of Metapyrone:

Results are presented in Tabs. 47 & 48; Figs. 47 & 48. *In vivo* administration of metapyrone significantly inhibited the respiratory rate of tissues (except liver of *Rana limnocharis* during winter) in both *Rana limnocharis* and *Rana cyanophlyctis* during winter and summer/rainy season (Tabs. 47 & 48; Figs. 47 & 48).

Discussion

The calorigenic effects of catecholamine hormones (norepinephrine and epinephrine have been investigated in only a few temperate and tropical amphibian species. In *Rana temporaria*, both adrenaline and nor-adrenaline stimulates whole body oxygen consumption (Harri and Hadenstam, 1972). The calorigenic effect of adrenaline has been reported to be season dependent in *Rana pipiens* (Farrar and Frye, 1977). Adrenaline and nor-adrenaline, when added *in vitro*, stimulated tissues respiration in *Rana limnocharis* (Gupta and Chakrabarty, 1990). In most of these studies, in general, very high doses were used and effects were observed on the rate of whole body oxygen consumption. Further, calorigenic effects of catecholamines were studied either *in vivo* or *in vitro*. Therefore, this study seems to be the first of its kind in which both *in vivo* and *in vitro* calorigenic effects of epinephrine (EP) and norepinephrine (NE) on tissues respiration were studied in two species of frogs exposed to natural climatic conditions of winter and summer. Earlier studies were conducted on frogs maintained under laboratory condition.

In vivo and *in vitro* stimulation of respiratory rate of vital tissues in *Rana limnocharis* and *Rana cyanophlyctis* during both summer and winter strongly suggest that catecholamines are actively involved in the regulation of the oxidative metabolism of amphibians. A single dose of catecholamine could significantly stimulate the metabolic rate of tissues only after 30 minutes during winter (Tabs. 32 & 33; Figs. 32 & 33) and after 10 minutes during summer/rainy season (Tabs. 34 & 35; Figs. 34 & 35). Depending upon the tissues, the stimulatory effect lasted for about 1h to 24 hours. The latent period for the calorigenic action of catecholamine was found to be more (30 min) during winter as compared to the latent period (10 min) during summer/rainy season. This might be due to slowing down of the circulatory system during winter leading to slow distribution of the exogenous hormones to different organs. The question whether the difference in the latent periods during winter and summer months was due to differences in the clearance rate/metabolism of catecholamines remains to be answered.

Since EP and NE were found to be stimulatory during both winter and summer, their calorigenic action seems to be independent of ambient temperature. Further, under both *in vivo* and *in vitro* conditions, the latent period for the calorigenic action of EP and NE depending upon tissues, species and seasons, ranged between 10 min to 30 min. It is important to mention here that during winter while thyroid hormones are calorigenically ineffective, testosterone produces calorigenic action only after 6 hours. It, thus, seems that due to their rapid and temperature-independent calorigenic action, catecholamines act as the major hormones for the regulation of the oxidative metabolism in amphibians at low temperature. Since the activities of sympathetic nervous system and the Chromaffin tissues are stimulated by low temperature, increased production of catecholamines might be responsible for maintaining the basal metabolic rate of the amphibians exposed to prolonged low natural temperature during cold winter months. Both EP and NE were found to produce the calorigenic action in a dose dependent manner (Tabs. 28, 29; Figs. 28, 29). Thus, increased secretions of EP and NE under decreasing ambient temperature might be helpful in maintaining the basal metabolic rate to keep the animals alive.

Norepinephrine was always found to be more potent than Epinephrine in stimulating the respiratory rate of the tissues. The higher potency of NE might be due to increased cellular sensitivity. Epinephrine is secreted mainly from the chromaffin tissues,while NE is secreted from the chromaffin tissues as well as from the sympathetic nervous system (Bentley, 1976; Gorbman *et al*., 1983b). The sympathetic nerve fibers are widely distributed as compared to the localized distribution of chromaffin tissues. Therefore, it seems that the tissues/organs have easy access to NE as compared to EP. This might has probably led to the selective development of responsiveness for EP and NE. More responsiveness of the oxidative machinery to NE seems to be of great adaptational significance.

L-T_3 did not potentiate the calorigenic action of catecholamines in both the species. But, the treatment with propyl thiouracil reduced the metabolic action of both EP and NE in both the species (Tab. 40; Fig. 40). Reduction in the stimulatory effects of catecholamines seems to suggest that endogenous thyroid hormones might be involved in the potentiation of the calorigenic action of the catecholamine hormones. It, thus, seems that the endogenous thyroid hormones potentiate the calorigenic action of catecholamines in frogs.

The mechanism of calorigenic action of catecholamines in mammals is well established. In mammalian brown adipose tissue, the catecholamines are found to stimulate 80% respiration through β_1-adrenergic component and 20% via alpha1-adrenergic component (Mohell et al., 1983a). β1-Adrenergic receptors stimulate respiratory rate using cAMP as a second messenger, while a1-adrenergic receptors mediate the hormonal response through Ca^{++} and /or phosphatidyl inositol metabolism (Mohell,et al., 1983a,b). Notwithstanding a large body of information on the mechanism of action of catecholamines in mammals, there is practically no information on the mechanism of action of catecholamines in lower vertebrates, especially in amphibians. The present findings of experiments involving a- and β-adrenergic agonists and antagonists clearly indicate the involvement of both alpha and beta adrenergic components in the regulation of calorigenesis in frogs. However, the degree of involvement of alpha and beta adrenergic mechanisms seems to depend upon the seasons, tissues and species. In Rana limnocharis, beta adrenergic mechanism seems to be more important for liver, while alpha-component seems to be comparatively more important in muscle. Unlike in Rana limnocharis, both alpha- and beta-adrenergic mechanisms seems to be equally important for both liver and muscle tissues in Rana cyanophlyctis. Similarly, inhibition of EP and NE-induced increase in the respiratory rate of tissues by alpha- and beta-adrenergic antagonists also confirm the involvement of both alpha- and beta-adrenergic components in the calorigenesis of amphibian species. Further, more effectiveness of the antagonists (Prazosin and Propranolol) together in inhibiting the stimulatory effect of EP and NE as compared to their individual inhibitory effect strongly suggests the involvement of both alpha- and beta-adrenergic mechanisms. The nature of the second messengers involved in the calorigenic action of the catecholamines might be similar to that of mammals.

In vivo and *in vitro* administration of corticosterone always stimulated the respiratory rate of tissues in both the species. However, cortisol did not alter the rate of oxygen consumption by tissues irrespective of species, season/temperature and mode of treatments (*in vivo*/*in vitro*). These findings seem to suggest that corticosterone, but not cortisol, is actively involved in the regulation of the oxidative metabolism in both the species. In amphibians, corticosterone and aldosterone are reported to be the main corticosteroid hormones (Jolivet-Jaudet and Ishi, 1985; Hanke, 1990). Further, both the steroids share common functions, i.e., regulation of carbohydrates, water and electrolyte (osmoregulation) metabolisms (Hanke, 1990). Therefore, the stimulatory effect of corticosterone on tissues respiration might be either due to the increased oxidation of glucose and/or due to increased active transport of electrolytes across the cellular membranes. Cortisol is reported to be present in very small amount only in *Bufo marinus* among anurans (Gorbman, *et al*., 1983b; Jolivet-Jaudet&[illegible]1985). Failure of our present attempt to find out the involvement in the oxidative metabolism suggests that probably amphibian tissues have not developed mechanism (receptors) for responding to cortisol. Thus, cortisol, if present in anurans, might be acting as an intermediate during the synthesis or degradation of other corticoids like corticosterone and aldosterone. In an apodan (*G*.

carnosus), corticosterone has been reported to stimulate few oxidative enzymes in a dose-dependent manner (Josekumar and Oommen, 1988a). It, thus, seems that in frogs also corticosterone might be stimulating the respiratory rate of tissues by stimulating activities of the oxidative enzymes.

On the basis of the present findings it can be concluded that catecholamines (EP and NE) play a major role in the regulation of the oxidative metabolism of frogs, especially at low temperature. These hormones seem to produce their calorigenic action via both alpha- and beta-adrenergic mechanisms involving cAMP and /or phosphatidyl inositol metabolites as second messengers. Due to their rapid and temperature-independent calorigenic actions, catecholamines seem to act also as emergency hormones for the energy metabolism of frogs to ensure their successful survival at low natural temperature. Since, corticosterone is reportedly involved in the regulation of water and electrolyte metabolism, its involvement in the energy metabolism might be associated with efficient supply of energy for the active process of osmoregulation in frogs due to their amphibious life style. Thus, the calorigenic action of catecholamines and corticosterone seems to be of great adaptational significance in amphibian species.

* * * * * * * * *

Table 27 : In vivo effects of norepinephrine and epinephrine on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during winter (Temperature : 6 - 14 °C)

Treatments	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	0.86 ± 0.05[*]	0.47 ± 0.04	0.67 ± 0.03
Norepinephrine	1.72 ± 0.09[c]	1.20 ± 0.07[c]	1.34 ± 0.11[b]
Epinephrine	1.24 ± 0.02[c]	0.70 ± 0.07[a]	0.97 ± 0.05[b]
	Rana cyanophlyctis		
Saline(Control)	0.74 ± 0.03	0.48 ± 0.02	0.76 ± 0.05
Norepinephrine	1.70 ± 0.09[c]	1.24 ± 0.07[c]	1.46 ± 0.03[c]
Epinephrine	1.29 ± 0.05[c]	0.76 ± 0.01[c]	1.12 ± 0.07[b]

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 28 : In vivo effects of norepinephrine on the rate of tissues respiration in <u>Rana limnocharis</u> and <u>Rana cyanophlyctis</u> during Summer (Temperature : 12 - 20 ^{o}C)

Treatments	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	<u>Rana limnocharis</u>		
Saline(Control)	2.02 ± 0.07*	1.36 ± 0.03	1.73 ± 0.05
Norepinephrine (2 µg)	2.60 ± 0.05^{c}	1.95 ± 0.04^{c}	2.28 ± 0.10^{b}
Norepinephrine (5 µg)	3.06 ± 0.09^{c,e}	2.19 ± 0.05^{c,e}	2.62 ± 0.07^{c,d}
	<u>Rana cyanophlyctis</u>		
Saline(Control)	1.98 ± 0.07	1.36 ± 0.05	1.72 ± 0.04
Norepinephrine (2 µg)	2.54 ± 0.12^{b}	1.90 ± 0.07^{c}	2.20 ± 0.10^{b}
Norepinephrine (5 µg)	3.01 ± 0.09^{c,d}	2.24 ± 0.08^{c,d}	2.68 ± 0.13^{c,d}

[*] Mean ± Standard error; n = 4.

[b,c] Differ from the saline treated control group : P < 0.01 and 0.001 respectively.

[d,e] Differ from norepinephrine (2 µg) group : P < 0.05 and 0.01 respectively.

Table 29 : In vivo effects of epinephrine on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

Treatments	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
		Rana limnocharis	
Saline(Control)	$2.02 \pm 0.07^{*}$	1.36 ± 0.03	1.73 ± 0.05
Epinephrine(2 ug)	2.44 ± 0.11^{a}	1.80 ± 0.03^{c}	2.20 ± 0.09^{b}
Epinephrine(5 ug)	$2.98 \pm 0.09^{c,e}$	$2.04 \pm 0.08^{c,d}$	2.42 ± 0.09^{c}
		Rana cyanophlyctis	
Saline(Control)	1.98 ± 0.07	1.36 ± 0.05	1.72 ± 0.04
Epinephrine(2 ug)	2.50 ± 0.14^{a}	1.81 ± 0.03^{c}	2.14 ± 0.09^{b}
Epinephrine(5 ug)	2.82 ± 0.10^{c}	$2.20 \pm 0.09^{c,e}$	2.39 ± 0.11^{b}

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

[d,e] Differ from the Epinephrine (2 ug) group : $P < 0.05$ and 0.01 respectively.

Table 30 : In vitro effects of epinephrine and norepinephrine on the rate of tissues respiration of <u>Rana limnocharis</u> and <u>Rana cyanophlyctis</u> during Winter (Temperature : 6 - 14 °C)

Treatments	Tissues oxygen consumption ($\mu l\ O_2$/mg wet tissue/h)	
	Liver	Muscle
	<u>Rana limnocharis</u>	
Saline (Control)	0.88 ± 0.07[*]	0.59 ± 0.03
Epinephrine	1.81 ± 0.13[c]	1.26 ± 0.09[c]
Norepinephrine	1.99 ± 0.10[c]	1.43 ± 0.11[c]
	<u>Rana cyanophlyctis</u>	
Saline (Control)	0.80 ± 0.07	0.40 ± 0.02
Epinephrine	1.61 ± 0.14[b]	1.13 ± 0.11[c]
Norepinephrine	1.82 ± 0.12[c]	1.42 ± 0.14[c]

[*] Mean ± Standard error; n = 4.

[b,c] Differ from the saline treated control group : $P < 0.01$ and 0.001 respectively.

Table 31 : In vitro effects of epinephrine and norepinephrine on the rate of tissues respiration of Rana limnocharis and Rana cyanophlyctis during Summer (Temperature : 14 - 20 ^{o}C)

Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline (Control)	1.81 ± 0.02[*]	1.65 ± 0.07
Epinephrine	2.19 ± 0.04[c]	1.85 ± 0.01[a]
Norepinephrine	2.22 ± 0.05[c]	1.89 ± 0.04[a]
	Rana cyanophlyctis	
Saline (Control)	1.69 ± 0.005	1.32 ± 0.04
Epinephrine	2.05 ± 0.03[c]	1.65 ± 0.05[b]
Norepinephrine	2.12 ± 0.05[c]	1.66 ± 0.07[b]

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 32 : Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of <u>Rana limnocharis</u> during Winter (Temperature : 8 - 15 °C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	1.58 ± 0.03[*]	1.39 ± 0.01
	Norepinephrine	1.59 ± 0.05	1.42 ± 0.05
30 Minutes :	Saline(Control)	1.58 ± 0.02	1.39 ± 0.03
	Norepinephrine	1.64 ± 0.02[a]	1.49 ± 0.03[a]
1 Hour :	Saline(Control)	1.58 ± 0.02	1.39 ± 0.05
	Norepinephrine	1.65 ± 0.01[a]	1.54 ± 0.03[a]
6 Hour :	Saline(Control)	2.15 ± 0.05	1.90 ± 0.05
	Norepinephrine	2.41 ± 0.09[a]	2.04 ± 0.01[a]
12 Hour :	Saline(Control)	1.70 ± 0.11	1.65 ± 0.07
	Norepinephrine	1.51 ± 0.03	1.60 ± 0.09
24 Hour :	Saline(Control)	1.53 ± 0.04	1.50 ± 0.02
	Norepinephrine	1.50 ± 0.03	1.44 ± 0.11

[*] Mean ± Standard error; n = 4.

[a] Differs from the respective saline treated control group : $P < 0.05$.

Table 33 : Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of <u>Rana</u> <u>cyanophlyctis</u> during Winter (Temperature : 5 - 14 ^{o}C)

Time	Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes	Saline(Control)	1.62 ± 0.03*	1.34 ± 0.05
	Norepinephrine	1.68 ± 0.03	1.39 ± 0.07
30 Minutes	Saline(Control)	1.62 ± 0.03	1.39 ± 0.01
	Norepinephrine	1.78 ± 0.01[b]	1.48 ± 0.01[c]
1 Hour	Saline(Control)	1.64 ± 0.01	1.38 ± 0.01
	Norepinephrine	1.76 ± 0.02[c]	1.48 ± 0.03[a]
6 Hour	Saline(Control)	2.10 ± 0.07	1.42 ± 0.03
	Norepinephrine	2.30 ± 0.05[a]	1.65 ± 0.04[b]
24 Hour	Saline(Control)	1.58 ± 0.04	1.30 ± 0.04
	Norepinephrine	1.55 ± 0.09	1.27 ± 0.01

* Mean ± Standard error; n = 4.

[a,b,c] Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 34 : Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of *Rana limnocharis* during Summer (Temperature : 14 - 24 ^{o}C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	$2.30 \pm 0.06^{*}$	1.92 ± 0.06
	Norepinephrine	2.55 ± 0.07^{a}	2.10 ± 0.04^{a}
30 Minutes :	Saline(Control)	2.30 ± 0.05	1.90 ± 0.03
	Norepinephrine	2.60 ± 0.05^{c}	2.25 ± 0.04^{c}
1 Hour :	Saline(Control)	2.30 ± 0.09	1.99 ± 0.07
	Norepinephrine	2.60 ± 0.02^{a}	2.30 ± 0.09^{a}
6 Hour :	Saline(Control)	2.21 ± 0.09	2.04 ± 0.03
	Norepinephrine	2.66 ± 0.03^{b}	2.30 ± 0.12
12 Hour :	Saline(Control)	2.25 ± 0.07	2.06 ± 0.08
	Norepinephrine	2.60 ± 0.03^{b}	2.30 ± 0.07
24 Hour :	Saline(Control)	2.25 ± 0.09	2.00 ± 0.06
	Norepinephrine	2.00 ± 0.11	1.89 ± 0.03

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 35: Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of <u>Rana cyanophlyctis</u> during Summer (Temperature : 16 - 24 °C)

Treatments		Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
Time		Liver	Muscle
10 Minutes :	Saline(Control)	2.35 ± 0.09[*]	1.49 ± 0.08
	Norepinephrine	2.49 ± 0.05	1.55 ± 0.07
30 Minutes :	Saline(Control)	2.47 ± 0.07	1.52 ± 0.03
	Norepinephrine	2.69 ± 0.04[a]	1.70 ± 0.06[a]
1 Hour :	Saline(Control)	2.45 ± 0.05	1.50 ± 0.03
	Norepinephrine	2.72 ± 0.10[a]	1.77 ± 0.08[a]
6 Hour :	Saline(Control)	2.45 ± 0.03	1.70 ± 0.07
	Norepinephrine	2.79 ± 0.05[b]	2.13 ± 0.08[b]
12 Hour :	Saline(Control)	2.39 ± 0.03	1.59 ± 0.02
	Norepinephrine	2.79 ± 0.05[c]	1.77 ± 0.01[c]
24 Hour :	Saline(Control)	2.39 ± 0.06	1.59 ± 0.07
	Norepinephrine	2.50 ± 0.03	1.70 ± 0.06

[*] Mean ± Standard error; n = 4.

[a,b,c] *Differ from the respective saline treated control* group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 36 : Time-dependent in vivo effects of epinephrine on the rate of tissues respiration of *Rana limnocharis* during Winter (Temperature : 4.6 - 14 ^{o}C)

Time	Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes	Saline(Control)	$1.48 \pm 0.03^{*}$	1.14 ± 0.01
	Epinephrine	1.50 ± 0.05	1.15 ± 0.09
30 Minutes	Saline(Control)	1.52 ± 0.01	1.22 ± 0.05
	Epinephrine	1.66 ± 0.03^{b}	1.49 ± 0.02^{b}
1 Hour	Saline(Control)	1.52 ± 0.03	1.24 ± 0.05
	Epinephrine	1.67 ± 0.04^{a}	1.50 ± 0.02^{b}
6 Hour	Saline(Control)	1.90 ± 0.03	1.70 ± 0.04
	Epinephrine	2.12 ± 0.07^{a}	1.85 ± 0.01^{b}
12 Hour	Saline(Control)	1.79 ± 0.09	1.52 ± 0.05
	Epinephrine	1.73 ± 0.01	1.46 ± 0.11
24 Hour	Saline(Control)	1.65 ± 0.05	1.20 ± 0.12
	Epinephrine	1.59 ± 0.04	1.20 ± 0.05

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the respective saline treated control group : $P < 0.05$ and 0.01 respectively.

Table 37 : Time-dependent in vivo effects of epinephrine on the rate of tissues respiration of <u>Rana</u> <u>cyanophlyctis</u> during Winter (Temperature : 4.6 - 14 °C)

Time	Treatments	Tissues oxygen consumption (ul O_2 /mg wet tissue/h) Liver	 Muscle
10 Minutes :	Saline(Control)	$1.61 \pm 0.03^{*}$	1.31 ± 0.09
	Epinephrine	1.64 ± 0.02	1.36 ± 0.12
30 Minutes :	Saline(Control)	1.60 ± 0.05	1.35 ± 0.01
	Epinephrine	1.75 ± 0.03^{a}	1.46 ± 0.02^{b}
1 Hour :	Saline(Control)	1.62 ± 0.03	1.34 ± 0.03
	Epinephrine	1.77 ± 0.05^{a}	1.50 ± 0.05^{a}
6 Hour :	Saline(Control)	2.05 ± 0.07	1.44 ± 0.07
	Epinephrine	2.27 ± 0.05^{a}	1.78 ± 0.09^{a}
12 Hour :	Saline(Control)	1.79 ± 0.10	1.33 ± 0.05
	Epinephrine	1.67 ± 0.07	1.34 ± 0.07
24 Hour :	Saline(Control)	1.62 ± 0.12	1.33 ± 0.11
	Epinephrine	1.60 ± 0.09	1.31 ± 0.05

[*] Mean ± Standard error; n = 4.

[a,b] Differs from the respective saline treated control group : $P < 0.05$, < 0.01.

Table 38 : Time-dependent *in vivo* effects of epinephrine on the rate of tissues respiration of Rana limnocharis during Summer (Temperature : 14 - 20 ^{o}C)

Time	Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	2.07 ± 0.05[*]	1.39 ± 0.07
	Epinephrine	2.29 ± 0.07[a]	1.50 ± 0.08
30 Minutes :	Saline(Control)	2.06 ± 0.03	1.38 ± 0.05
	Epinephrine	2.35 ± 0.09[a]	1.59 ± 0.01[b]
1 Hour :	Saline(Control)	2.09 ± 0.07	1.40 ± 0.05
	Epinephrine	2.40 ± 0.04[b]	1.65 ± 0.07[a]
6 Hour :	Saline(Control)	2.10 ± 0.08	1.44 ± 0.09
	Epinephrine	2.42 ± 0.03[b]	1.70 ± 0.09
12 Hour :	Saline(Control)	2.08 ± 0.07	1.44 ± 0.05
	Epinephrine	2.40 ± 0.05[b]	1.68 ± 0.11
24 Hour :	Saline(Control)	1.98 ± 0.03	1.40 ± 0.07
	Epinephrine	2.35 ± 0.10[a]	1.40 ± 0.11

[*] Mean ± Standard error; n = 4.

[a,b] Differ from the respective saline treated group : $P < 0.05$ and 0.01 respectively.

Table 39 : Time-dependent in vivo effects of epinephrine on the rate of tissues respiration of <u>Rana cyanophlyctis</u> during Summer (Temperature : 16 - 24 °C)

Time	Treatments	Tissues oxygen consumption (µl O_2/mg wet tissue/h)	
		Liver	Muscle
10 Minutes :	Saline(Control)	2.38 ± 0.01[*]	1.75 ± 0.05
	Epinephrine	2.52 ± 0.07	1.80 ± 0.06
30 Minutes :	Saline(Control)	2.35 ± 0.05	1.75 ± 0.07
	Epinephrine	2.65 ± 0.04[b]	1.92 ± 0.06
1 Hour :	Saline(Control)	2.35 ± 0.03	1.80 ± 0.09
	Epinephrine	2.70 ± 0.09[b]	1.92 ± 0.06
6 Hour :	Saline(Control)	2.42 ± 0.07	1.85 ± 0.06
	Epinephrine	2.70 ± 0.07[a]	2.09 ± 0.03[a]
12 Hour :	Saline(Control)	2.39 ± 0.03	1.86 ± 0.01
	Epinephrine	2.79 ± 0.05[c]	2.00 ± 0.07
24 Hour :	Saline(Control)	2.35 ± 0.08	1.80 ± 0.06
	Epinephrine	2.59 ± 0.09	1.80 ± 0.10

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 40 : In vivo effects of epinephrine, norepinephrine and propyl thiouracil (PTU) on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 21 °C).

Treatments	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	2.05 ± 0.07[*]	1.44 ± 0.09	1.82 ± 0.03
Epinephrine (E)	2.40 ± 0.10[a]	1.88 ± 0.07[b]	2.20 ± 0.07[b]
Norepinephrine(NE)	2.62 ± 0.04[c]	1.94 ± 0.06[b]	2.27 ± 0.03[c]
PTU	1.62 ± 0.09[b]	1.28 ± 0.005[a]	1.71 ± 0.01[a]
PTU + E	2.09 ± 0.04[d,l]	1.58 ± 0.10[d,k]	1.92 ± 0.09[d]
PTU + NE	2.15 ± 0.07[e,l]	1.50 ± 0.01[f,m]	1.99±0.05[a,e,l]
	Rana cyanophlyctis		
Saline(Control)	2.01 ± 0.07[*]	1.36 ± 0.05	1.72 ± 0.04
Epinephrine (E)	2.50 ± 0.14[a]	1.81 ± 0.03[c]	2.14 ± 0.09[b]
Norepinephrine(NE)	2.54 ± 0.12[b]	1.90 ± 0.07[c]	2.30 ± 0.10[b]
PTU	1.75 ± 0.10	1.16 ± 0.04[a]	1.60 ± 0.005[a]
PTU + E	2.10 ± 0.05[d,k]	1.42 ± 0.07[c,k]	1.85 ± 0.08[k]
PTU + NE	2.29 ± 0.09[a,l]	1.49 ± 0.11[d,k]	1.93 ± 0.08[d,l]

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[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

[d,e] Differ from Epinephrine/norepinephrine group : $P < 0.05$ and 0.01 respectively.

[k,l] Differ from PTU group : $P < 0.05$ and 0.01 respectively.

Table 41 : In vitro effects of a- and B-adrenergic agonists and antagonists on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 11 - 23 °C)

Treatments	Rate of oxygen consumption (µl O_2/mg wet tissue/h)			
	Rana limnocharis		*Rana cyanophlyctis*	
	Liver	Muscle	Liver	Muscle
Saline	0.88±0.04[*]	0.54+0.08	0.78+0.03	0.41+0.07
Phenylephrine(PHE)	1.00±0.03[a]	0.83±0.03[a]	0.98+0.07[a]	0.62+0.01[a]
Isoproterenol(ISO)	1.02±0.01[a]	0.84±0.09[a]	1.03±0.09[a]	0.64±0.03[a]
PHE + ISO	1.30±0.07[b]	1.06±0.03[c]	1.31±0.12[b]	0.82±0.09[a]
Prazosin(PRAZ)	0.52±0.09[a,e]	0.52±0.07[e]	0.70±0.03	0.32±0.05[e]
Propranolol(PROP)	0.46±0.11[a,e]	0.45±0.09[e]	0.70±0.05[d]	0.31±0.05[e]
PRAZ + PROP	0.37±0.18[a,e]	0.34±0.03[a,f]	0.39±0.10[b,f]	0.27±0.03[f]
PHE + PRAZ	0.97±0.03[l]	0.67±0.02[e]	0.84±0.05[k]	0.49±0.01[f,k]
ISO + PROP	0.94±0.02[e,l]	0.63±0.05	0.82±0.07	0.42±0.03[f]
ISO + PROP +PRAZ	0.57±0.09[a,e]	0.44±0.07[e]	0.55±0.02[c,e]	0.32±0.07[e]
PHE + PROP + PRAZ	0.60±0.07[a,e]	0.56±0.03[f]	0.58±0.08[d]	0.35±0.03[f]

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated group : P < 0.05, 0.01 and 0.001 respectively.

[d,e,f] Differ from respective Isoproterenol or Phenylephrine group : P < 0.05, 0.01, 0.001 respectively.

[k,l] differ from respective antagonist (Prazosin or Propranolol) group : P < 0.05 and 0.01 respectively.

Table 42 : In vitro effects of catecholamines and adrenergic antagonists on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature : 12 - 22 °C)

Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
	Liver	Muscle
Saline(Control)	$1.20 \pm 0.02^{*}$	0.76 ± 0.08
Epinephrine (EP)	1.47 ± 0.05^{b}	1.01 ± 0.05^{a}
Norepinephrine (NEP)	1.67 ± 0.05^{c}	1.23 ± 0.04^{b}
Propranolol (PROP)	0.99 ± 0.09^{a}	0.60 ± 0.07
Prazosin (PRAZ)	1.05 ± 0.04^{b}	0.69 ± 0.03
EP + PROP	1.31 ± 0.09^{k}	0.78 ± 0.04^{e}
EP + PRAZ	1.39 ± 0.10^{k}	$0.89 \pm 0.05^{d,k}$
EP + PROP + PRAZ	$0.99 \pm 0.08^{a,e}$	0.41 ± 0.03^{f}
NEP + PROP	$1.40 \pm 0.07^{a,d,k}$	$0.89 \pm 0.03^{f,l}$
NEP + PRAZ	1.44 ± 0.12^{k}	$0.91 \pm 0.04^{f,l}$
NEP + PROP + PRAZ	1.15 ± 0.06^{f}	0.73 ± 0.08^{e}

* Mean ± Standard error; n = 4,

^{a,b,c} Differ from the saline treated group : $P < 0.05$, 0.01 and 0.001 respectively.

^{d,e,f} Differ respectively from Epinephrine or norepinephrine group : $P < 0.05$, 0.01 and 0.001 respectively.

^{k,l} Differ from respective Propranolol or Prazosin group : $P < 0.05$, and 0.01 respectively.

Table 43 : In vivo effects of corticosterone and cortisol on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 – 14 $^{\circ}$C)

Treatments	Tissue oxygen consumption (µl O_2 /mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	0.86 ± 0.05[*]	0.47 ± 0.04	0.67 ± 0.03
Corticosterone	1.10 ± 0.05[a]	0.70 ± 0.04[b]	0.98 ± 0.03[c]
Cortisol	1.05 ± 0.08	0.59 ± 0.05	0.89 ± 0.12
	Rana cyanophlyctis		
Saline(Control)	0.74 ± 0.03	0.48 ± 0.02	0.76 ± 0.05
Corticosterone	1.13 ± 0.07[b]	0.89 ± 0.05[c]	1.00 ± 0.03[c]
Cortisol	0.80 ± 0.03	0.61 ± 0.09	0.78 ± 0.02

[*] Mean + Standard error: n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 44 : In vivo effects of corticosterone and cortisol on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

Treatments	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	$2.02 \pm 0.07^{*}$	1.36 ± 0.03	1.73 ± 0.05
Corticosterone	2.40 ± 0.06^{b}	1.78 ± 0.04^{c}	2.08 ± 0.02^{c}
Cortisol	2.06 ± 0.05	1.50 ± 0.09	1.74 ± 0.06
	Rana cyanophlyctis		
Saline(Control)	1.98 ± 0.07	1.36 ± 0.05	1.72 ± 0.04
Corticosterone	2.36 ± 0.09^{a}	1.68 ± 0.02^{c}	2.12 ± 0.05^{c}
Cortisol	2.04 ± 0.05	1.42 ± 0.09	1.70 ± 0.70

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

Table 45 : In vitro effects of corticosterone and cortisol on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 - 14 °C)

Treatments	Tissues oxygen consumption (μl O_2/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline (Control)	0.88 ± 0.07[*]	0.59 ± 0.03
Corticosterone	1.21 ± 0.09[a]	0.80 ± 0.02[c]
Cortisol	0.89 ± 0.07	0.59 ± 0.09
	Rana cyanophlyctis	
Saline (Control)	0.80 ± 0.07	0.40 ± 0.02
Corticosterone	1.06 ± 0.05[a]	0.51 ± 0.04[a]
Cortisol	0.79 ± 0.05	0.44 ± 0.01

[*] Mean ± Standard error: n = 4.

[a,c] Differ from the saline treated control group : $P < 0.05$ and 0.001 respectively.

Table 46 : In vitro effects of corticosterone and cortisol on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

Treatments	Tissues oxygen consumption (μl O$_2$/mg wet tissue/h)	
	Liver	Muscle
	Rana limnocharis	
Saline (Control)	1.81 ± 0.02[*]	1.65 ± 0.07
Corticosterone	2.10 ± 0.03[c]	1.89 ± 0.05[a]
Cortisol	1.80 ± 0.04	1.59 ± 0.04
	Rana cyanophlyctis	
Saline (Control)	1.69 ± 0.005	1.32 ± 0.04
Corticosterone	1.90 ± 0.04[b]	1.57 ± 0.03[b]
Cortisol	1.59 ± 0.09	1.30 ± 0.07

[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from the saline treated control group : P < 0.05, 0.01 and 0.001 respectively.

Table 47 : In vivo effects of metapyrone on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 7 -15 °C)

Treatments	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Oil	1.14 ± 0.07*	0.90 ± 0.07	1.03 ± 0.04
Metapyrone	0.89 ± 0.09	0.66 ± 0.05[a]	0.79 ± 0.06[a]
	Rana cyanophlyctis		
Oil	1.60 ± 0.09	1.24 ± 0.07	1.46 ± 0.05
Metapyrone	1.22 ± 0.07[a]	0.94 ± 0.09[a]	1.10 ± 0.10[a]

* Mean ± Standard error; n = 4.

[a] Differs from oil treated control group : $P < 0.05$.

Table 48 : *In vivo effects of metapyrone on the rate of tissues* respiration in <u>Rana limnocharis</u> and <u>Rana cyanophlyctis</u> during Summer (Temperature : 14 - 24 ^{o}C)

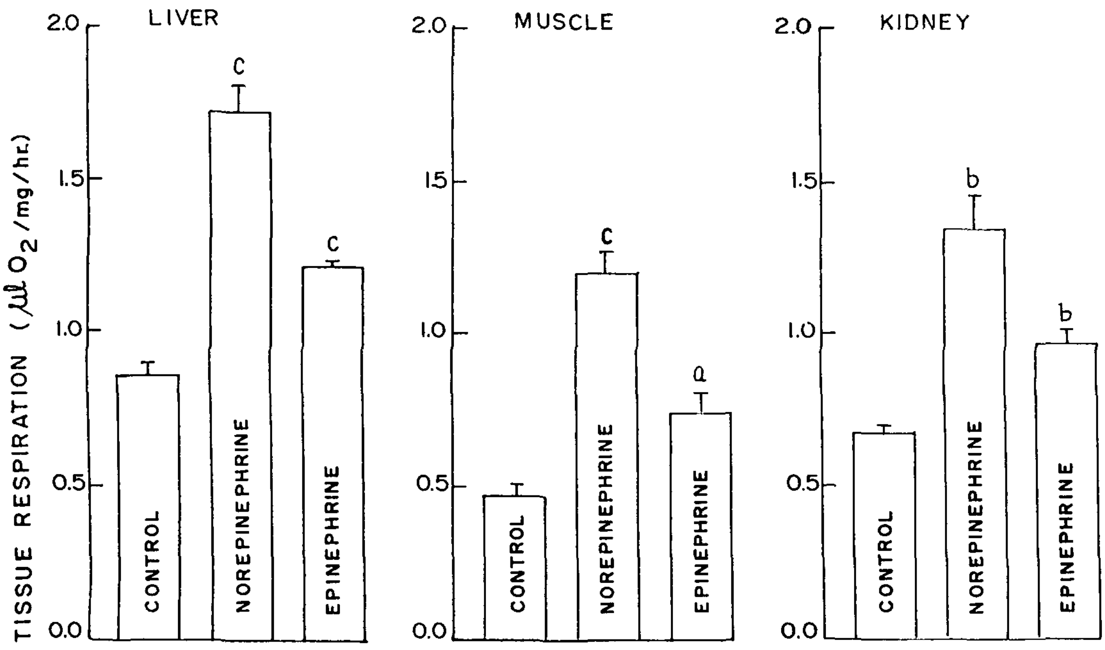
Treatments	Tissue oxygen consumption (μl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
		<u>Rana limnocharis</u>	
Oil	1.98 ± 0.03[*]	1.80 ± 0.07	1.85 ± 0.03
Metapyrone	1.80 ± 0.05[a]	1.59 ± 0.005[a]	1.68 ± 0.04[a]
		<u>Rana cyanophlyctis</u>	
Oil	2.09 ± 0.09	1.82 ± 0.05	1.99 ±0.09
Metapyrone	1.83 ± 0.09[a]	1.60 ± 0.03[a]	1.70 ± 0.60[a]

[*] Mean ± Standard error; n = 4.

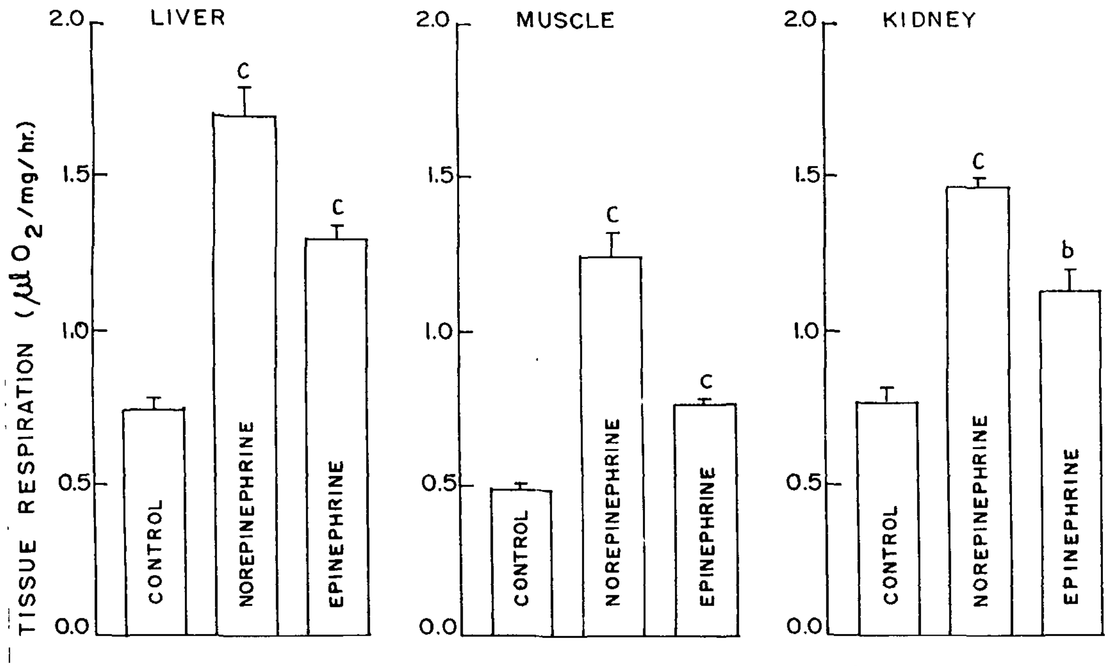
[a] Differs from the Oil treated control group : $P < 0.05$.

Fig. 27 : In vivo effects of norepinephrine and epinephrine on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during winter (Temperature : 6 - 14 °C)

^{a,b,c} Differ from the saline treated control group $P < 0.05$, 0.01 and 0.001 respectively.



<u>Rana</u> <u>limnocharis</u> (Winter)

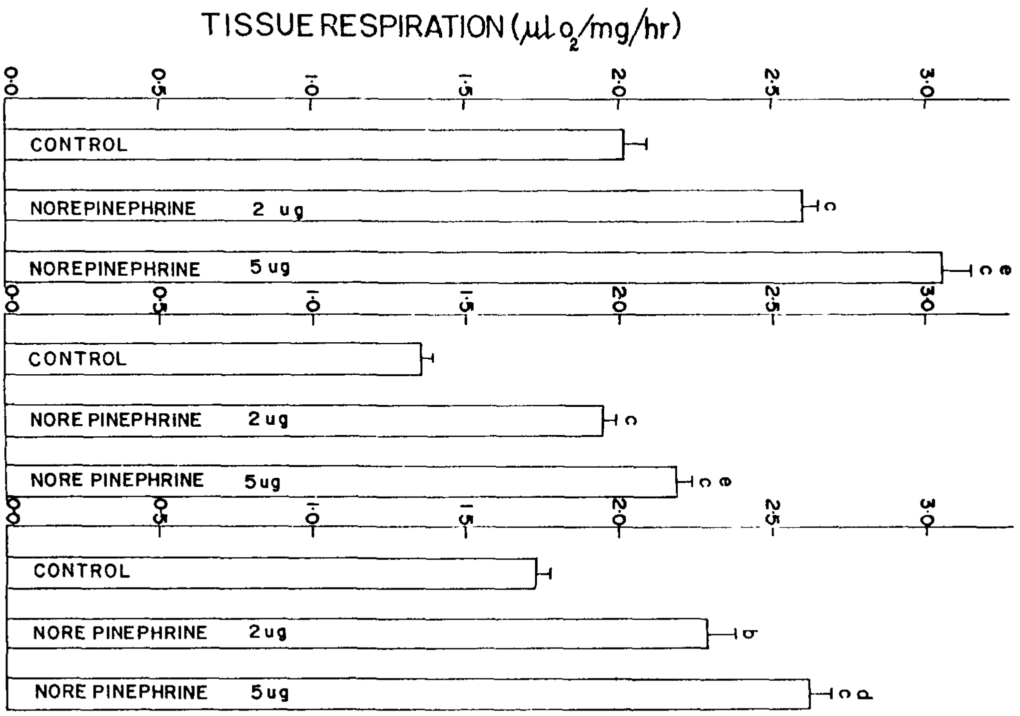


<u>Rana</u> <u>cyanophlyctis</u> (Winter)

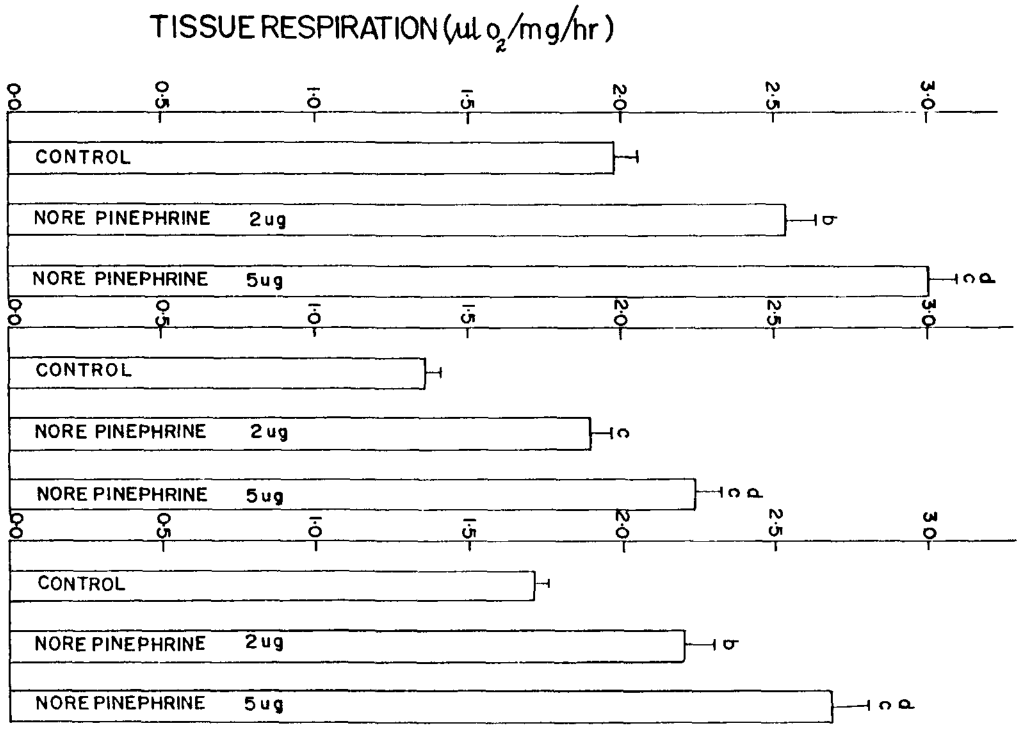
Fig. 28 : In vivo effects of norepinephrine on the rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during Summer (Temperature : 12 - 20°C)

^{b,c} Differ from the saline treated control group : $P < 0.01$ and 0.001 respectively.

^{d,e} Differ from norepinephrine (2 ug) group : $P < 0.05$ and 0.01 respectively.



Rana limnocharis (Summer)

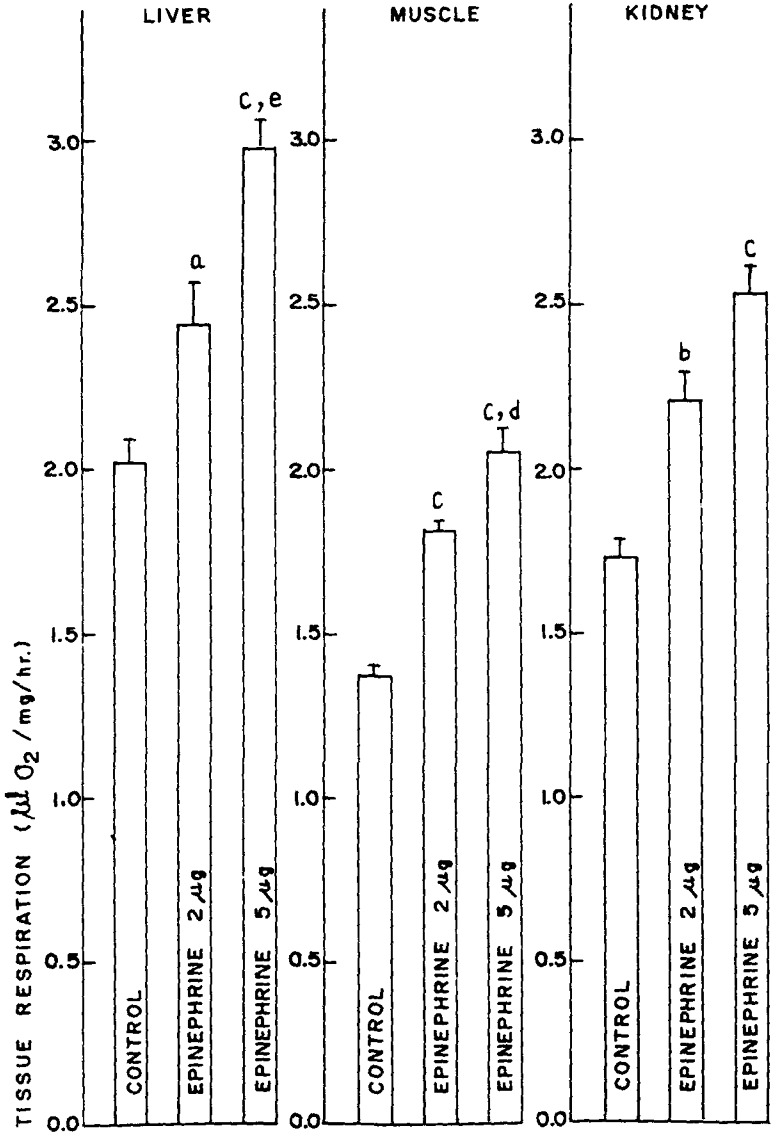


Rana cyanophlyctis (Summer)

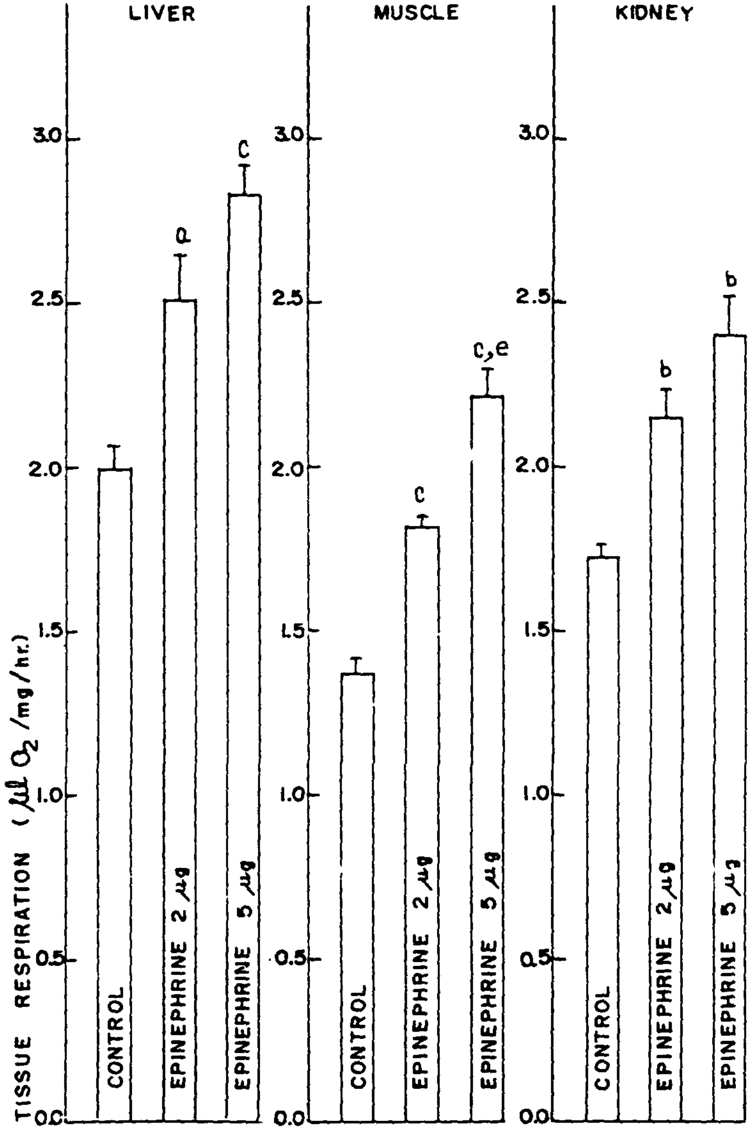
Fig. 29 : In vivo effects of epinephrine on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

^{a,b,c} Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

^{d,e} Differ from the Epinephrine (2 µg) group : $P < 0.05$ and 0.01 respectively.



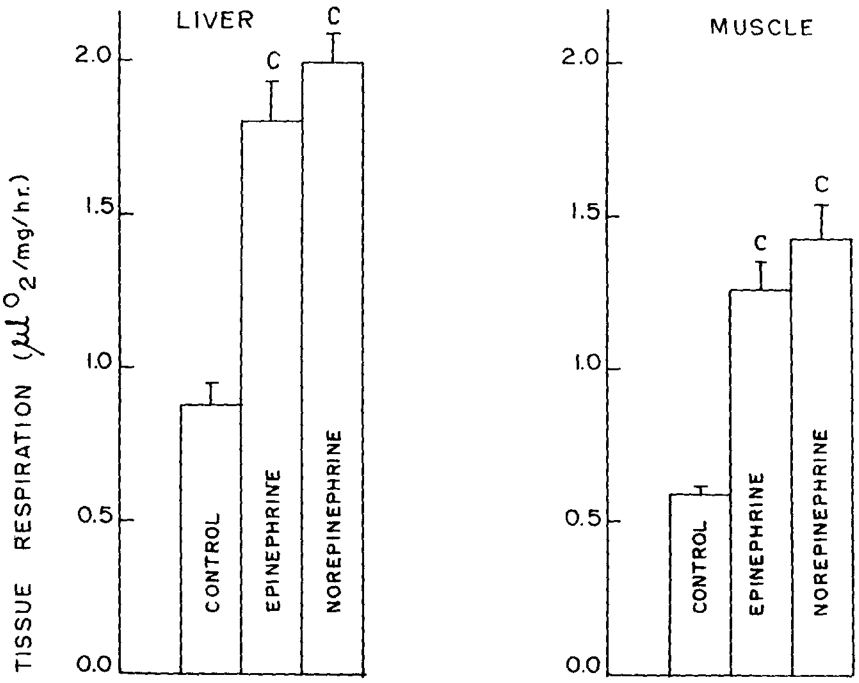
Rana limnocharis (Summer)



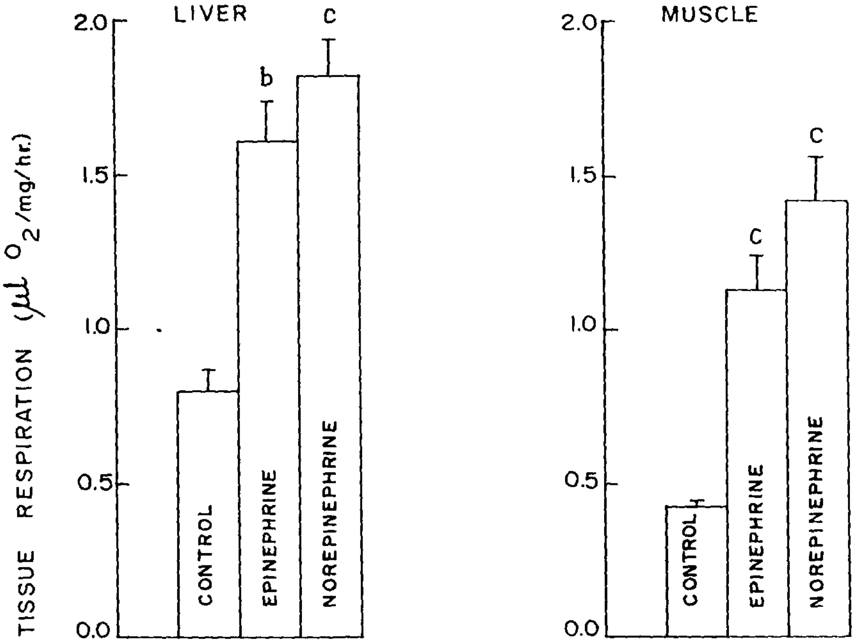
Rana cyanophlyctis (Summer)

Fig. 30 : In vitro effects of epinephrine and norepinephrine on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 - 14 °C)

[b,c] Differ from the saline treated control group : $P < 0.01$ and 0.001 respectively.



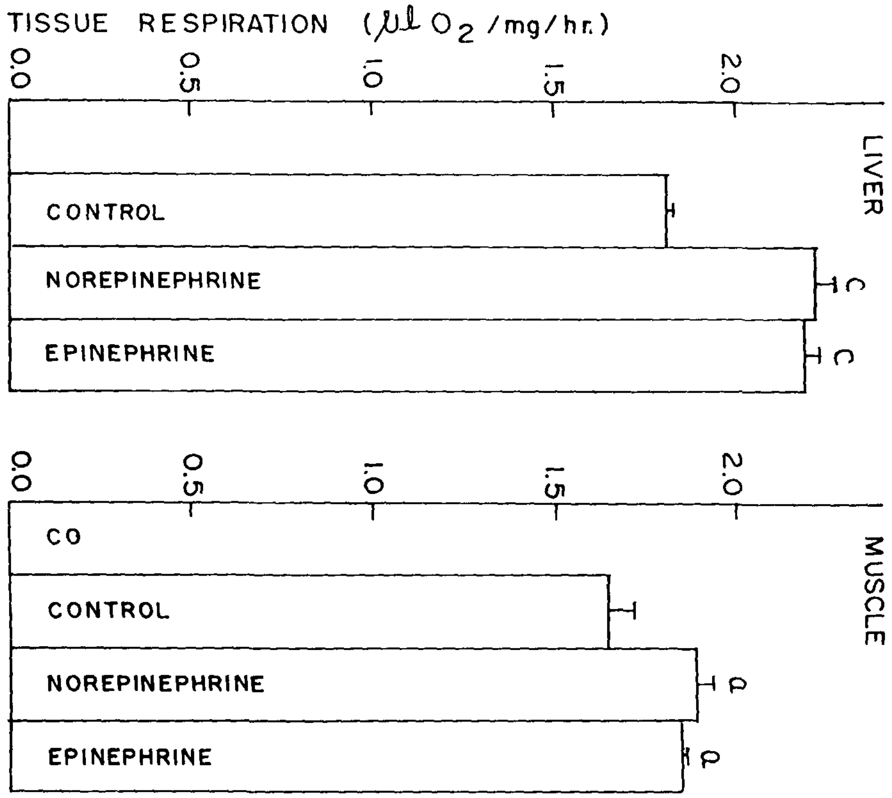
Rana limnocharis (Winter)



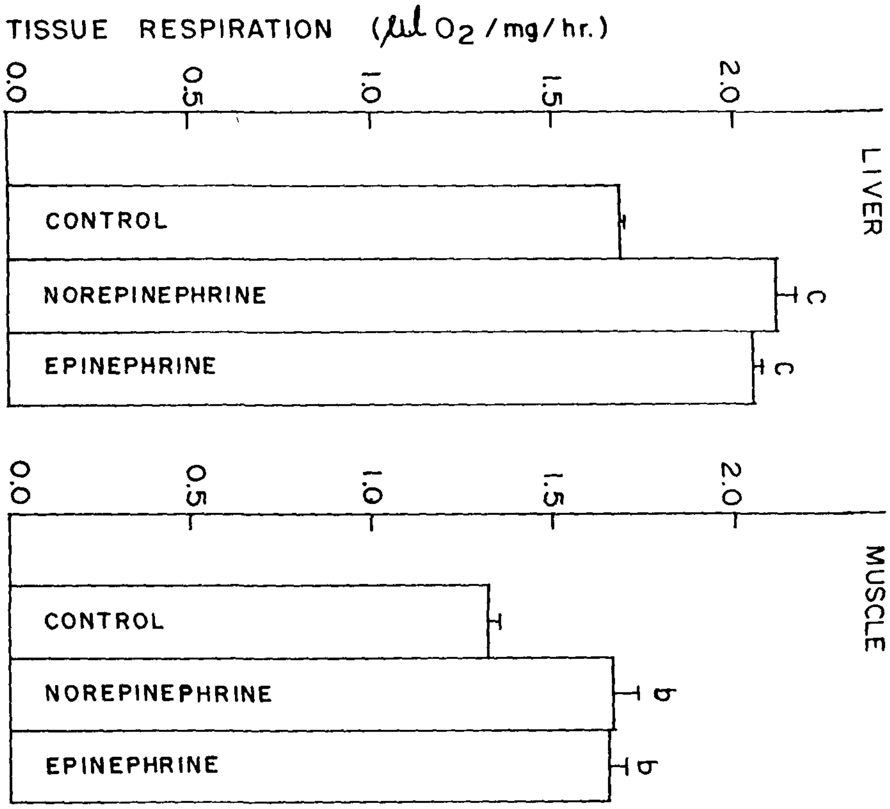
Rana cyanophlyctis (Winter)

Fig. 31 : In vitro effects of epinephrine and norepinephrine on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 14 - 20 °C)

^{a,b,c} Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.



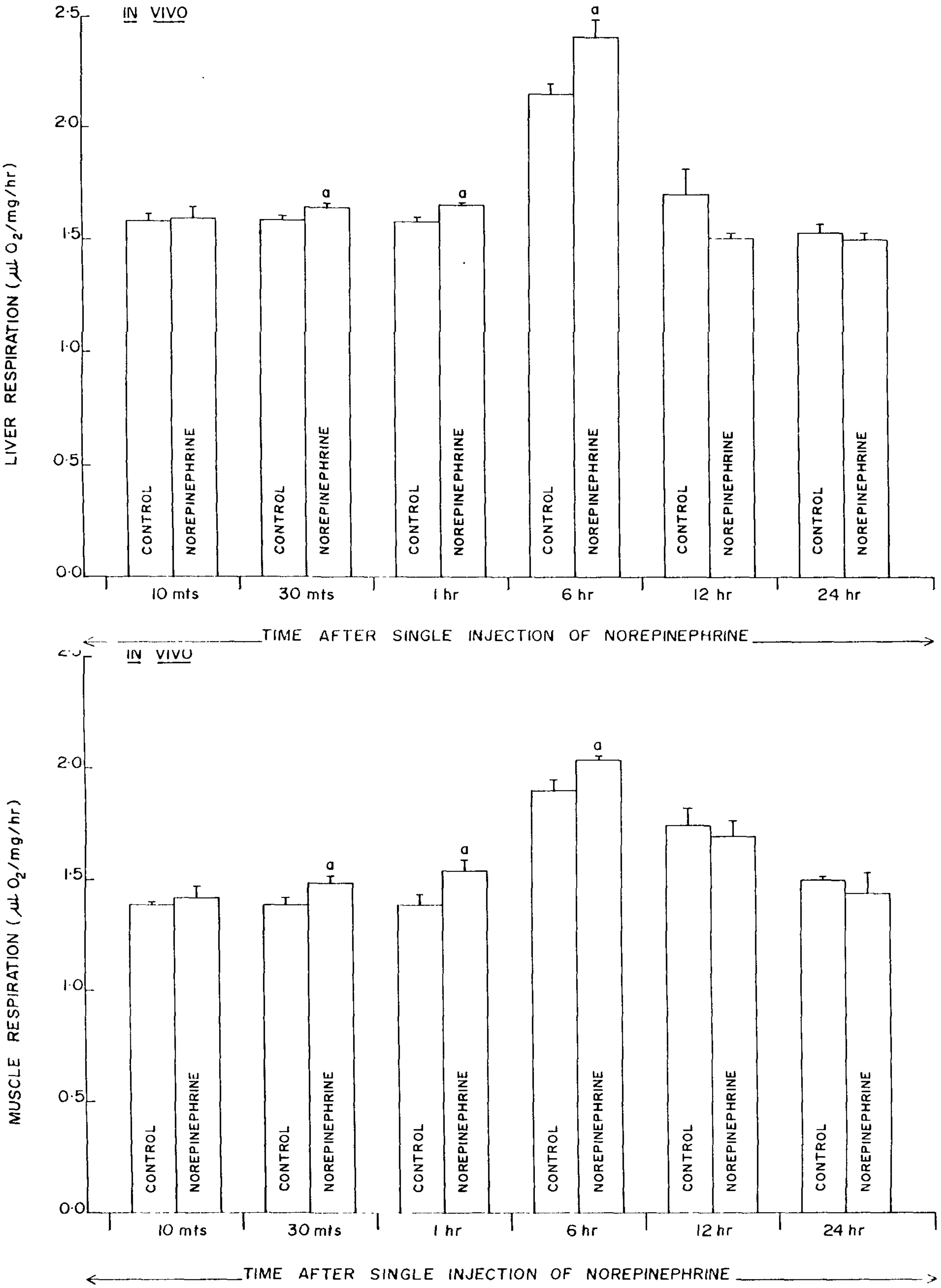
Rana limnocharis (Summer)



Rana cyanophlyctis (Summer)

Fig. 32 : Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of <u>Rana limnocharis</u> during Winter (Temperature : 8 - 15 °C)

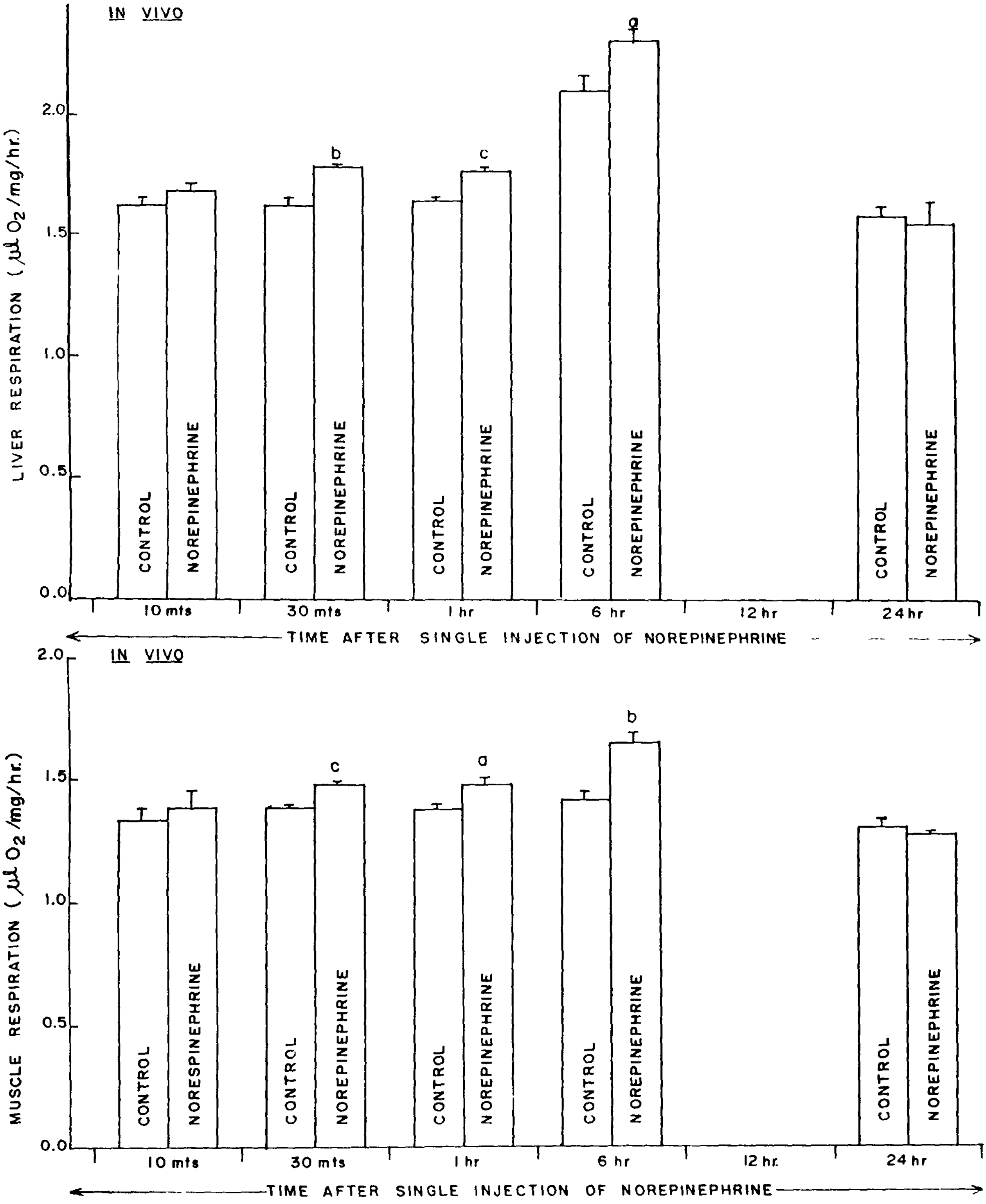
[a] Differs from the respective saline treated control group : $P < 0.05$.



Rana limnocharis (Winter)

Fig. 33 : Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of <u>Rana</u> <u>cyanophlyctis</u> during Winter (Temperature : 5 - 14 ^{o}C)

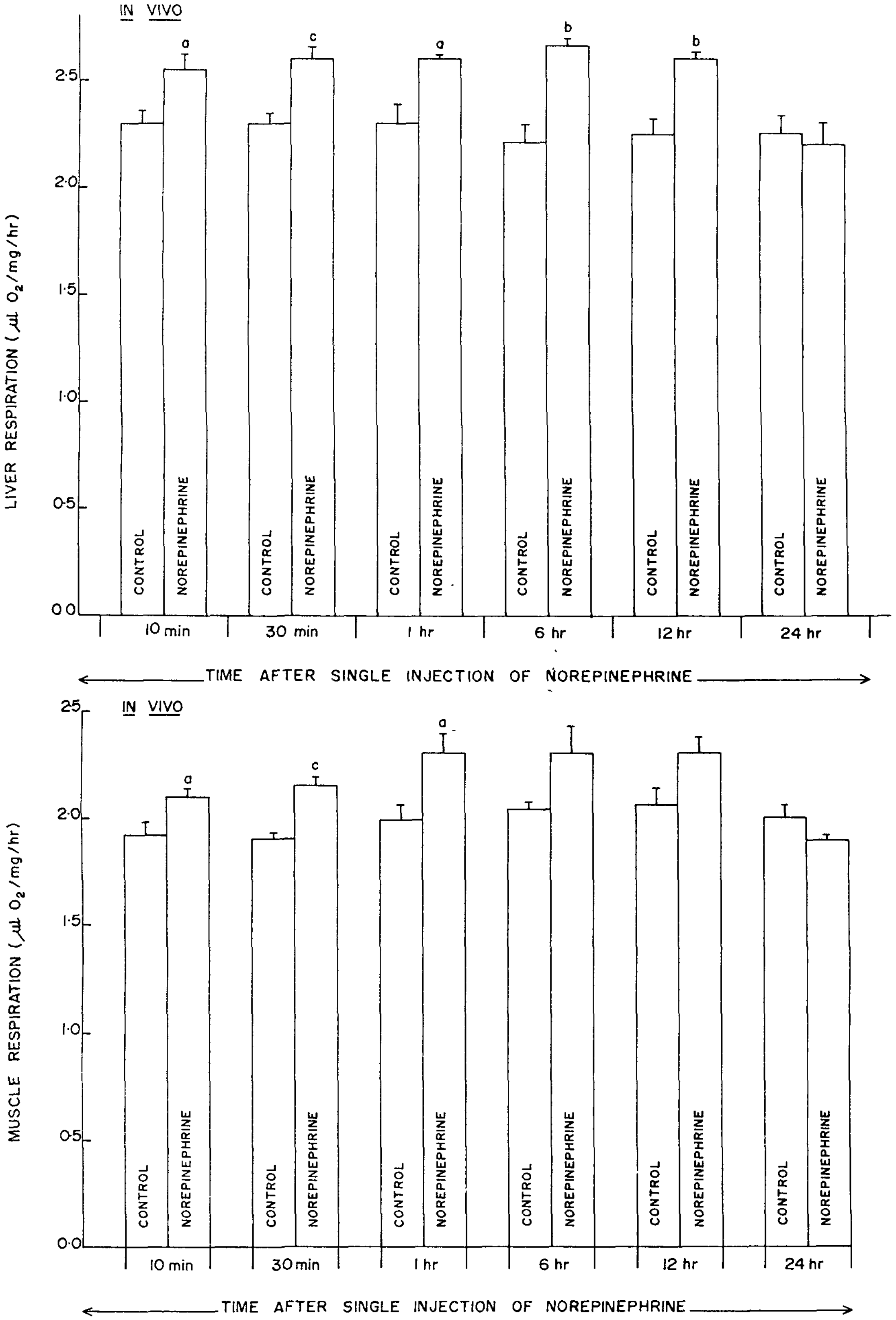
^{a,b,c} Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.



Rana cyanophlyctis (Winter)

Fig. 34 : Time-dependent in vivo effects of norepinephrine on the rate of tissues respiration of <u>Rana limnocharis</u> during Summer (Temperature : 14 - 24 ^{o}C)

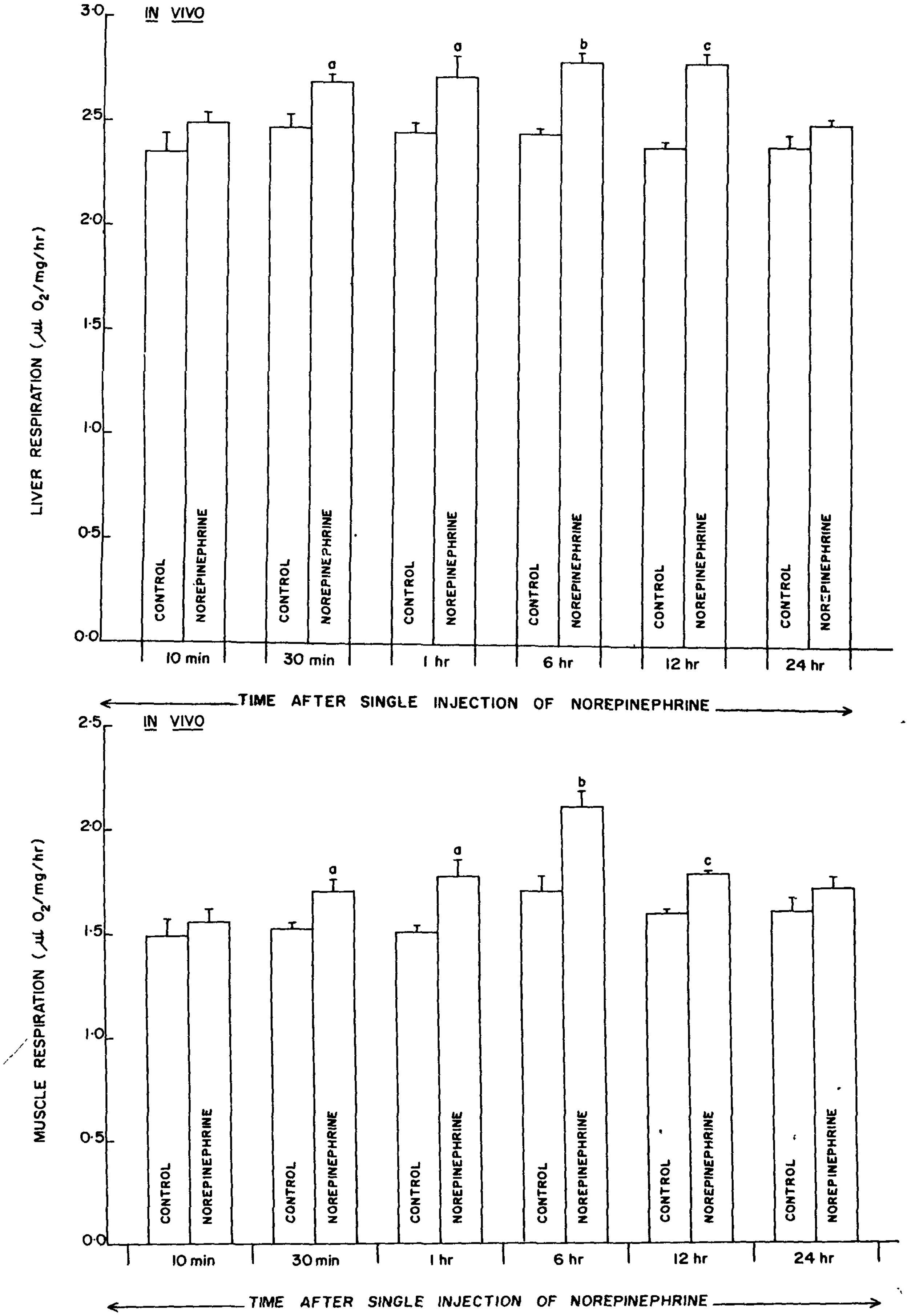
^{a,b,c} Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.



Rana limnocharis (Summer)

Fig. 35: Time-dependent *in vivo* effects of norepinephrine on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature : 16 - 24 °C)

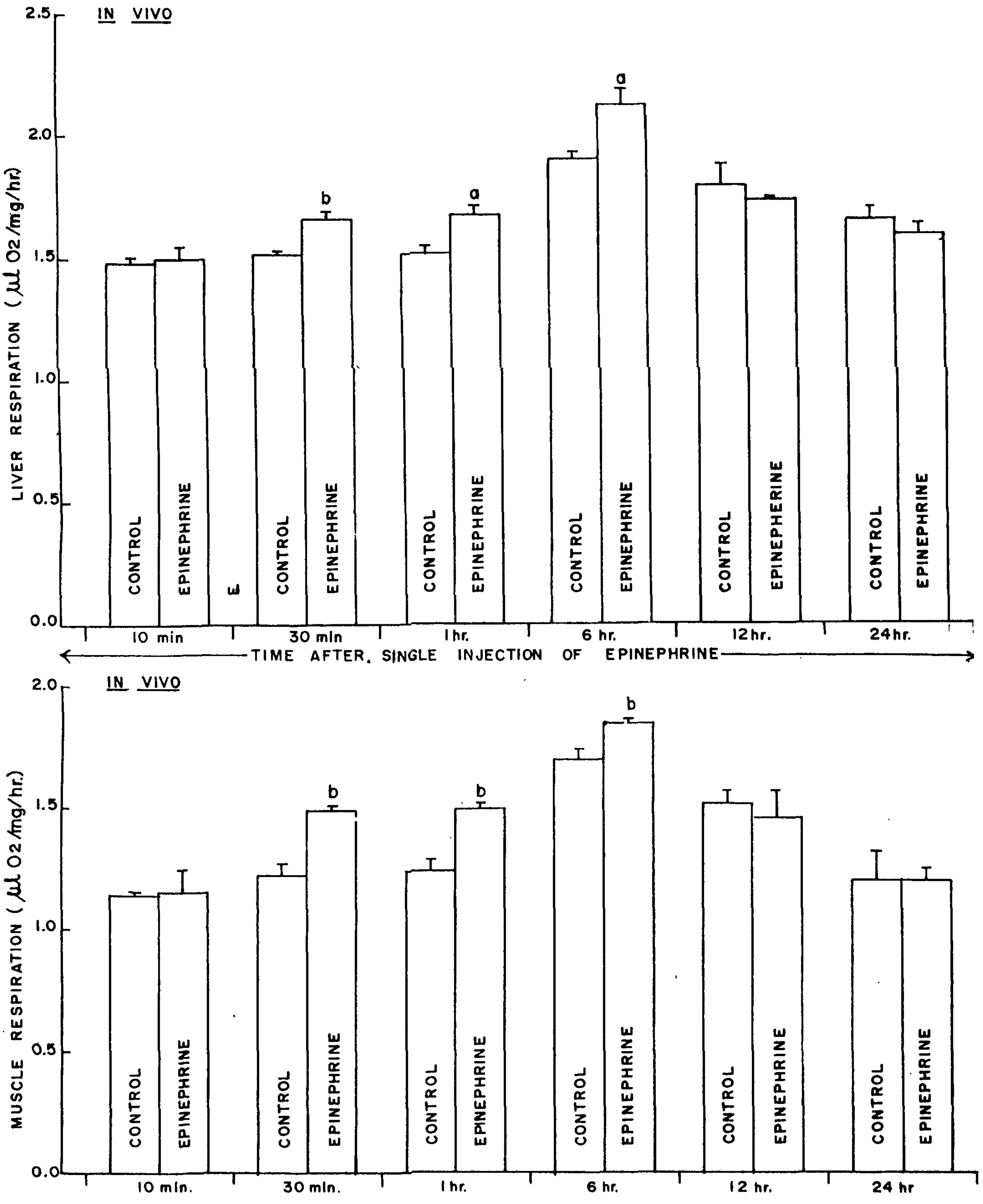
[a,b,c] Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.



Rana cyanophlyctis (Summer)

Fig. 36 : Time-dependent in vivo effects of epinephrine on the rate of tissues respiration of *Rana limnocharis* during Winter (Temperature : 4.6 – 14 °C)

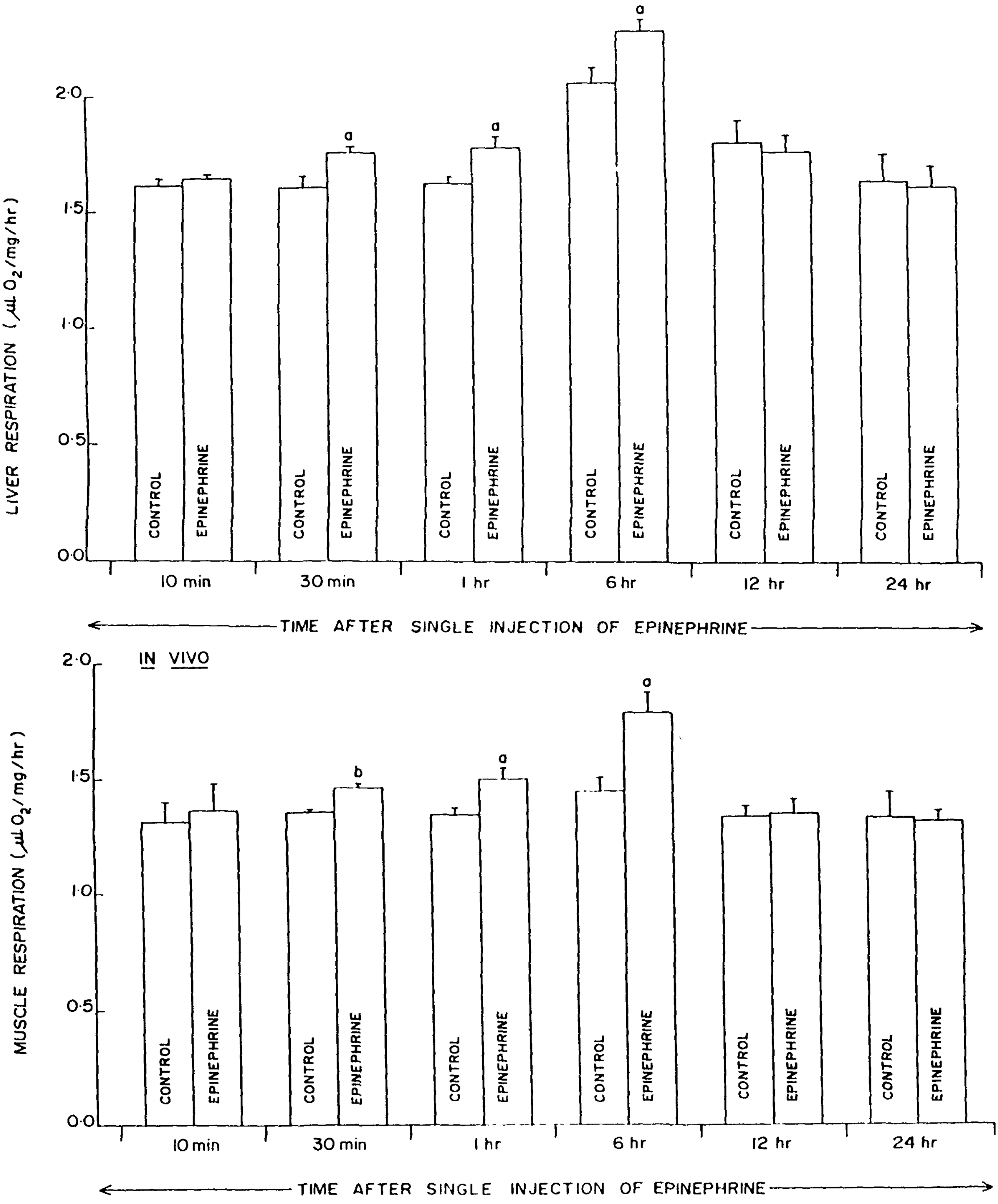
[a,b] Differ from the respective saline treated control group : $P < 0.05$ and 0.01 respectively.



<u>Rana</u> <u>limnocharis</u> (Winter)

Fig. 37 : Time-dependent in vivo effects of epinephrine on the rate of tissues respiration of Rana cyanophlyctis during Winter (Temperature : 4.6 - 14 °C)

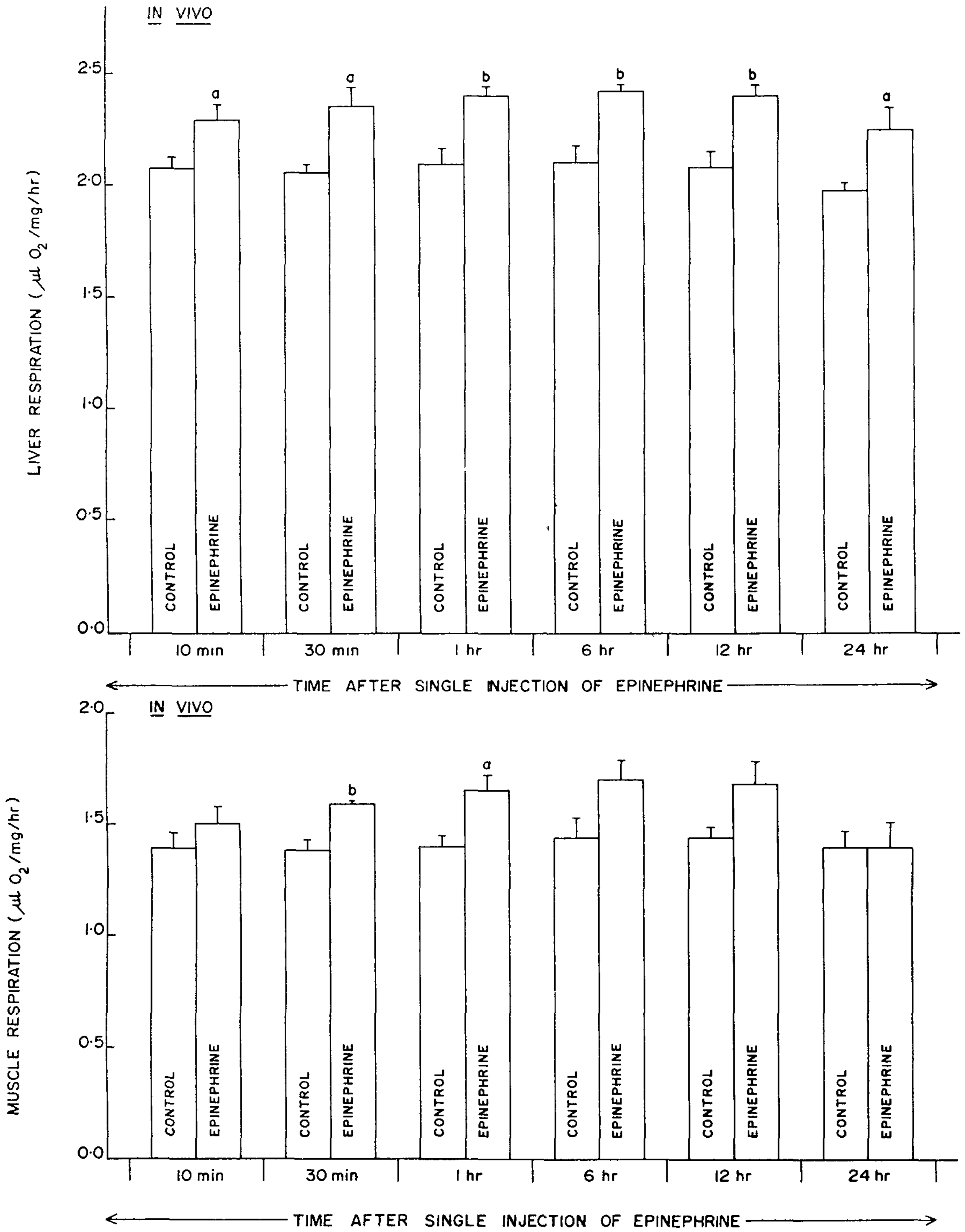
^{a,b}Differs from the respective saline treated control group : $P < 0.05, < 0.01$.



Rana Cyanophlyctis (Winter)

Fig. 38 : Time-dependent in vivo effects of epinephrine on the rate of tissues respiration of *Rana limnocharis* during Summer (Temperature : 14 → 20 °C)

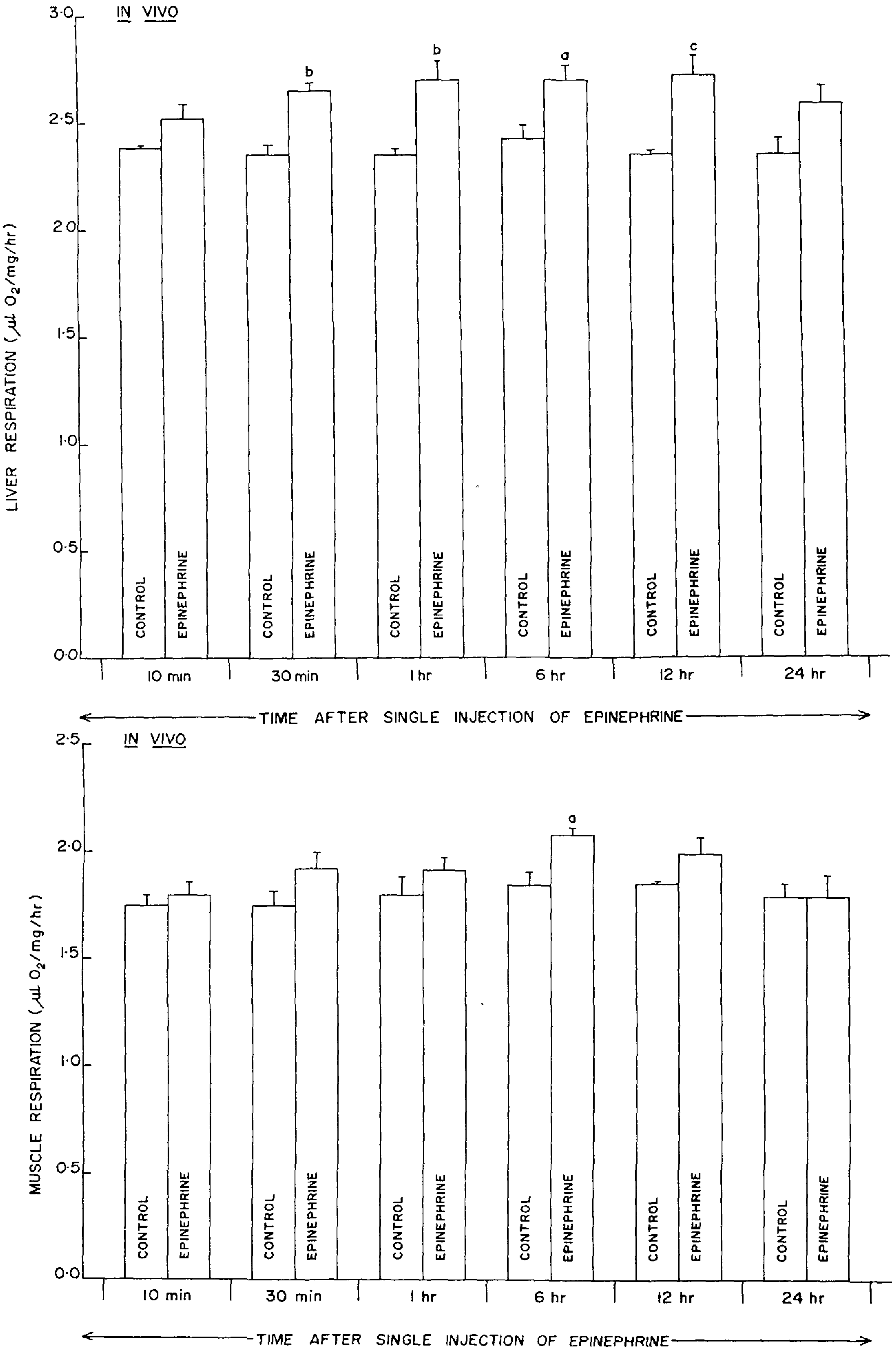
^{a,b} Differ from the respective saline treated group : $P < 0.05$ and 0.01 respectively.



Rana limnocharis (Summer)

Fig. 39 : Time-dependent *in vivo* effects of epinephrine on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature : 16 - 24 °C)

^{a,b,c} Differ from the respective saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

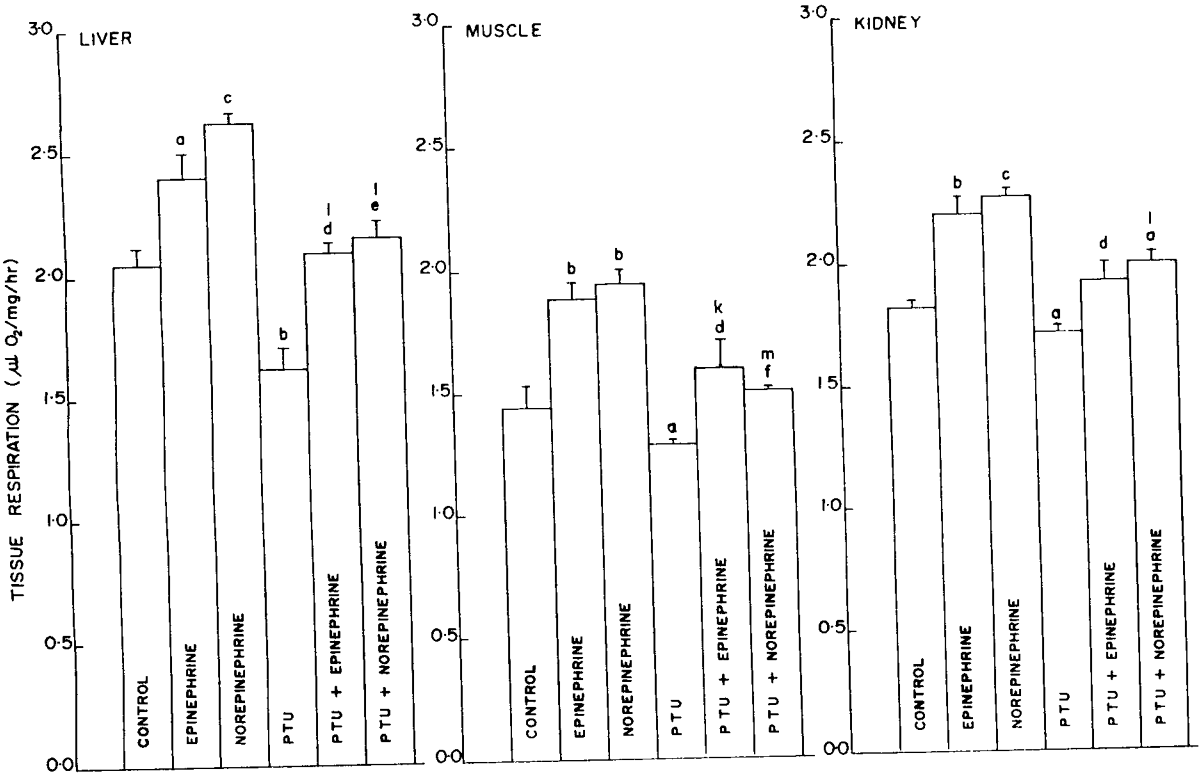


Rana Cyanophlyctis (Summer)

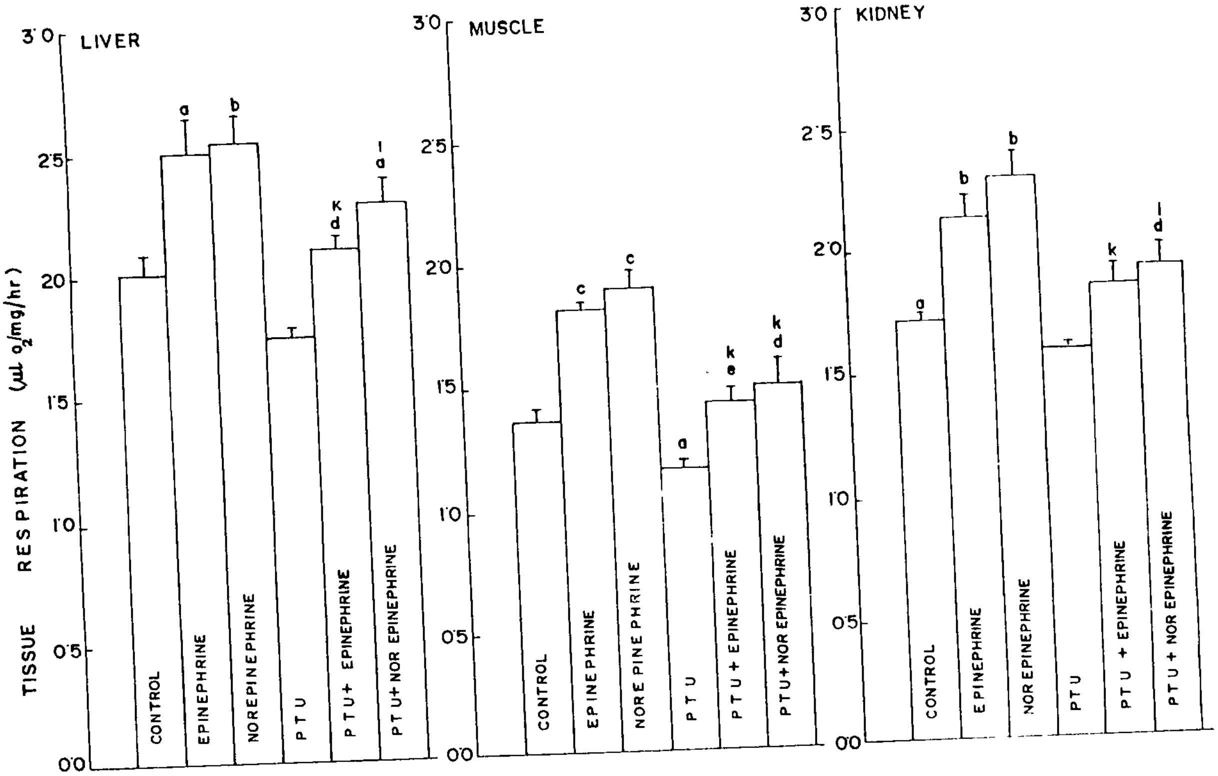
Fig. 40 : In vivo effects of epinephrine, norepinephrine and propyl thiouracil (PTU) on the rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during Summer (Temperature : 12 - 21 °C).

[a,b,c] Differ from saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

[d,e] Differ from Epinephrine/norepinephrine group : $P < 0.05$ and 0.01 respectively.



Rana limnocharis



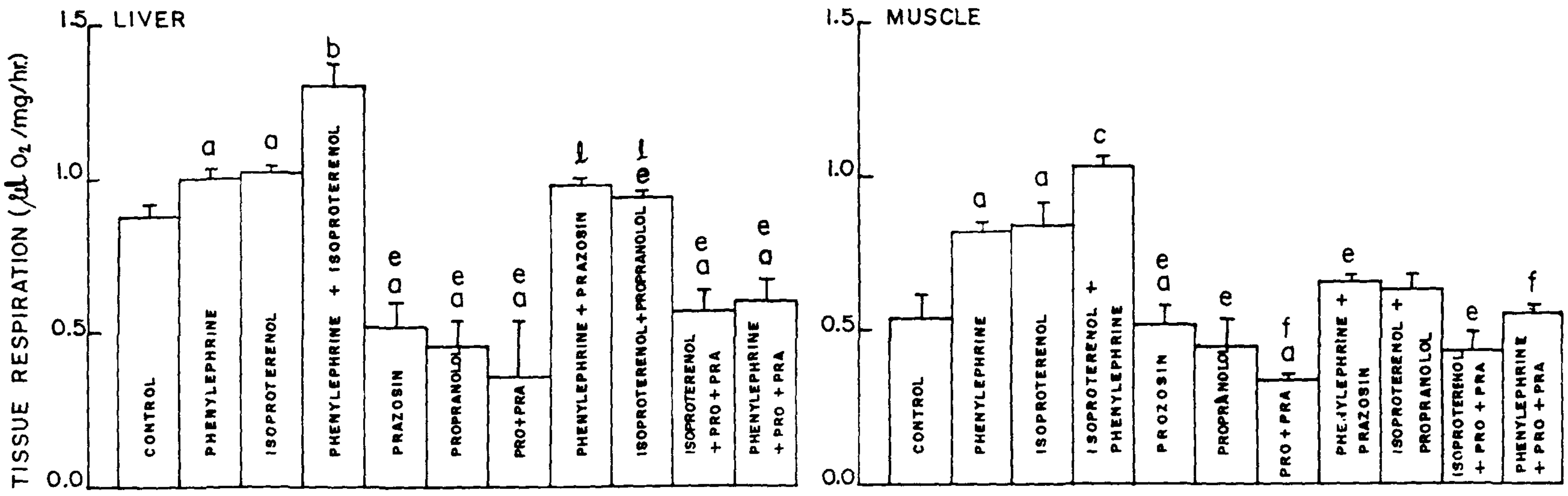
Rana cyanophlyctis
(Summer)

Fig. 41 : In vitro effects of a- and β-adrenergic agonists and antagonists on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 11 - 23 °C)

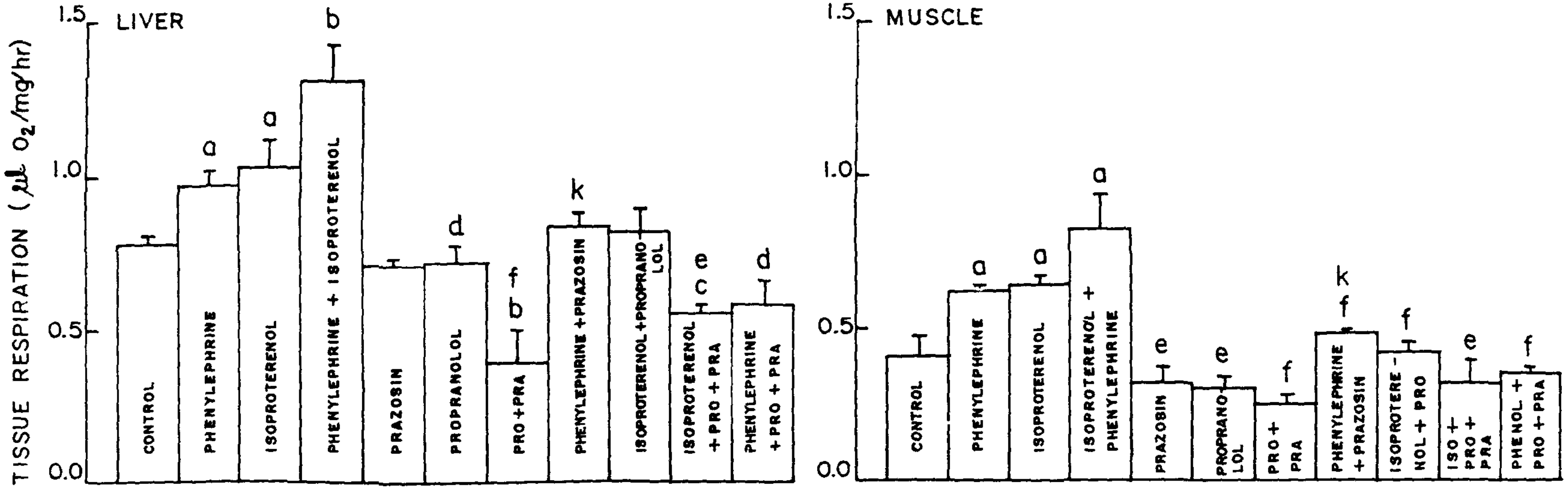
[a,b,c] Differ from the saline treated group : $P < 0.05$, 0.01 and 0.001 respectively.

[d,e,f] Differ from respective Isoproterenol or Phenylephrine group : $P < 0.05$, 0.01 and 0.001 respectively.

[k,l] differ from respective antagonist (Prazosin or Propranolol) group : $P < 0.05$ and 0.01 respectively.



Rana limnocharis
(Summer)



Rana cyanophlyctis
(Summer)

Fig. 42 : In vitro effects of catecholamines and adrenergic antagonists on the rate of tissues respiration of *Rana cyanophlyctis* during Summer (Temperature : 12 - 22 °C)

[a,b,c] Differ from the saline treated group : $P < 0.05$, 0.01 and 0.001 respectively.

[d,e,f] Differ respectively from Epinephrine or norepinephrine group : $P < 0.05$, 0.01 and 0.001 respectively.

[k,l] Differ from respective Propranolol or Prazosin group : $P < 0.05$, and 0.01 respectively.

Rana cyanophlyctis

(Summer)

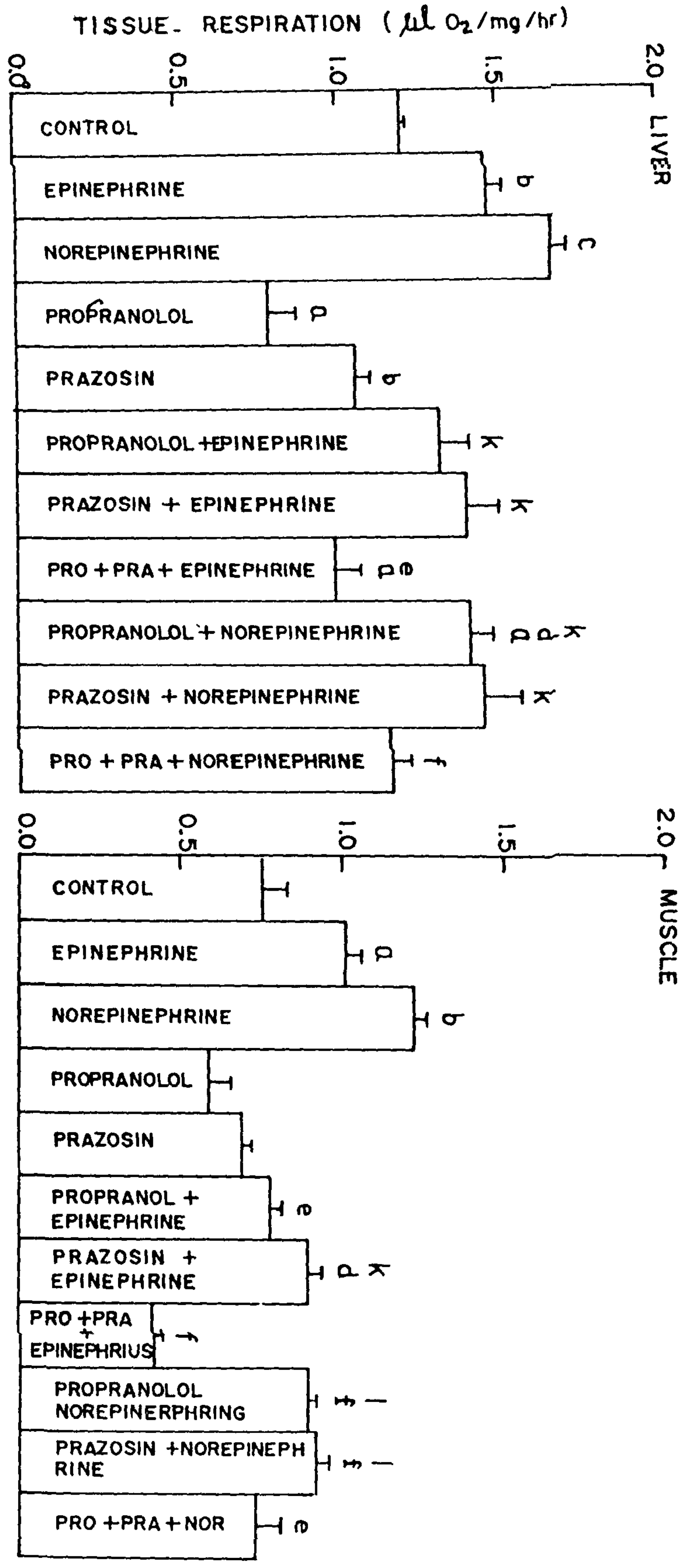


Fig. 43: In vivo effects of corticosterone and cortisol on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Winter (Temperature : 6 - 14 °C)

[a,b,c] Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.

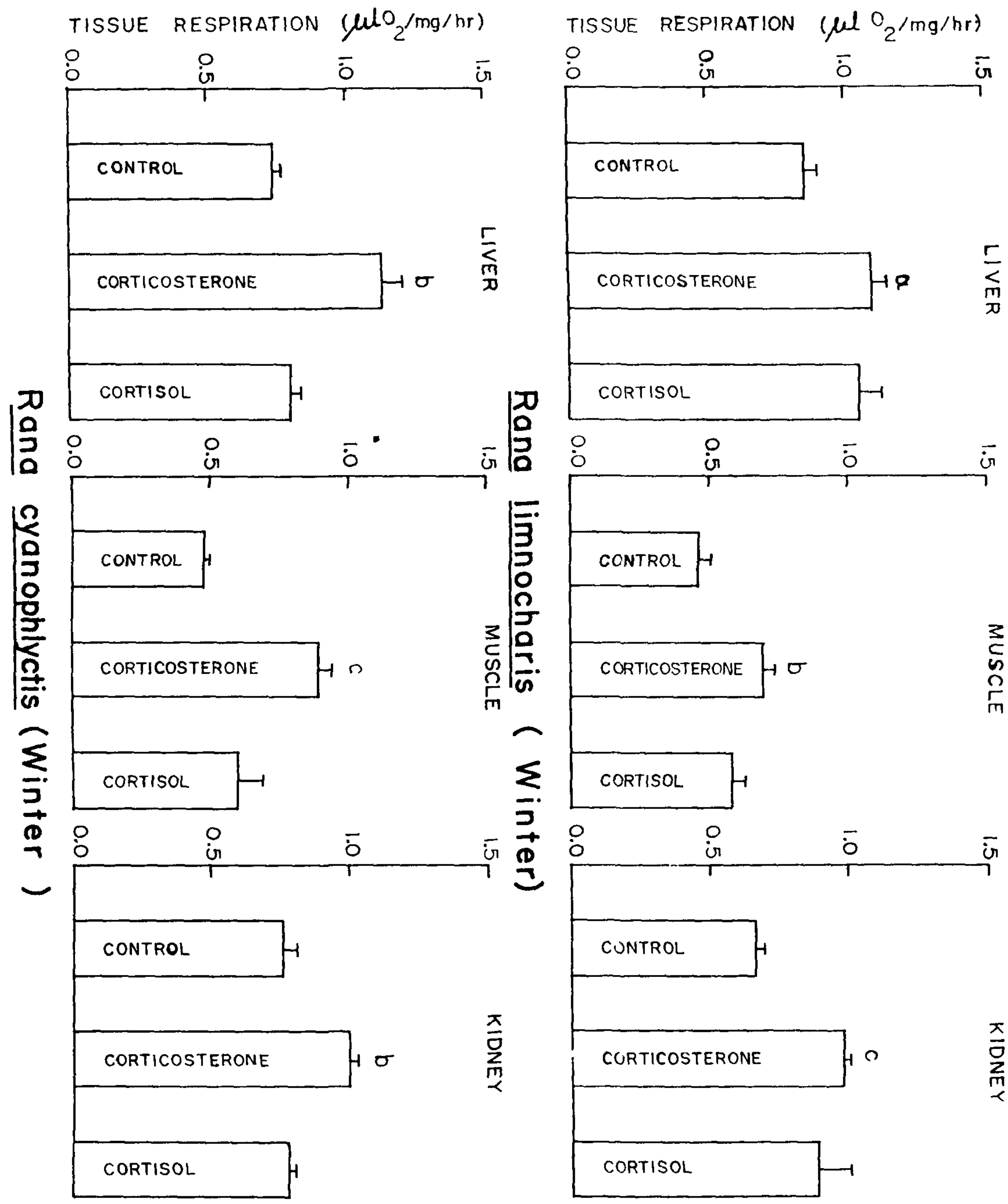
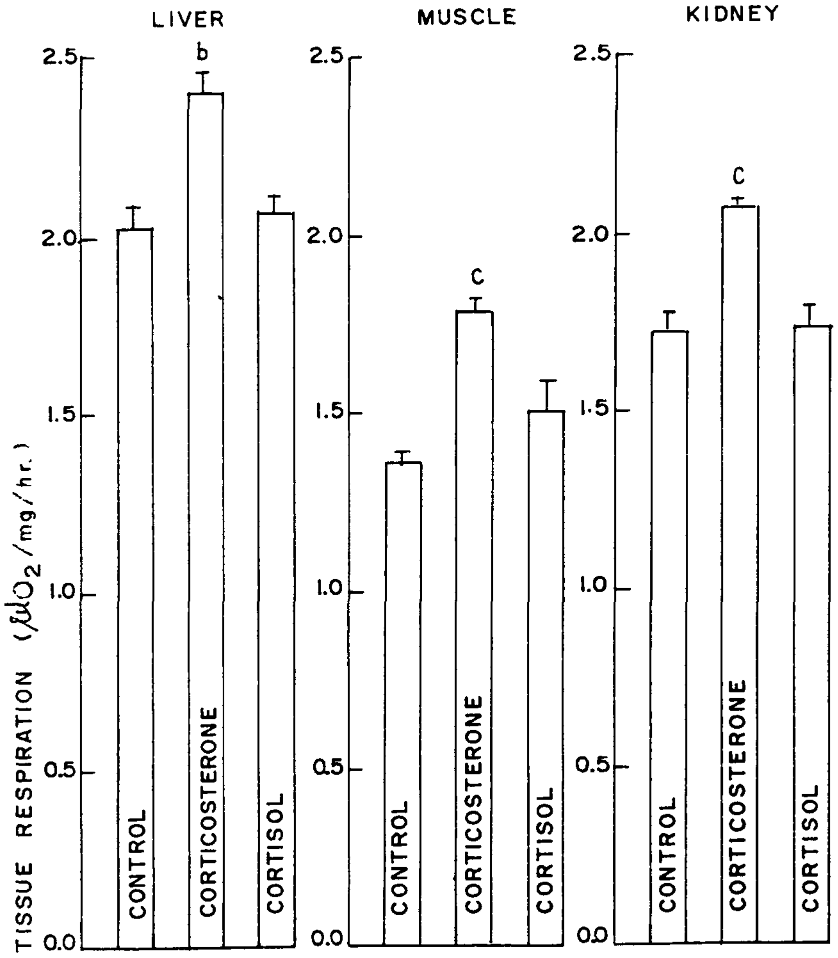
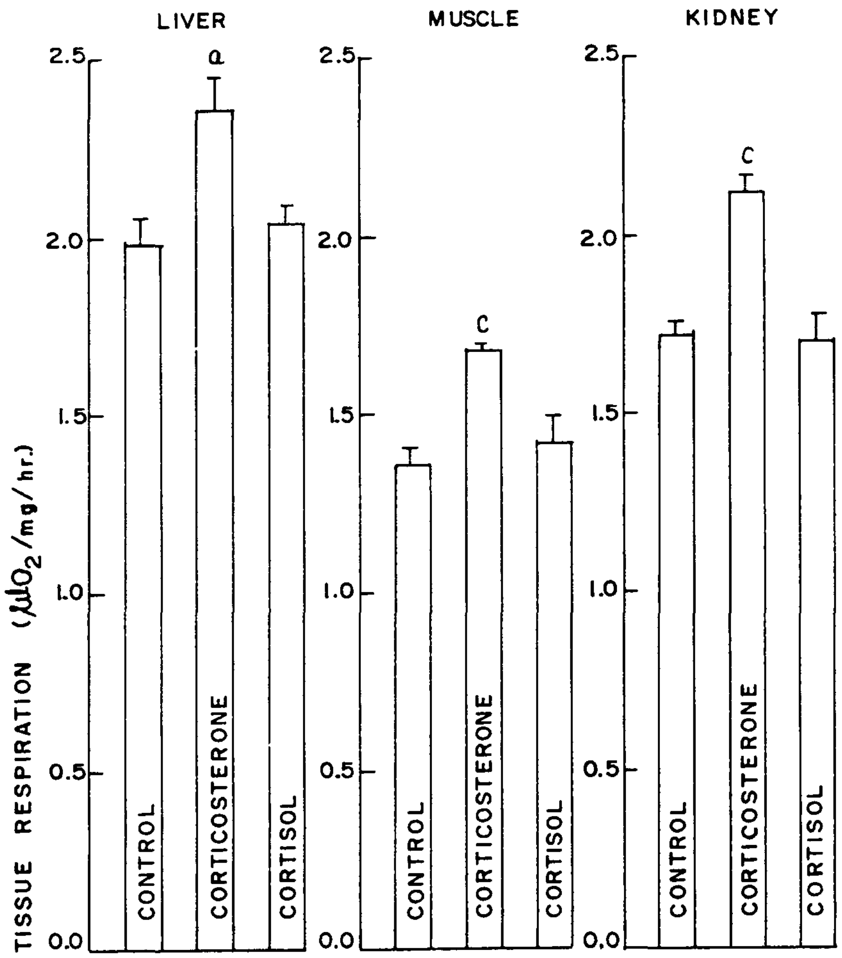

Rana limnocharis (Winter)
TISSUE RESPIRATION (μl O_2/mg/hr)
0.0
0.5
1.0
1.5
LIVER
CONTROL
CORTICOSTERONE
a
CORTISOL
MUSCLE
CONTROL
CORTICOSTERONE
b
CORTISOL
KIDNEY
CONTROL
CORTICOSTERONE
c
CORTISOL
Rana cyanophlyctis (Winter)
TISSUE RESPIRATION (μl O_2/mg/hr)
0.0
0.5
1.0
1.5
LIVER
CONTROL
CORTICOSTERONE
b
CORTISOL
MUSCLE
CONTROL
CORTICOSTERONE
c
CORTISOL
KIDNEY
CONTROL
CORTICOSTERONE
b
CORTISOL

Fig. 44 : In vivo effects of corticosterone and cortisol on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

[a,b,c] Differ from the saline treated control group $P < 0.05$, 0.01 and 0.001 respectively.



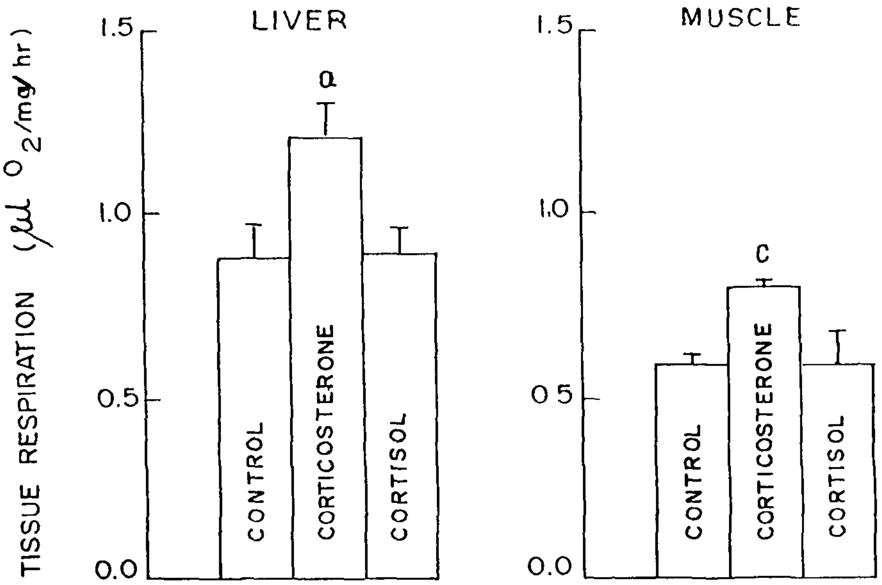
Rana limnocharis (Summer)



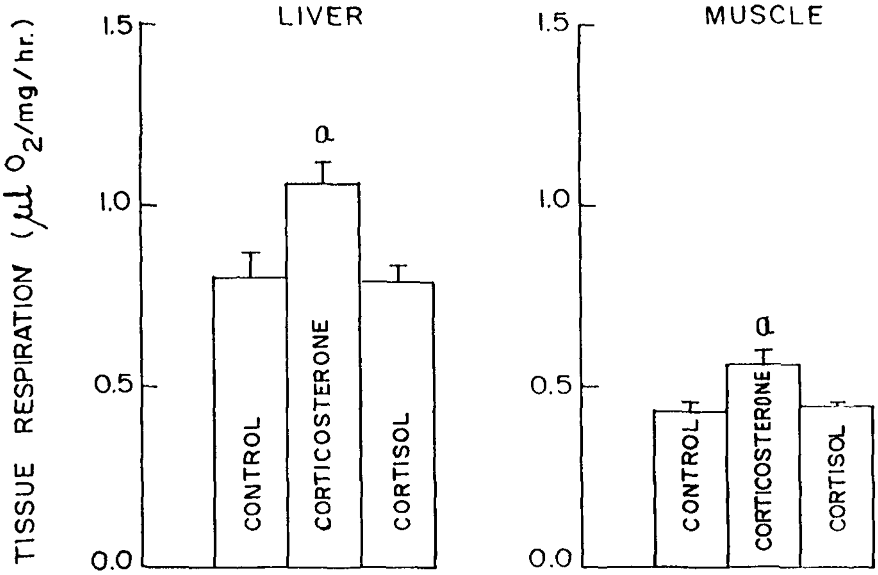
Rana cyanophlyctis (Summer)

Fig. 45 : In vitro effects of corticosterone and cortisol on the rate of tissues respiration of *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 - 14 ^{o}C)

^{a,c}Differ from the saline treated control group : $P < 0.05$ and 0.001 respectively.



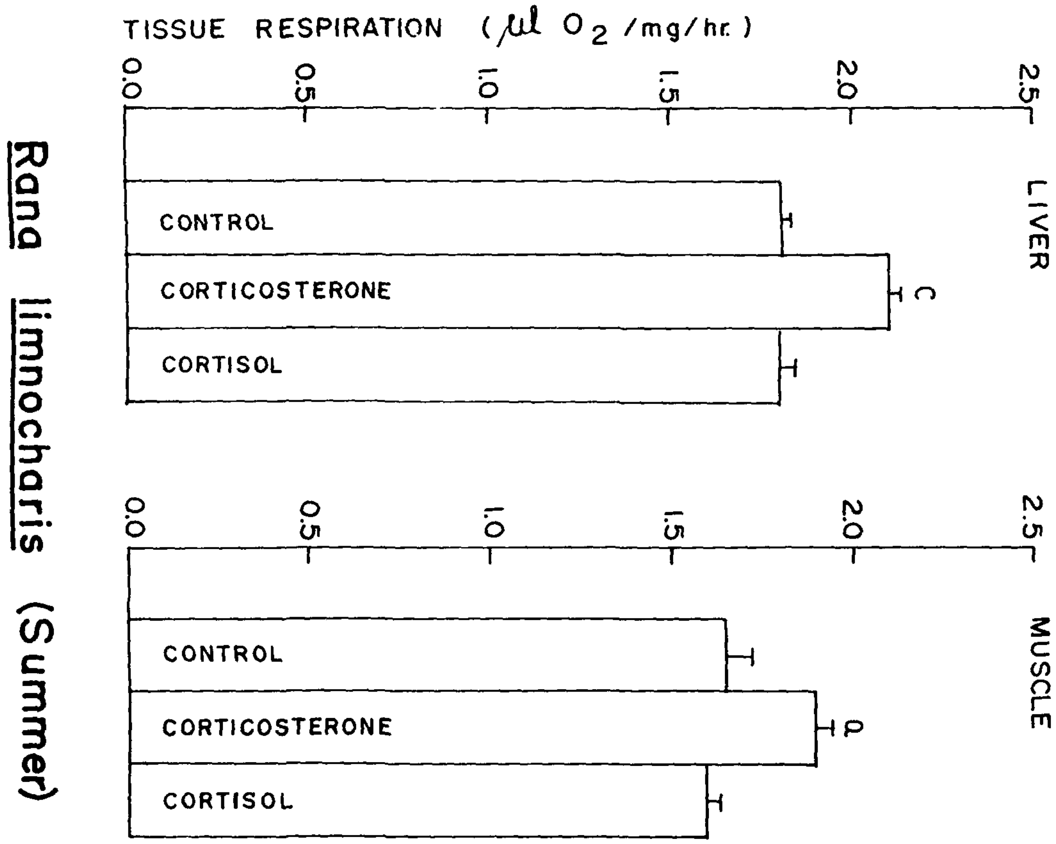
Rana limnocharis (Winter)



Rana cyanophlyctis (Winter)

Fig. 46 : In vitro effects of corticosterone and cortisol on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature : 12 - 20 °C)

^{a,b,c} Differ from the saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.


TISSUE RESPIRATION (μl O2 /mg/hr.)
0.0
0.5
1.0
1.5
2.0
2.5
LIVER
CONTROL
CORTICOSTERONE
c
CORTISOL
MUSCLE
CONTROL
CORTICOSTERONE
a
CORTISOL
Rana limnocharis (Summer)

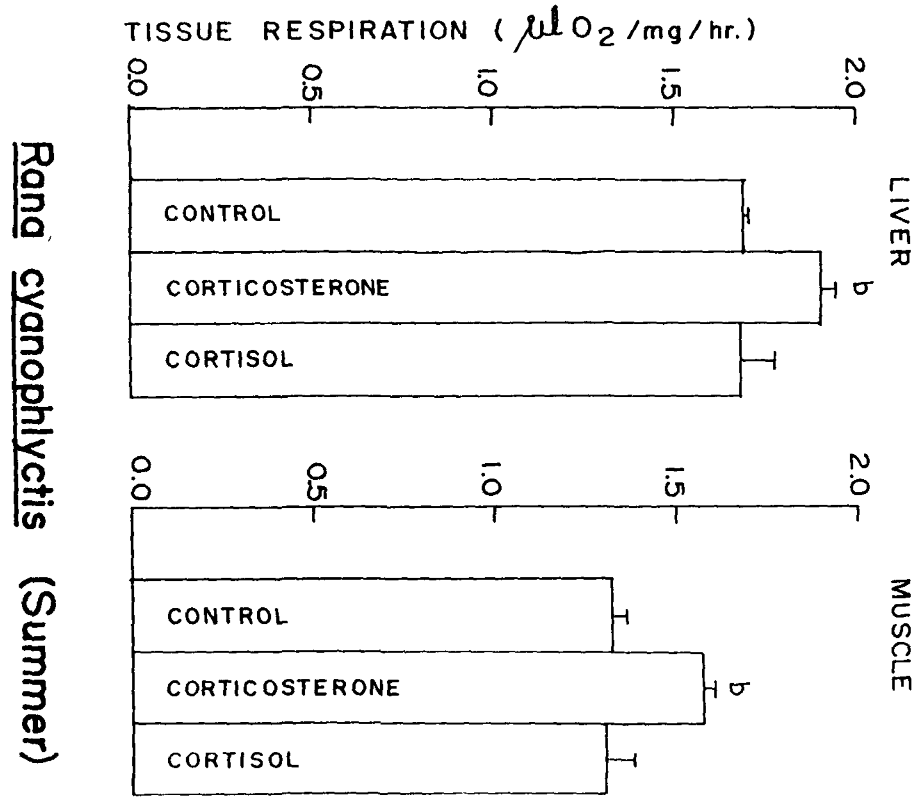
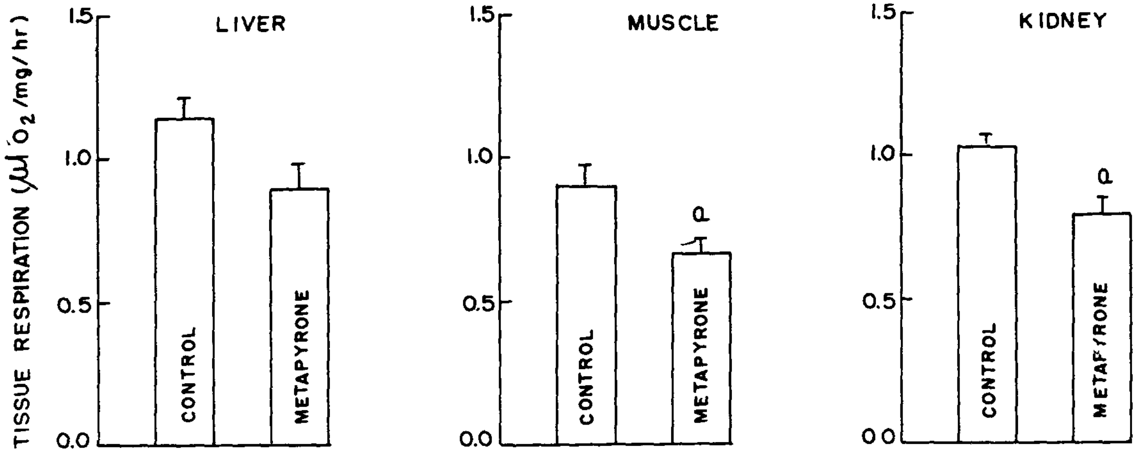
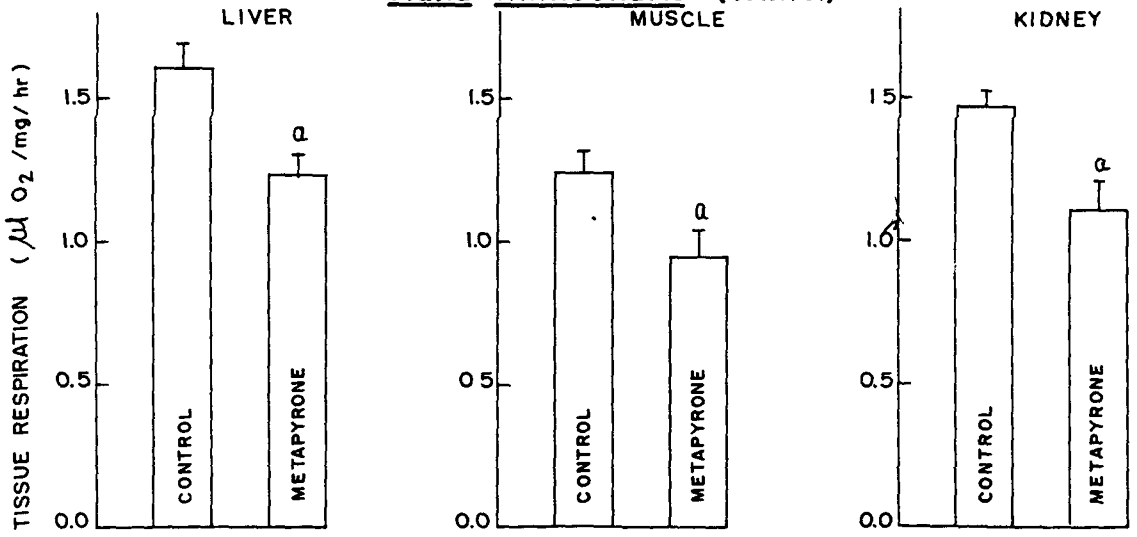

TISSUE RESPIRATION (μl O2 /mg/hr.)
0.0
0.5
1.0
1.5
2.0
LIVER
CONTROL
CORTICOSTERONE
b
CORTISOL
MUSCLE
CONTROL
CORTICOSTERONE
b
CORTISOL
Rana cyanophlyctis (Summer)

Fig. 47 : In vivo effects of metapyrone on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 7 -15 °C)

[a] Differs from oil treated control group : $P < 0.05$.



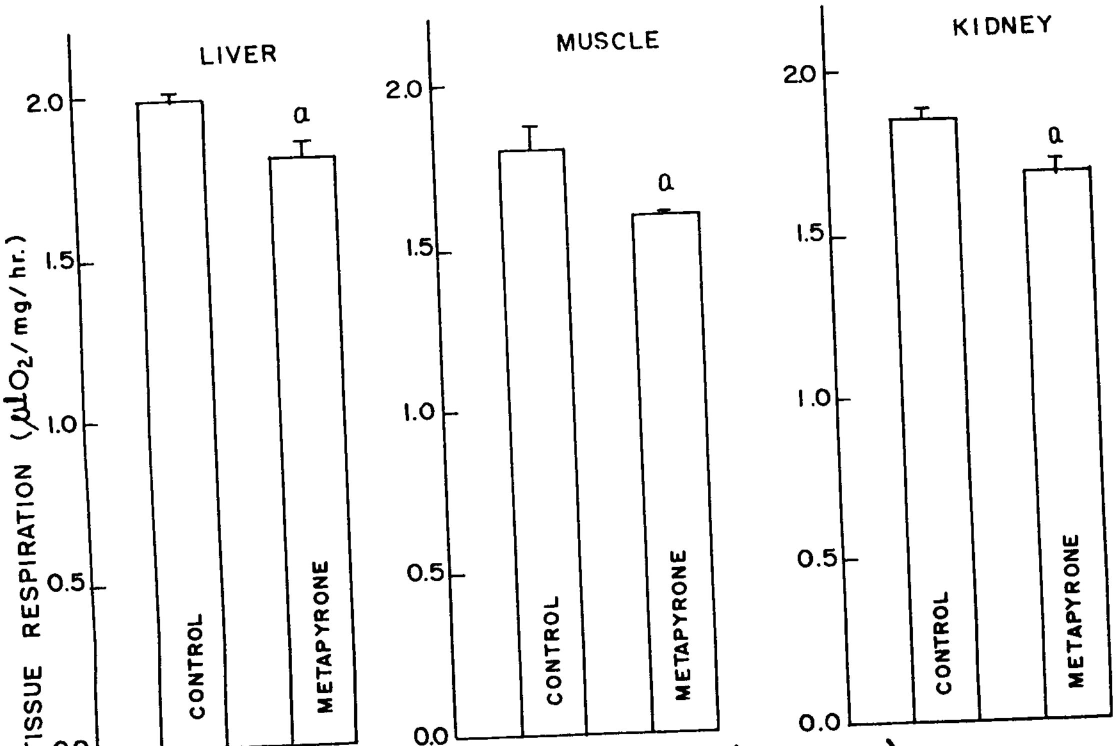
Rana limnocharis (Winter)



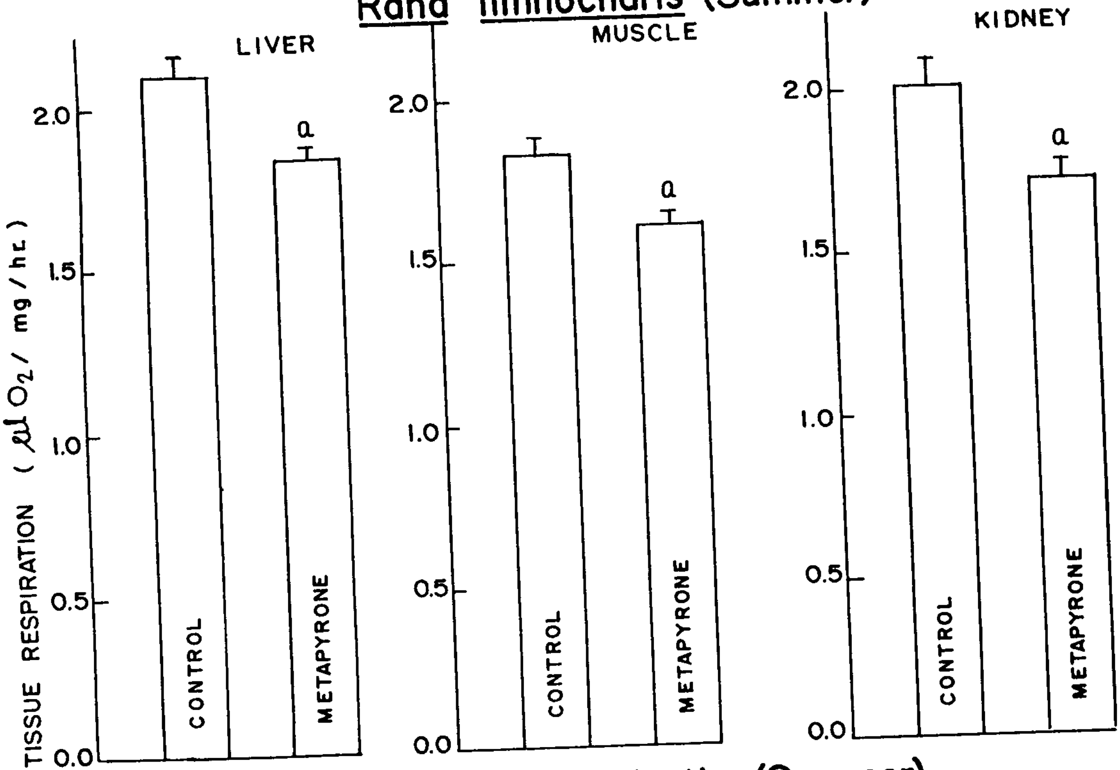
Rana cyanophlyctis (Winter)

Fig. 48 : In vivo effects of metapyrone on the rate of tissues respiration in <u>Rana limnocharis</u> and <u>Rana cyanophlyctis</u> during Summer (Temperature : 14 - 24 °C)

[a] Differs from the Oil treated control group : $P < 0.05$.



Rana limnocharis (Summer)



Rana cyanophlyctis (Summer)

CHAPTER 6

Chapter 6

Effects of Melatonin on the Oxidative Metabolism in *Rana limnocharis* and *Rana cyanophlyctis*

Introduction

In lower vertebrates, the pineal complex acts as a photoreceptor organ as well as an endocrine gland (Vollrath, 1981). Photoreceptor function of the pineal complex might be helpful in synchronizing the feeding activity and breeding phase. Recent reports suggest that the pineal hormone (melatonin) is involved in the process of thermoregulation in mammals and birds (Heldmaier, *et al.*, 1989). Melatonin is reported to increase non-shivering thermogenesis and to improve the thermogenic capacity of homeotherms. There are a few reports on temperate reptilian species which suggest a role of the pineal complex in thermoregulation and energy metabolism (Hutchinson and Koch, 1974; Engbretson and Hutchinson, 1976; Bartholomew, 1982). The pineal complex is also reported to be involved in the lipid metabolism in a tropical/sub-tropical reptile (Halder-Misra *et al.*, 1984). Notwithstanding these reports, so far no attempt has been made to study the involvement of melatonin in the energy metabolism of any amphibian species (Gupta and Thapliyal, 1991). Therefore, we decided to study the calorigenic action of melatonin in two species of frogs. The findings of the present study suggest that melatonin might be involved in the energy metabolism of amphibian species.

Materials and Methods

All experiments were conducted on adult male *Rana limnocharis* (Body weight : 8-10 g) and *Rana cyanophlyctis* (Body weight: 10-12 g) which were captured locally. *In vivo* and *in vitro* experiments were conducted during winter and summer/rainy months on both the species until/unless specified. For *in vivo* experiments, frogs were maintained under natural climatic conditions in plastic cages and acclimatized for 10 days before the treatments were started. For *in vitro* experiments frogs were killed immediately after collection from the nature. The rate of tissues respiration was measured with the help of an Oxygen electrode (for details, please see "Chapter I"). The details of experimental protocol are given below.

Experimental Protocol:

Expt. No	Treatment(s)	In vivo/vitro	Month (Temperature)	Dose	Duration
1.	Control	In vivo	January (6-14 ^{0}C)		
	Melatonin			2 and 5 µg/f/Day	4 days
	-do-		July (12-20 ^{0}C)		
2.	Control	In vivo	July (12-20 ^{0}C)		
	L-T$_3$			2 µg/f/day	4 days
	Melatonin			-do-	-do-
	L-T$_3$ + Melatonin			2 µg + 2 µg/f/day	-do-

Twenty-four hours after the last *in vivo* injection, control and treated frogs were decapitated and tissues (liver, skeletal muscle and kidney) were rapidly removed, rinsed in ice-cold frog Ringer's solution and stored in a refrigerator. The rate of tissue respiration was measured with the help of an oxygen electrode (for details, please see "Chapter 1"). The data were analyzed with the help of student's "t" test (Snedecor, 1961).

Results:

In vivo effects of melatonin:

In vivo administration of melatonin significantly increased the respiratory rate of tissues (liver, skeletal muscle and kidney) in a dose dependent way in both *Rana limnocharis* and *Rana cyanophlyctis* during winter and summer/rainy seasons(Tabs. 49 & 50 ; Figs. 49 & 50). Administration of L-T$_3$ had no effect on the melatonin-induced increase in the metabolic rate of tissues during summer/rainy seasons (Tabs. 49 & 50; Figs. 49 & 50).

Discussion

In the present study, melatonin increased the rate of tissues oxygen consumption in a dose-dependent way. It seems that alterations in the circulating level of melatonin may alter the energy metabolism. In lower vertebrates, temperature and photoperiod both are reportedly involved in the regulation of pineal activity (Vivien-Roels and Rivet, 1986). Therefore, temperature -induced alteration in the oxidative metabolism of frogs might be due to alterations in the circulating level of melatonin. L-T_3 did not alter the metabolic action of melatonin (Tab. 51; Fig 51.). Therefore, the calorigenic action of melatonin is not through the thyroid hormones in frogs. However, at present it is not known whether melatonin influences the metabolic rate directly or indirectly by potentiating the calorigenic action of catecholamines. In mammals also, it remains to be established whether melatonin affects the oxidative metabolism directly or potentiates the calorigenic action of catecholamines (McElroy *et al.*, 1986; Vishwanathan *et al.*, 1986; Heldmeir *et al.*, 1989). Since, melatonin is reported to influence lipid metabolism in reptiles, melatonin might be directly involved in the stimulation of the oxidative metabolism. However, this suggestion remains to be supported by *in vitro* experiments. Further, in the present study pharmacological doses of melatonin were used. Therefore, it is difficult to assess the physiological importance of melatonin in the oxidative metabolism of frogs. This study forms a base for further study on the role of melatonin in the regulation of the oxidative metabolism of amphibians and other lower vertebrates. The peak level of melatonin is recorded during the mid-night hours which are generally associated with the lowest temperature. Therefore, it is possible that the mid-night increase in melatonin might be of great importance for maintaining the basal metabolic rate of frogs at the lowest temperature of nights. If so, as the catecholamines, the calorigenic action of melatonin may be proved to be of great adaptational significance in frogs.

* * * * * * *

Table 49 : Dose-dependent In vivo effects of melatonin on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Winter (Temperature : 6 - 14 ^{o}C)

Treatments	Tissue oxygen consumption (µl O$_2$/mg wet tissue/h)		
	Liver	Muscle	Kidney
Rana limnocharis			
Saline(Control)	0.86 ± 0.05[*]	0.47 ± 0.04	0.67 ± 0.03
Melatonin(2 µg)	1.50 ± 0.07[c]	1.20 ± 0.05[c]	1.31 ± 0.08[c]
Melatonin(5 µg)	2.01 ± 0.05[c,f]	1.71 ± 0.07[c,f]	1.81 ± 0.09[c,e]
Rana cyanophlyctis			
Saline(Control)	0.74 ± 0.03	0.48 ± 0.02	0.76 ± 0.05
Melatonin(2 µg)	1.32 ± 0.04[c]	1.04 ± 0.10[b]	1.41 ± 0.09[b]
Melatonin(5 µg)	1.78 ± 0.09[c,e]	1.40 ± 0.08[c,d]	1.56 ± 0.07[c,e]

[*] Mean ± Standard error; n = 4.

[b,c] Differ from saline treated control group: $P < 0.01$ and 0.001 respectively.

[d,e,f] Differ from melatonin (2 µg) group : $P < 0.05$, 0.01 and 0.001 respectively

Table 50 : In vivo effects of different doses of melatonin on the rate of tissues respiration in *Rana limnocharis* and *Rana cyanophlyctis* during Summer (Temperature : 12 - 20 °C)

Treatments	Tissue oxygen consumption(µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline(Control)	2.02 ± 0.07*	1.36 ± 0.03	1.73 ± 0.05
Melatonin(2 µg)	3.01 ± 0.10^{c}	1.60 ± 0.07^{a}	2.60 ± 0.07^{c}
Melatonin(5 µg)	3.30 ± 0.09^{c}	2.10 ± 0.03^{c,f}	3.02 ± 0.07^{c,e}
	Rana cyanophlyctis		
Saline(Control)	1.98 ± 0.07	1.36 ± 0.05	1.72 ± 0.04
Melatonin(2 µg)	2.40 ± 0.07^{b}	1.65 ± 0.05^{b}	2.11 ± 0.09^{b}
Melatonin(5 µg)	2.74 ± 0.03^{c,e}	1.81 ± 0.10^{b}	2.60 ± 0.13^{c,d}

* Mean ±Standard error; n = 4.

^{b,c} Differ from saline treated control group : $P < 0.01$ and 0.001 respectively.

^{d,e} Differ from melatonin(2 µg) group : $P < 0.05$ and 0.01 respectively.

Table 51 : In vivo effects of T_3 and melatonin on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanoplyctis_ during Summer (Temperature : 12 - 20 °C)

Treatments	Tissue oxygen consumption (µl O_2/mg wet tissue/h)		
	Liver	Muscle	Kidney
	Rana limnocharis		
Saline (Control)	2.02 ± 0.07[*]	1.36 ± 0.03	1.73 ± 0.05
Melatonin	3.01 ± 0.10[c]	1.60 ± 0.07[a]	2.16 ± 0.07[b]
L-T3	2.08 ± 0.09	1.41 ± 0.10	1.77 ± 0.02
L-T3 + Melatonin	3.10 ± 0.09[c]	1.69 ± 0.03[c]	2.29 ± 0.09[b]
	Rana cyanophlyctis		
Saline(Control)	1.98 ± 0.07	1.36 ± 0.05	1.72 ± 0.04
Melatonin	2.40 ± 0.07[b]	1.65 ± 0.05[b]	2.11 ± 0.09[b]
l-T3	2.09 ± 0.02	1.50 ± 0.04	1.94 ± 0.07[a]
l-T3 + Melatonin	2.54 ± 0.09[b]	1.76 ± 0.05[b]	2.42 ± 0.11[c]

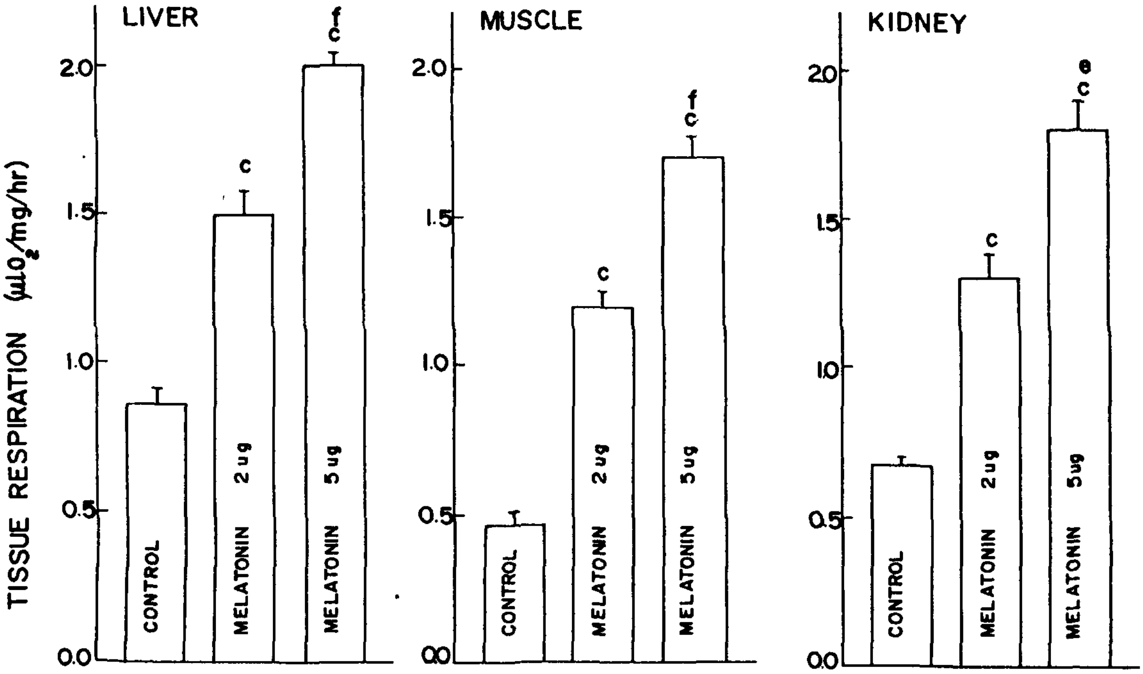
[*] Mean ± Standard error; n = 4.

[a,b,c] Differ from saline treated control group : P < 0.05, 0.01 and 0.001 respectively.

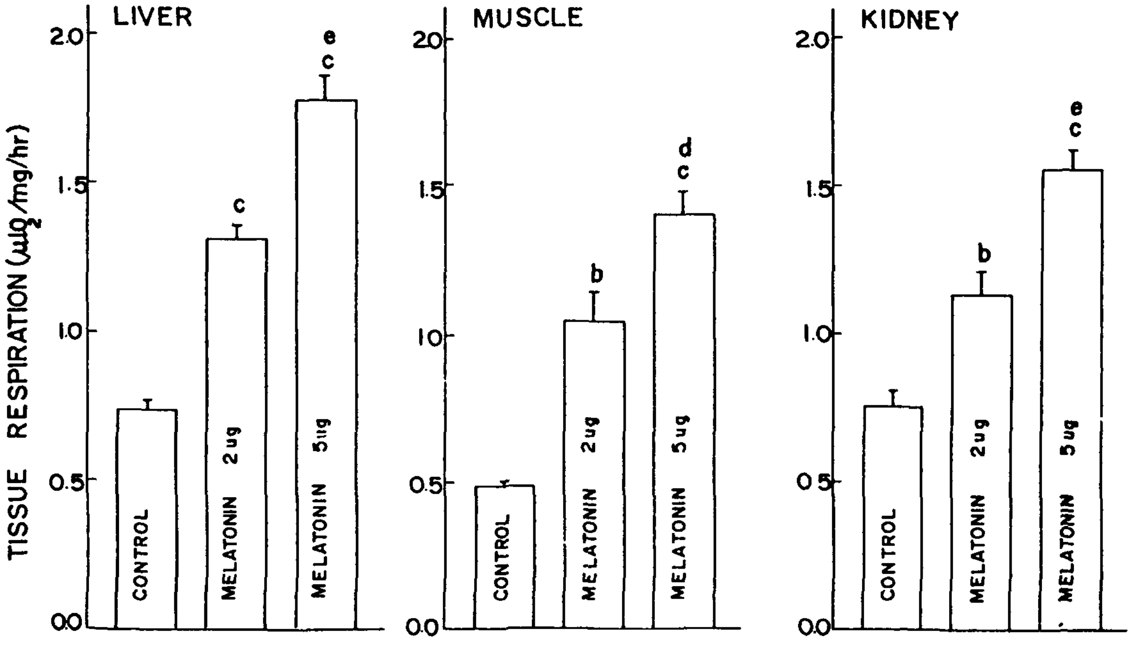
Fig. 49 : Dose-dependent In vivo effects of melatonin on the rate of tissues respiration in Rana limnocharis and Rana cyanophlyctis during Winter (Temperature : 6 - 14 °C)

[b,c] Differ from saline treated control group: $P < 0.01$ and 0.001 respectively.

[d,e,f] Differ from melatonin (2 µg) group : $P < 0.05$, 0.01 and 0.001 respectively



Rana limnocharis (Winter)

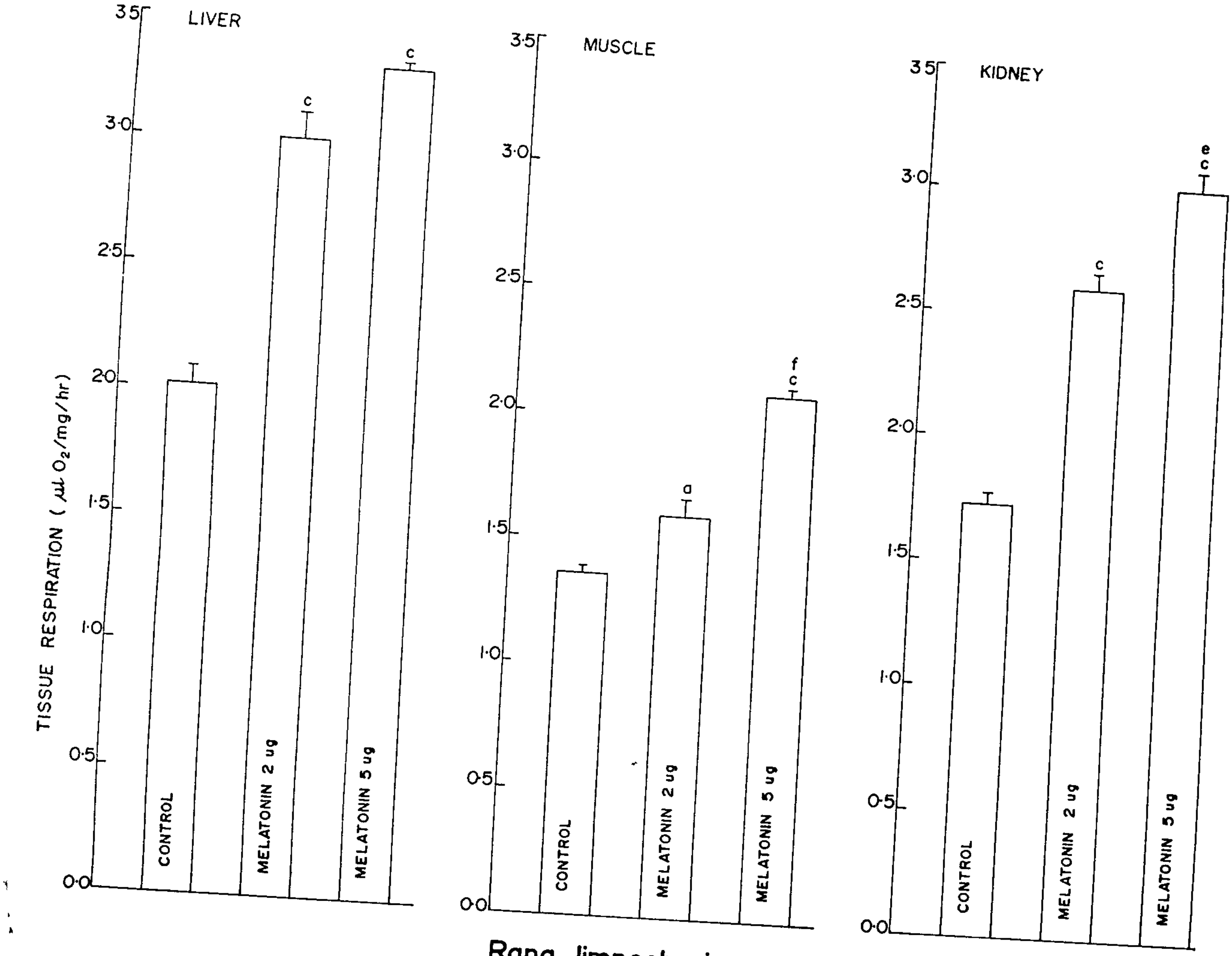


Rana cyanophlyctis (Winter)

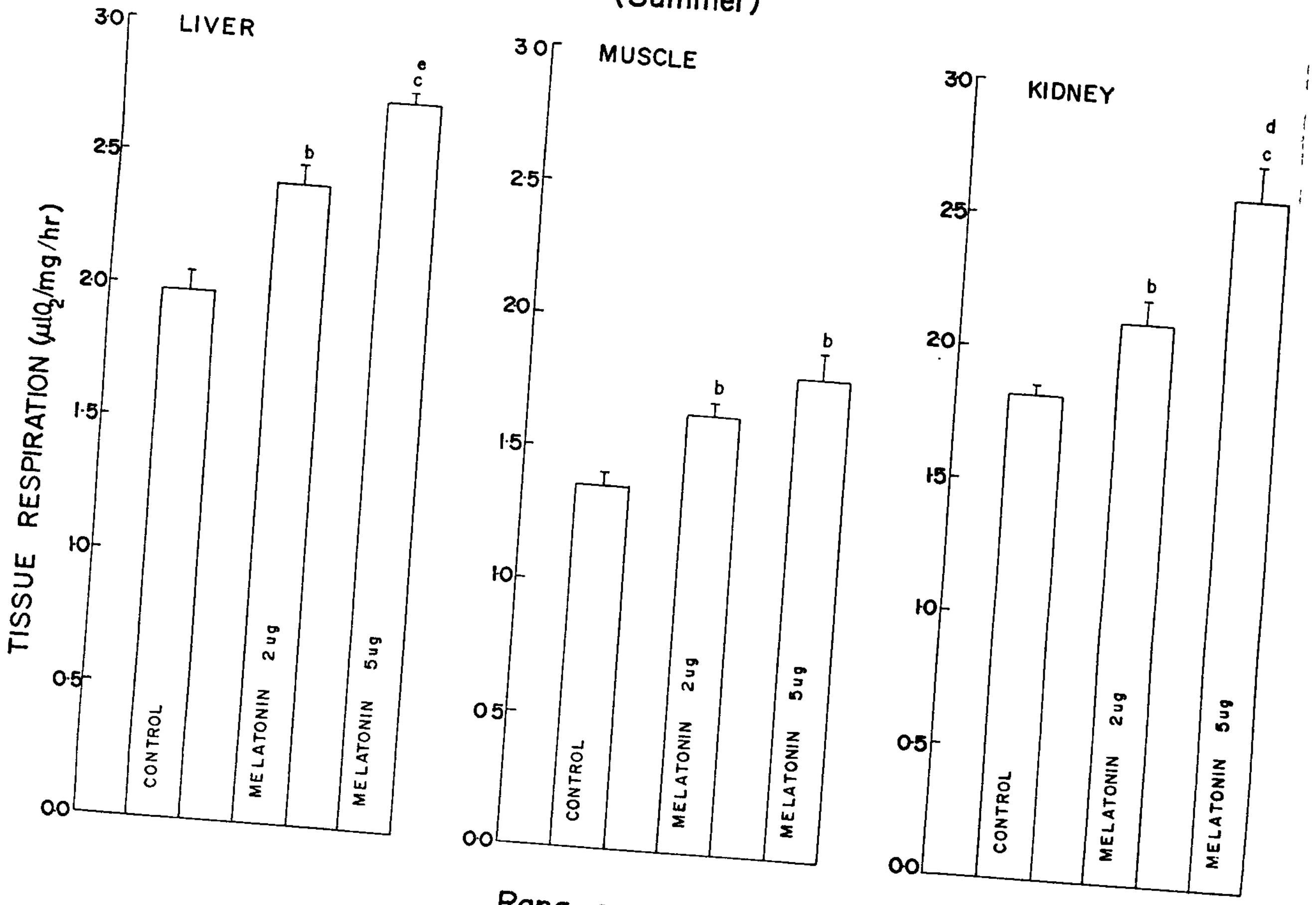
Fig. 50 : In vivo effects of different doses of melatonin on the rate of tissues respiration in _Rana limnocharis_ and _Rana cyanophlyctis_ during Summer (Temperature : 12 - 20 °C)

^{b,c}Differ from saline treated control group : $P < 0.01$ and 0.001 respectively.

^{d,e}Differ from melatonin(2 µg) group : $P < 0.05$ and 0.01 respectively.



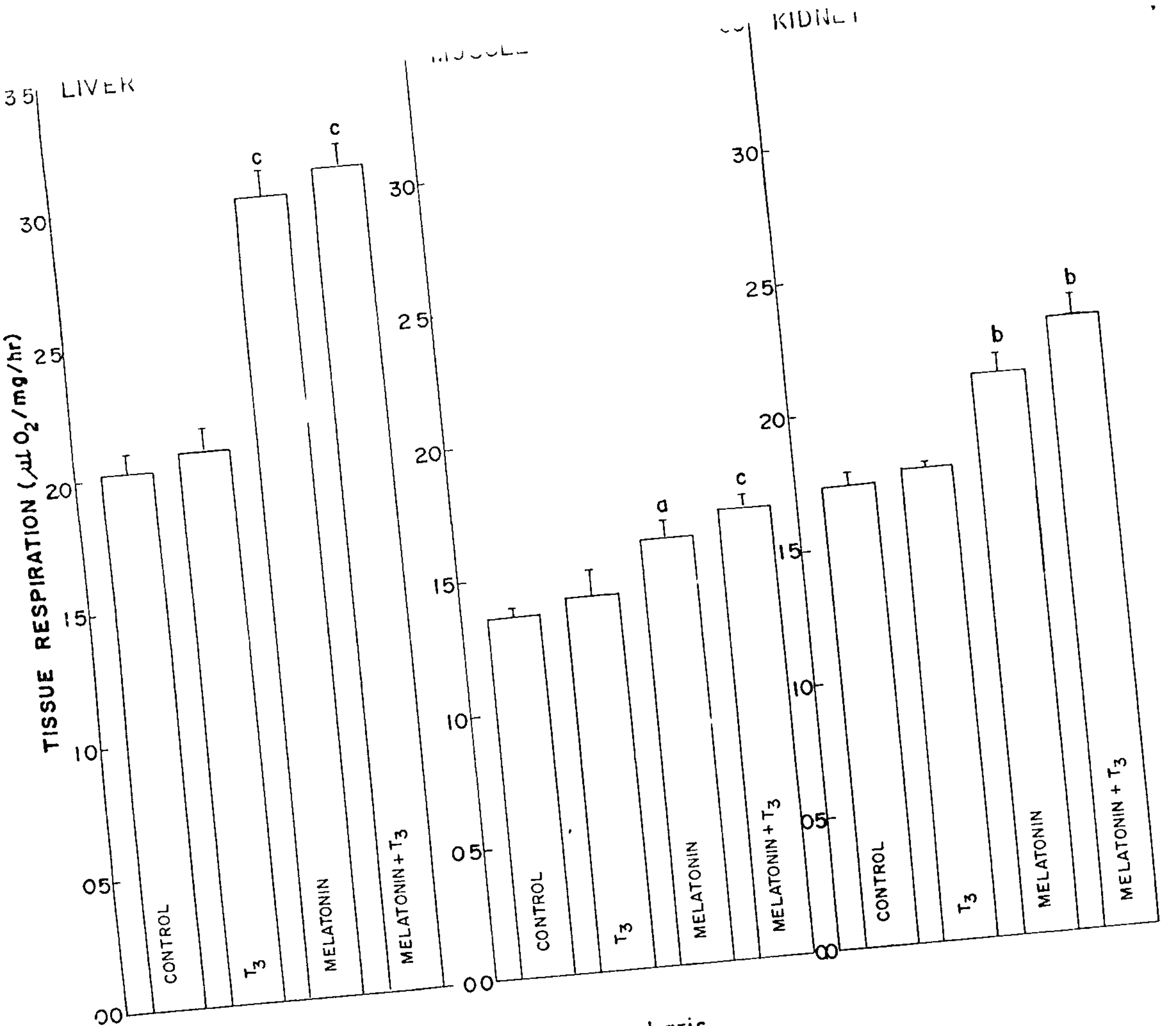
Rana limnocharis
(Summer)



Rana cyanophlyctis
(Summer)

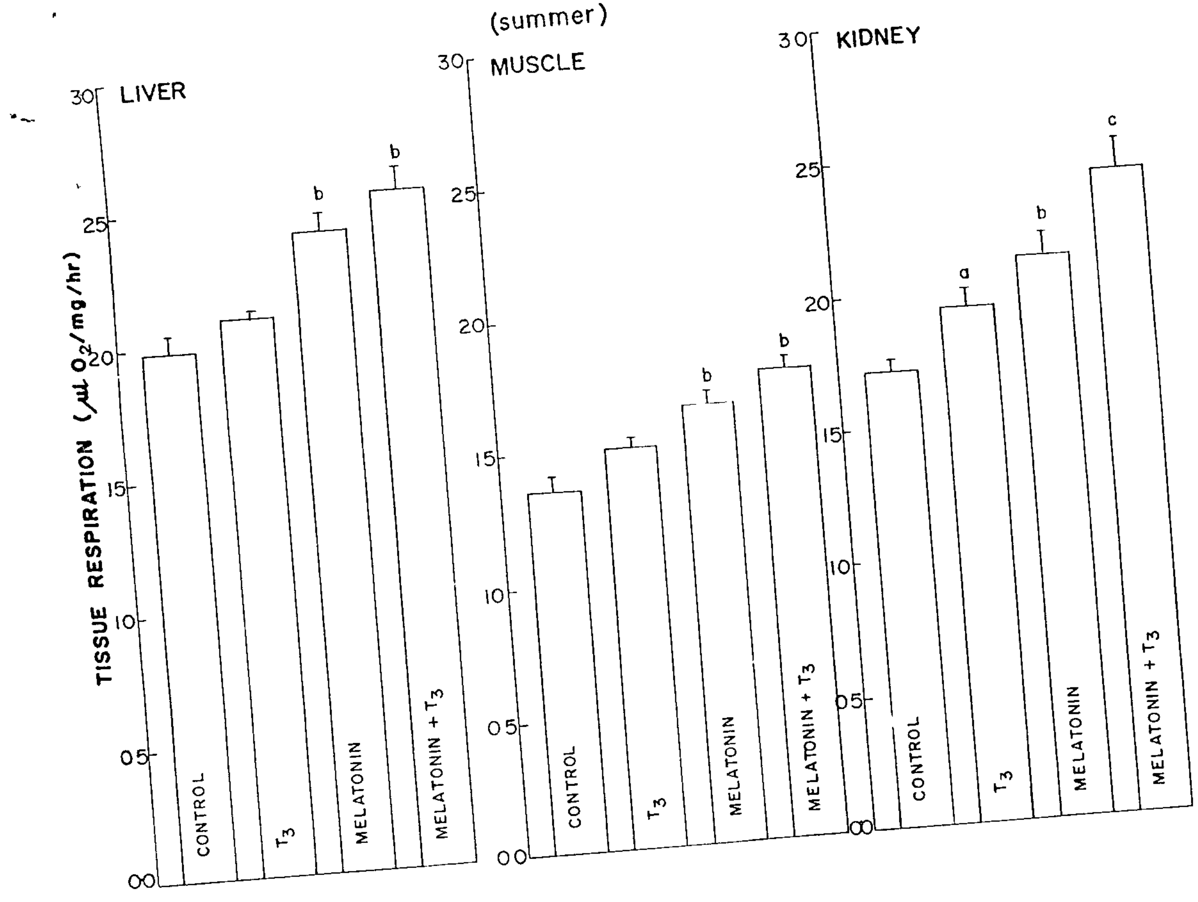
Fig. 51 : In vivo effects of T_3 and melatonin on the rate of tissues respiration of _Rana limnocharis_ and _Rana cyanoplyctis_ during Summer (Temperature : 12 - 20 °C)

[a,b,c] Differ from saline treated control group : $P < 0.05$, 0.01 and 0.001 respectively.



Rana limnocharis

(summer)



Rana cyanophlyctis

(Summer)

CHAPTER 7

Chapter 7

Summary and Conclusions

The Indian streaked frog, Rana limnocharis which is available in and around Shillong is a hibernating species, and the Indian skipper frog, Rana cyanophlyctis does not hibernate even at low temperature of winter months at Shillong. Very little is known about the involvement of hormones and their mechanism(s) of action in the control of metabolic rate of vital tissues like liver, skeletal muscle and kidney. Experiments were planned to investigate the relative roles of thyroidal, gonadal, adrenal and pineal hormones during winter and summer/rainy months in the regulation of the oxidative metabolism in Rana limnocharis and Rana cyanophlyctis.

The major findings and conclusions are given below:

Chapter 1 : Materials and Methods

This chapter deals with the details of the materials used, experimental conditions and methods used for maintaining the experimental animals, for conducting in vivo and in vitro experiments, and measurement of the rate of tissues oxygen consumption.

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

1. Both *Rana limnocharis* and *Rana cyanophlictis* exhibit annual variations in the rate of tissues oxygen consumptions.

2. During winter months,the average metabolic rate of tissues in *Rana cyanophlyctis* remained significantly higher than that of *Rana limnocharis*.

3. The average rate of oxygen consumption of all the tissues in *Rana limnocharis* was found to be significantly low during winter (hibernation phase) as compared to the summer/rainy months (active phase).

4. The average respiratory rate of muscle in *Rana cyanophlyctis* was significantly higher during summer/rainy seasons as compared to winter months but the average respiratory rate of kidney and liver did not change significantly between winter and summer/rainy months.

5. In both *Rana limnocharis* and *Rana cyanophlyctis*, the rate of liver oxygen consumption was always higher than that of muscle and kidney and the rate of muscle oxygen consumption was found to be always lower than that ofliver and kidney.

6. *Rana cyanophlyctis* feeds during winter months and the feeding status might be associated with the higher metabolic rate of tissues in *Rana cyanophlyctis* than *Rana limnocharis* which does not take food during winter months/hibernation.

7. The metabolic rate of muscle tissue seems to be associated with the activity level and/or temperature. However, the rate of oxygen consumption of liver seems to be associated with food intake.

8. Significant decrease in the rate of tissues respiration in *Rana limnocharis* during winter months seems to be directly related to its decreased physical activity (lethargy) and prolonged fasting.

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

1. L-T_3 and L-T_4 never stimulated the respiratory rate of tissues . in *Rana limnocharis* irrespective of ambient temperature.

2. In *Rana cyanophlyctis*, only *in vivo* administration of L-T_3 significantly increased the respiratory rate of muscle and

kidney during winter while both L-T$_3$ and L-T$_4$ stimulated only kidney respiratory rate during summer/rainy season.

3. *In vitro* treatments with L-T$_3$ and L-T$_4$ did not influence the tissue respiratory rate in *Rana limnocharis* and *Rana cyanophlyctis* irrespective of season and temperature.

4. *In vivo* stimulatory effect of L-T$_3$ and L-T$_4$ in *Rana cyanophlyctis* during winter and summer/rainy seasons seems to be indirect via other hormones (Catecholamines).

5. Stimulatory effect of thyroid hormones seems to be dependent on species, tissues, seasons and/or temperature and the mode of treatments (*in vivo*/*in vitro*).

6. Administration of PTU significantly decreased the respiratory rate of liver, muscle and kidney in both the species during summer/rainy and winter months.

7. PTU-induced decreased in thyroid hormones might be responsible for significant decline in the respiratory rate of tissues. Endogenous thyroid hormones seem to be indirectly involved in the oxidative metabolism of frogs.

8. It seems that thyroid hormones do not play a significant role in the regulation of the oxidative metabolism of frogs at comparatively low temperature.

Chapter 4: Rate of testicular hormones in the regulation of oxidative metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

1. Testosterone invariably stimulated the rate of tissues respiration in both the species irrespective of seasons and ambient temperature.

2. Testosterone is ineffective in stimulating the tissue respiratory rate in the presence of Actinomycin D and cyclohexamide.

3. Cyproterone acetate always inhibited the rate of tissues respiration in both the species.

4. Testicular hormones seem to be directly involved in the regulation of the oxidative metabolism in the frogs, and the calorigenic effect of testosterone seems to be independent of seasons/ambient temperature.

5. The stimulatory effect of testosterone is brought through the process of transcription .

6. During early evolution, testicular hormones might have possessed calorigenic functions to meet energy demand at low temperature to ensure successful reproduction.

Chapter 5 : Role of adrenal hormones in the regulation of the oxidative metabolism in *Rana limnocharis* and *Rana cyanophlyctis*.

1. Fpinephrine and norepinephrine invariabl~~e~~y increased the rate y/ of tissues respiration significantly in both the species *in vivo* as well as *in vitro* experiments irrespective of seasons and ambient temperature

2. Epinephrine (EP) and norepinephrine (NE) stimulated the respiratory rate of tissues in a dose-dependent way.

3. NE is more potent than EP in stimulating respiratory rate of the tissues. The higher potency of NE might be due to the increased cellular sensitivity. Norepinephrine is secreted from the chromaffin tissues as well as from the sympathetic nervous system. The sympathetic nerve fibers are widely

distributed. Therefore, it seems that tissues/organs have easy access to NE as compared to EP. This might has probably led to the selective development of responsiveness.

4. Both alpha-, and beta-adrenergic receptors are involved in the calorigenic action of catecholamines.

5. *In vitro* stimulation of the rate of tissues respiration strongly suggest that the calorigenic action of catecholamines is direct.

6. Administration of corticosterone always stimulated the respiratory rate of tissues in both the species.

7. Cortisol does not have any stimulatory effect on the rate of tissues respiration irrespective of season/temperature and species.

8. *In vivo* administration of metapyrone significantly inhibited the respiratory rate of tissues (except in liver of *Rana limnocharis* during winter) in both the species during winter and summer/rainy season.

9. The degree of involvement of alpha-, and beta-adrenergic mechanism seem to depend upon tissues, seasons and species.

10. Failure of cortisol in stimulating the rate of tissues respiration indicates that probably amphibian tissues have not developed mechanism (receptor) for responding to cortisol.

Chapter 6 : Effect of melatonin in the oxidative metabolism in Rana limnocharis and Rana cyanophlyctis.

1. Melatonin increased the rate of tissues oxygen consumption in a dose dependent manner in both the species during winter and summer/rainy months.

2. L-T_3 did not alter the metabolic action of melatonin.

   Thus, the calorigenic action of melatonin is not mediated by thyroid hormones in frogs.

3. The mechanism of calorigenic action of melatonin and its physiological significance remains to be investigated.

On the basis of these findings, it can be concluded that the endocrine regulatory mechanism for the oxidative metabolism has evolved during the course of evolution. In frogs, especially at low temperature, thyroid hormones are not directly and actively involved in the regulation of the oxidative metabolism. Testicular hormones seem to be actively and directly involved in the energy metabolism of frogs. The acquisition of calorigenic function by testicular hormones in amphibians and other poikilotherms might be an adaptation to meet the energy demand associated with various activities to ensure a successful reproduction and survival under adverse climatic conditions where thyroid hormones are calorigenically ineffective. Due to their temperature-independent, direct and rapid calorigenic actions, catecholamines seem to act as emergency hormones for regulation of the energy metabolism in frogs. The catecholamines might be responsible for the successful survival of frogs at very low temperature.

Corticosterone (but not cortisol) also seems to be directly involved in the regulation of the metabolic rate of tissues. The pharmacological doses of melatonin also seem to be calorigenic, but the mechanism of action and its physiological significance remain to be unknown.

It seems that frogs (anurans) have developed a multi-hormonal mechanism for the regulation of the oxidative metabolism, probably to ensure a successful survival under diverse habitat and climatic conditions.

We have also compared the responses of the two species to various hormonal treatments to understand the differences between the physiology of the hibernating and the non-hibernating species. A comparative account of their dissimilar responses to major treatments (based on the data presented in the Chapters 1 to 6) is given below :-

Rana limnocharis	*Rana cyanophlyctis*
1. Average respiratory rate of all three tissues during winter months was found to be significantly low as compared to that during Summer/ rainy months.	Only average respiratory rate of muscle tissue during winter months was found to be lower than that during Summer/rainy months.
2. Tissues did not respond to L-T_3 and L-T_4.	L-T_3 significantly increased muscle and kidney respiratory during winter, and both L-T_3 and L-T_4 stimulated kidney respiration during summer.
3. PTU decreased the respiratory rate of muscle (7%) and kidney (10%) during summer months.	PTU decreased respiratory rate of muscle (14%) & Kidney (20%) during summer months.
4. Testosterone stimulated respiratory rate of liver (26%) & muscle (25%) during winter months.	Testosterone stimulated liver respiratory rate (62%) & the rate of muscle oxygen uptake (69%) during winter months

5. Cyproterone acetate decreased the respiratory rate of muscle (10%) & of kidney (13%) during winter.	Cyproterone acetate decreased the respiratory rate of muscle (20%) and of kidney (25%) during winter months.
6. During winter months, norepinephrine and epinephrine stimulated liver respiration by 98% and 43% respectively.	During winter months, norepinephrine and epinephrine stimulated liver respiratory rate by 128% and 73% respectively.

On the basis of these responses, it may be concluded that the extent of involvement of endogenous thyroid hormones in the oxidative metabolism is comparatively more in *Rana cyanophlyctis* than in *Rana limnocharis*. The tissues sensitivity to testosterone during winter months seems to be greater in *Rana cyanophlyctis* than in Rana limnocharis. Further, the tissues of Rana cyanophlyctis seem to be comparatively more responsive/sensitive to catecholamines as compared to the tissues of Rana limnocharis during winter. Thus, it seems obvious that the higher levels of androgens and the greater sensitivity of tissues to testosterone and the catecholamines in *Rana cyanophlyctis* might be responsible for keeping this species active even at very low temperature of the winter months.

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