INTRODUCTION
Environment is labile, as such there is a pervasive reciprocity between organisms and environment. In the global ecosystem, every organism is dependent upon the environmental factors. Hence, organisms have developed physiological, biochemical, morphological and distributional adaptations. These adaptions help the organisms to survive in ambient labile conditions. All multicellular organisms are supposed to have their own physiological clock and all of them perhaps operate in a coordinated fashion. All factors should act in appropriate proportions for organisms to be conducive for environment. But one factor alone cannot bring necessary living conditions for an organism. Rhythmic physiological processes in numerous organisms, representing all the major groups of plants and animals have been described (Halberg, 1969). But there is a season for everything and a time for every purpose under the Heaven.
Biological rhythms:

Biological rhythms have been identified since earliest times. Rhythmicity is inherited from birth (Akimushkin, 1970) and is ubiquitous. Plants and microorganisms in their natural habitat exhibit rhythmic behaviour. These rhythmic or periodic activities related to routine functions, vary with the time of the day and also of the year. For example, butterflies and most birds are active during the day (diurnal) whereas, moths, cockroaches, scorpions and toads are active during night (Nocturnal). Many animals restrict their activities to certain times of the day and are inactive or relatively lethargic during the remaining periods of the day. Thus, some are diurnal and others are nocturnal (Cloudsley-Thompson, 1957). Organisms unconsciously adopt to sun clock mechanisms in their metabolism and physiology. The source of these rhythms is the "Biological clock" (Harker, 1964; Brown et al., 1970). The biological clocks are innate, very sensitive to environmental stimuli and show phase with light, temperature etc. and impart changes in the activities of the organisms. A number of biological processes do vary in direct responses to the environmental cycles. The rhythmic biological processes were found to permit (Cockroaches, rats, mice etc) in the absence of natural day-night cycles (light, temperature, humidity etc.).

Rhythms are distinguished by biologists into two main categories. They are exogenous (external timing hypothesis) and endogenous (autonomous internal timing hypothesis).

Exogenous rhythms:

The exogenous hypothesis postulates that in the standard laboratory conditions, the organisms use subtle rhythmic geophysical factors to maintain natural rhythmicity (Brown and Park, 1967; Brown, 1971, 1972; Brown and Chow, 1973, 1974, 1976). They are analogous to forced oscillations of passive systems.
Endogenous rhythms:

There are a variety of endogenous rhythms with periodicities ranging from milliseconds to years (Moore-Ede et al., 1982). Rapp (1979) listed over four hundred cellular oscillations. However, the endogenous hypothesis is not accepted by all workers. The endogenous clock hypothesis, postulates that the organisms are independent of environmental factors. This hypothesis is supported by Harker (1954), Pittendrigh (1961) and Aschoff (1964).

Chronon hypothesis is proposed by Ehret and Trucco (1967) in support of endogenous hypothesis. But they failed to explain the differences occurring in circadian times. Endogenous rhythms are analogous to self-sustaining oscillations of active systems. The general properties of the rhythms are so remarkably similar among all of the wide spectrum of living creatures. The differences among the rhythms involve chiefly the nature of the species-specific biochemical, physiological and behavioural phenomena that comprise the rhythmic variations. The recurring cycles of the rhythms of organisms in their natural environment results from two simultaneous kinds of sources (a) a continuous direct response to the fluctuating, periodic environment, both living and non-living and (b) an inherent tendency of an organism to repeat the successive cycles.

Types of Rhythms:

Biological clocks of various frequencies or durations are found at all biological levels such as ecosystem, population, individual organ, tissue and cell. Halberg (1959) has coined the terms to explain meaningfully the various types of rhythms. These rhythms are used as "Clocks" or "Calenders" to enable the organism to live in harmony with their rhythmic environment.
Solar day Rhythms:

The most extensively investigated of the biological rhythms are those that are correlated with the 24 hour solar day. Under natural conditions, all the factors that contribute to the recurring pattern display in common the 24 hour period. A major effect of the 24 hour environmental rhythm in illumination is therefore, to relate adaptively to the day-night changes the phase of an animals genetically determined solar day behavioural pattern.

Lunar Related Rhythms:

Another terrestrial period, the lunar day of 24 hours and 50 minutes is associated with rhythmic variations. The periods of the Ocean tides are determined chiefly by the rotation of the earth. The tidal pattern of any given area typically change in amplitude through the synodic month. The temporal patterns of the activities of the seashore organisms follow the tidal changes. Animals display the maxima in their activity cycles during low tides.

The biological rhythms are classified into three categories: 1) high frequency; 2) medial frequency and 3) low frequency oscillations (Halberg, 1969). The high frequency oscillations are those which occur at the order of seconds such as heart beat. The low frequency oscillations are characterized by oscillations occurring at the order of more than 7 days to months eg. menstrual cycles, seasonal reproductive cycles etc. The medial frequency oscillations are of the order of 0.5 hours to 6 days approximately. They are shown by locomotor, sleeping, feeding etc.

Annual and sidereal rhythms:

These annual and sidereal rhythms are very conspicuous in physical environment. Annual variations have been reported even for organisms inhabiting equatorial regions. In a constant environment, except with a
steady 12L:12D regimen, weaver finches have been observed to continue through two nearly normal annual reproductive cycles (Lofts, 1964).

Circadian rhythms:

The oscillations ranging around 24 hours are called "Circadian rhythms" (Halberg, 1959). The word circadian (Latin: circa = about; dies = day) was coined by Franz Halberg (1959). Circadian rhythmicity has been documented as a prominent and pervasive feature of animal and plant physiology (Bunning, 1973; Aschoff, 1981; Moore-Ede, et al., 1982; Sweeny, 1987; Edmunds, 1988). Such rhythms have been reported in all major eukaryotic taxa, and at levels of biological complexity ranging from protozoans to mammals. Circadian rhythms have not generally been found to occur in prokaryotes (Sweeny, 1987; Hardeland, and Balzer, 1988). But there have been several reports of rhythmicity having circadian properties in nitrogen fixing cyanobacteria (Grobbelaar, 1986; Mitsusi, 1986 and Sweeny, 1989).

Circadian rhythmicity is based on an endogenous biological mechanism with clock like properties. Under light-dark (LD) cycles, circadian systems exhibit daily rhythms in many different functions. In most living organisms, recurring changes are found in the physiological state during the 24 hours of a day (circadian) (Menaker, 1971 and Bunning, 1977). As such, carbohydrate rhythms have been reported in larval and adults of several insects (Novočerki and Patton, 1964; John and Muraleedharan, 1988). All circadian rhythms in nature follow a strict 24 hours periodicity of their geophysical environment owing to the ubiquitous entraining influence of the LD cycles caused by sunrise/sunset. All living organisms express daily rhythms in their living behaviour, biochemistry and physiology (Bunning, 1973; Aronson et al., 1993). The ubiquitous nature points to an early origin of circadian rhythms. The importance of circadian timing system in the temporal co-ordination of various cellular, physiological and behavioural events are elaborated by various authors (Enright, 1970; Kramn, 1980; Daan and
Aschoff, 1982; Edmunds, 1988; Sweeny and Borgese, 1989; Gerkema, 1993).

**Entrainment:**

The mechanism of entrainment related to the responses of light pulses by LD cycles was put forward by Rawson (1956). According to Bruce (1960), it is the phenomenon whereby a periodic repletion of light and dark cycles causes an endogenous rhythms to become periodic with the same period as the entraining cycle. All circadian rhythms are entrained by light and dark cycles (Roelfsema, 1987; Hastings et al., 1991). Entrainment has not been extensively studied, but there is clear evidence in both birds and mammals (Hastings et al., 1985). Recently phase response curves have been compiled for diverse species ranging from unicellular organisms to human beings and for a variety of perturbations (Johnson, 1990, 1992 and Dawson et al., 1993). Circadian oscillators can entrain only a limited range of "Zeitgeber" periods. The fact that circadian rhythms can entrain to sinusoidal light cycles suggest that entrainment can also be achieved through a continuous action of light. In contrast, many nocturnal animals spend most of the day light hours in light excluding shelters emerging at dusk and regaining their shelters at dawn. Such animals are only exposed to light for brief durations. A remarkable example of this process is provided in a recent study of entrainment in the nocturnal flying squirrel, *Glaucophis volans* (DeCoursey, 1986)

**Zeitgeber:**

Aschoff (1951) coined the term "Zeitgeber" which means the external periodicity entrains a self sustaining endogenous rhythms. The role of "Zeitgebers" in synchronizing circadian rhythms has been investigated such as light and darkness (Czeisler et al., 1981 and Gerkema et al., 1993), food availability cycles (Boulos et al., 1980; Jilge, 1991, Tokura and Aschoff, 1980; North, 1993), noise silence cycles (Meyer,
1968; Sulzman et al., 1977), atmospheric pressure (Hayden and Lindberg, 1969) and water availability cycles (Moore, 1980 and Mistelberger, 1992). All circadian rhythms are entrained by light and dark cycles.

**Diurnal rhythms:**

Diurnal rhythms or diurnal adaptation means "doing right thing at the right time and thereby making use of the most favourable and avoiding the unfavourable". This purpose could perhaps be achieved by a pure reaction of the organisms to the factor which has prime adaptive value (Aschoff, 1964).

Diurnal rhythms were studied in metabolic activities in vertebrates (Agren et al., 1931; Vogel, 1948; Georgi, 1949; Bander, 1950; Koehler, 1955; Sollberger, 1955; Albrecht et al., 1956; Koudda et al., 1969; Rajarami Reddy et al., 1980; Venugopal, 1980). Many animals restrict their activities to certain times of the day and are inactive or relatively lethargic during the remaining periods of the day. Thus, some are diurnal and others are nocturnal (Cloudsley-Thompson, 1957). Body temperature and metabolism are highly correlated. But this correlation changes with time of the day. In birds and humans, the correlation is higher during the daily rest phase than during the active phase (Aschoff and Pohl, 1970).

Some diurnal changes in the activity and metabolic rate in *Octopus vulgaris* (Wells et al., 1983), the effect of environmental factors on the rhythmicity of the benthic gobid fish, *Glossogobius giurus* (Gheevarghese and John, 1984), seasonal changes in the behaviour of tropical bat (Marimuthu, 1984), nocturnal behaviour and food selection in the land snail, *Monadenia hillbrandimariiposa* (Szlavecz, 1986) have been studied in relation to circadian rhythms.

**Biological rhythms in invertebrates and vertebrates:**

Few investigators have examined the rhythms in oxygen consumption in amphibians. The seasonal cycles of gas exchange in *Rana*
temporaria (Krogh, 1904; Dolk and Postma, 1927) and Rana pipens (Fromm and Johnson, 1955) were reported.

Cole and Allison (1929) found that a sudden increase in illumination intensity produced a higher rate of pharyngeal breathing movements in frogs. The locomotor rhythms in the slimy salamander, Plethodon glutinosus and the green frog, R. clamitans can be shifted in phase by the environmental high cycle (Adler, 1969).

Brown et al. (1955) reported that amphibians and other organisms maintained at a constant temperature and pressure had endogenous rhythms of oxygen consumption which are correlated with average daily changes in ambient atmospheric pressure.

The slugs are reported to be more active during night than during the day time (Kulkarni and Nagabhushanam, 1973) and hence there could be corresponding variations in the various physiological processes of the organisms. Ralph (1957) found that the oxygen consumption rhythms of earthworms, Lumbricus terrestris were not merely reflections of motor activity rhythms.

Amphibians have highly permeable skin through which they can both take up and lose water. In comparison with reptiles, amphibians tend to be more nocturnal. The daily activity pattern of the toad, Bufo boreas changes drastically with the seasons. Diurnal rhythms of body temperature recorded in early March, late April and mid June showed considerable differences in daily range (5, 20 and 10 °C respectively). B. boreas showed little evidence of daily rhythmicity in temperature preference (Smith, 1984). Other amphibians (Claussen, 1973; and Licht and Brown, 1967) also show little diurnal change in temperature preference. In Rana pipens tadpoles, the diurnal temperature preference pattern is bimodal with one peak in the first half of the night dark phase and one near the middle of the light (Casterlin and Reynolds, 1978).
Biological rhythms have been chiefly oriented in two directions (1) Overt activity rhythms (2) Internal physiological rhythms. Studies on activity rhythms in vertebrates were reported in humans (Kleitman, 1939; Gesell, 1943; Kleitman and Engelmann, 1953; Hellbriigge, 1960), in rats and mice (Wolf, 1930; Vogel, 1955; Brown and Terracini, 1959; Pittendrigh, 1960; Rawson, 1960; Subbarao, 1967).

Overt rhythms among invertebrates have been mostly reported in insects (Lutz, 1932; Gunn, 1940; Cloudsley-Thompson, 1953; Harker, 1953, 1954, 1956; Saunders, 1976) for crustaceans and arachnids (Naylor, 1958; Gopalakrishna Reddy, 1967; Sailaja, 1989).

Pioneering work on rhythms in locomotion was carried out on rats (Bellamy, 1970). Overt rhythms among vertebrates are mostly reported in mammals especially rodents (Glick and Cohen, 1964; Hoffman, 1965; Pittendrigh, 1967; Kavaneau, 1969; Miselis and Walcot, 1970; Stephen, 1973; Kramm, 1975; Vijayalakshmi, 1977a,b; Natalni, 1978; and Alexander and Neuhaus, 1978). Free running physiological rhythms in the field mouse, Mus booduga was reported (Venugopal, 1980). Physiological functions of the blood of field crab, Oziotelphusa senex senex (Sailaja, 1989), maintenance of a circadian rhythmic pattern of carbohydrate reserves in developing pupal forms of Achaea janatalinn (Annie John and Muralidharan, 1994) circadian rhythms in the biting behaviour of mosquito, Armigeres subalbatus (Selvaraj Pandian, 1994), dial variations in antibody response to bovine serum albumin in Oreodromis mossaambicus (Dinakaran Michael and Priscilla, 1994) circadian rhythms in the locomotor activity of the field mouse, Mus booduga (Kumarasamy, 1994) circadian rhythms of blood pressure in school age children (Grossman, 1990) nature of circadian rhythms of photoperiodic photosensitivity in the black headed buntings, Emberiza melanocephala (Vinod Kumar et al., 1994) were reported in relation to dial variations. Investigation of the diurnal variations of organic and elemental carbon (Turpin and Barbara, 1990) and splitting circadian rhythms have been
observed to occur in a variety of vertebrates, such as, hamsters, rats, mice, palm squirrels, monkeys, starlings and lizards (Gwinner, 1974; Underwood, 1977; Boulos and Terman, 1979; Fuller et al., 1979; Ellis et al., 1982; Turek et al., 1982; Valli, 1985; Navaneethakannan, 1987; Ruis et al., 1991). In rats and hamsters, split circadian rhythms were recorded in feeding, drinking, electrical brain self-stimulation and body temperature rhythms (Boulos and Terman, 1979; Pickaard et al., 1984).

Some authors reported that the property of the circadian system is its apparent capacity, at least in rare instances, to have its phase adaptively adjusted to local time even in the absence of any obvious environmental cues. The rate of biological activity varies with the local time of day or hour angle of the sun. The living system appears steadily to derive information about fluctuations in the terrestrial atmosphere. The light and dark (photo-scotophases) were probably the dominant environmental regulators of the seasonally, diurnally changing metabolism of the poikilothersms.

Brown et al. (1954) reported that the fiddler crab, Ucapugilator, is mainly active at night upto eight times higher than the values recorded during the day. During late spring and summer, potatoes and fiddler crabs possessed mean metabolic rates for the 5-7 AM and 5-7 PM periods of the day with the mean 2 to 6 AM and 2 to 6 PM rates of barometric pressure change respectively (Brown, 1962).

Programme of the present study

From the foregone literature, it is obvious that extensive work has been carried out on circadian and other rhythms in various animals. But only few investigators have examined rhythms in metabolism and physiology in amphibians. Therefore, the present study was undertaken to investigate the daily rhythms in the physiology, haematology and metabolism of the South Indian toad, Bufo melanostictus. This study may
give some additional information in relation to the 4 hour intervals of the 24 hours day.

The following parameters were chosen for the present programme of the study.

Respiration:

Respiration is an essential physiological activity of living organisms by which they obtain energy for all metabolic activities (Roberts, 1975). Amphibians use gills, lungs, skin and the buccal cavity for gaseous exchange. The importance of a given respiratory surface differs between species, between developmental stages of the same species, and in a given individual under different environmental conditions. The whole animal oxygen consumption is an index for aerobic metabolism (Prosser, 1973). It provides information on to the ability of the animal to extract oxygen from the environment. Oxygen consumption represents the basal metabolism (Prosser and Brown, 1977) and leads to some compensatory metabolic adjustments in the biochemical machinery of the organism (Parvatheswara Rao, 1968). Metabolism can be known through oxygen consumption.

According to Tyler et al (1982), larval and adult type red cells of *R. catesbeiana* (at least from some geographic locations) can be distinguished using dark-field illumination. The developmental history of the frogs and toads (order anura) is unique among the vertebrates. Frogs of the family, *Ranidae* are the most successful, widely distributed and mostly studied of all the amphibians.

Although the literature on circadian and other rhythms is voluminous, few investigators have examined rhythms of oxygen consumption in amphibians. Fromm and Johnson (1955) found seasonal differences in *R. pipiens*. Whitford and Hutchison (1965) showed that photoperiod had a significant influence on oxygen consumption of *Amblystoma maculatum* and Vinegar and Hutchison (1965) found similar
results with *R. clamitans*. There are also few reports of daily cycles of gas exchange in amphibians. Brown *et al.* (1955) reported that *Notophthalmus viridescens* had a persistent biomodel daily rhythms of oxygen consumption which may have been correlated with average daily fluctuations of barometric pressure. Kasbohm (1967) found daily rhythms of oxygen consumption in *R. temporaria* which were directly correlated with photoperiod. Chugunov and Kispoev (1968) measured the oxygen consumption of *R. temporaria* in the field and found that the average at night was significantly higher than during the day. Kayser and Schieber (1969) found a bimodal daily rhythm of metabolism in *Rana esculenta* under LD 12:12 and at 15 °C. They also found that total oxygen consumption was greater under a photoperiod than under conditions of LL or DD. Penaeid prawns *P. indicus* and *Metapenaeus monoceros* were reported to be nocturnal with reference to motor activities (Menon and Raman, 1961). Janakiram *et al.* (1985) reported that oxygen uptake of these animals is more in evening hours and early dark hours in all salinities.

There is an extensive literature on the oxygen consumption with respect to light and temperature in the crab, *Paratelphusa hydrodromus* (Ramamurthi and SainathJanak, 1973) in *O. senex senex* (Sailaja, 1989) in the scorpion, *Heterometrus fulvipes* (Gopalakrishna Reddy, 1967) and also in relation to seasonal variations in *O. senex senex* (Jayamma, 1988).

In view of the lack of literature on the rhythmicity studies in oxygen consumption in *Bufo melanostictus*, the present study was undertaken to unravel the relationship between the metabolism and oxygen consumption in toad, *B. melanostictus*.

**Haematology**

Haematological indices vary from animal to animal and in the same animal at different stages of life. At birth, the haemoglobin content is higher than during any other stage of life. Amphibian erythrocytes are
usually flat, elliptical discs with a nuclear bulge in the centre. Sizes vary considerably. Anurans have small, less elongated and numerous red cells. Haematological parameters are used as valuable diagnostic tools in medicine (Dabrowska and Wlasow, 1986). Besides this, other intrinsic factors such as sex, size and weight are known to influence the haematological parameters (Banerjee, 1957; Pradhan, 1961; Srivastava, 1968; Dube and Dutta, 1974; Siddiqui and Naseem, 1979). In mammals the haematological parameters are reported to vary with season (Preston, 1960; Natvig et al., 1963; Sealandar, 1964), sex (Rosalin et al., 1934; Sealandar, 1964; Mulcahy, 1970; Gilman, 1972; Srihari and Shakuntala Sridhara, 1980), age (Ostrumova, 1960; Sealandar, 1964), body weight (Panday, 1977; Chanchal et al., 1978) habitat (Haws and Goodnight, 1962) and size (Preston, 1960; Pradhan, 1961).

Pesticide poisoning on various haematological parameters was studied in various animals such as fresh water fishes (Koundinya, 1978; Koundinya and Ramamurthi, 1979; Natarajan, 1981; Jayantha Rao, 1982; Madhu, 1983; Srinivasan and Radhakrishnamurthy, 1983; Ramamurthi, 1988), birds (Gupta and Paul, 1972; Mandal and Lahiri, 1985), rodents (Prigge et al., 1977; Judd, 1979; Philip, 1984; Mita Das et al., 1987; Rajani et al., 1987) and in rats (Janardhana Reddy, 1988; Sudhakar, 1991; Vani, 1991; Anil Kumar, 1994).

The studies on amphibian (especially toads) haematological parameters in relation to dial variations is lacking. Hence, in the present investigation an attempt has been made to identify the haematological changes in relation to dial variations at 4 hour intervals of *B. melanostictus*.

**Ionic composition of the blood:**

Blood plays an important role in the osmotic and ionic regulations of the body. This is made possible by the presence of various inorganic and organic constituents of the blood. The ionic composition of the blood
was influenced by season, humidity of the environment (Price and Holdich, 1980), body size (Padmanabha Naidu, 1966a), salinity (Castille and Addison, 1981) and temperature (Houston and Koss, 1984; Pramoda Kumari, 1985). Blood besides transporting nutrients and waste products, maintains pH and fluid balance. Thus blood coordinates the functions of various individual cells in a well-integrated organism.

**Inorganic constituents:**

Inorganic ions are necessary for maintaining the osmolarity within the cells. They are powerful modulators of cellular activities (Bygrave, 1978) and play an important role in the metabolic adjustments accompanying stress conditions (Berish, 1973). In addition to their osmotic role, inorganic ions such as sodium ($\text{Na}^+$), Potassium ($\text{K}^+$), Calcium ($\text{Ca}^{2+}$), Magnesium ($\text{Mg}^{2+}$), chlorides ($\text{Cl}^-$) and bicarbonates ($\text{HCO}_3^-$) play a prominent role in the regulation of cellular homeostasis (Baskin et al., 1981).

The inorganic composition of blood of several arthropods and molluscs have been extensively studied. (Robertson, 1957; Jeuniaux, 1971; Edwards, 1982; Robertson, 1966; Colin, 1968; Matsumoto et al., 1974; Krogh, 1939; Lockwood, 1962; Parry, 1966; Holmes and Donaldson, 1969; Conte, 1969; Prosser, 1973; Fleming, 1974).

Among the major ions viz. sodium, potassium and chlorides, the metabolism of sodium received considerable attention by many workers (Maetz, 1970; Fleming, 1974). It has been shown that pesticides inhibit $\text{Na}^+\text{-K}^+$ ATPase activity in fishes (Janicki and Kunter, 1971; Campbell et al., 1974; Leadon et al., 1974). Sodium serves as an essential activating ion for specific enzyme systems.

Potassium is the principal cation of the intracellular fluid and it is excreted by the kidneys. Hyperkalemia occurs frequently in renal failure when the kidney is not capable of excreting exesssive potassium (Martin, 1981). Any remarkable increase or decrease in potassium level may be
accompanied by serious disturbances in muscular irritability, myocardial function and respiration (Coles, 1967). Potassium together with some other inorganic ions and organic compounds plays a pivotal role in the intracellular osmotic regulation (Degain and Warburg, 1984).

Calcium is the important osmotic effector and conferring stability to the cell membrane. Magnesium is a widely distributed bivalent cation in animal systems. It is a co-factor for many enzymes. Hypomagnesemia in farm animals produce excitement, convulsions and finally death due to respiratory failure (Jones and Hunt, 1983).

**Organic constituents:**

The organic constituents of the blood are glucose, proteins, Total ninhydrin positive substances(TNPS), Lipids and glycogen. These are used for the various vital physiological activities. Glucose is the major sugar. Tandon and Joshi (1974) reported that the blood glucose was higher during the winter months than in summer months in teleosts. Protein fractions are influenced by several factors such as diet, age (Alikhan, 1971; Alikhan and Lysenko, 1973), sex (Peters and Long, 1973), and Season (Djangmah and Grove, 1970). Lipids are involved in the production of energy during prolonged and sustained activities such as muscular exercise (Hoar and Cottle, 1952), spawning and osmotic stress (Bilinski, 1974). Lipids are important storage materials and are found to accumulate before hibernation (Giese, 1966) and aestivation (Krishnamoorthy, 1968; Krupanidhi, 1978).

Dial variations in the blood composition was studied in the fresh water field crab, *Oziotelphusa senex senex* (Sailaja, 1989). Thorough literature survey reveals the fact that the role of blood constituents during dial variations in amphibians (with special reference to toad) was lacking. In order to explain the physiological transport functions of the blood at various times of the day, the present study was undertaken to
ascertain a role to the organic and inorganic constituents of the blood of
the toad, *Bufo melanostictus* at regular intervals during daily variations.

**Metabolic enzymes**

Metabolic pathways fall into three categories.

1. Anabolic pathways are those involved in the synthesis of the
   compounds constituting the body structure and machinery. Protein
   synthesis is such a pathway.

2. Ketabolic pathways involve oxidative processes that release free
   energy, usually in the form of high energy phosphate, e.g. the respiratory
   chain and oxidative phosphorylation.

3. Amphibolic pathways have more than one function and occur at
   the "cross roads" of metabolism, acting as a link between the anabolic and
   ketabolic pathways, e.g. the citric acid cycle. The food that is digested,
   absorbed is metabolised to energy molecule in the presence of enzymes is
   one example (Mayes, 1988).

Enzymes are structurally proteins whose biological function is the
catalysis of chemical reaction in living organisms. Enzymes are
macromolecules with a molecular weight of above 100,000 and a diameter
of 40Å. These are very specific in their action. Oxidative enzymes widely
occur in different tissues of amphibians (Zweifel, 1957; Hutchinson and
Rowlan, 1975).

Dial rhythms were reported in enzymatic and other metabolic
activities in various animals by several workers(Glick and Cohen, 1964; 
Nishiitruje *et al.*, 1967; Venkatachari and Muralikrishna Dass, 1968; 
Cymborowski *et al.*, 1970; Devarajulu Naidu, 1973; Ramamurthi and 
Sainathjanak, 1973; Chengalraju *et al.*, 1973; Vasantha *et al.*, 1975; 
Rajaram Reddy *et al.*, 1978; Vijayalakshmi *et al.*, 1977a,b; Sasira Babu *et 
al.*, 1976; Chandrasekhara Reddy and Padmanabha Naidu, 1977; 
Koundinya, 1978; Kurisaki and Nagamori, 1981; Rashatwar and Ilyas,
Lactate dehydrogenase (LDH) catalyses the interconversion of lactate and pyruvate and has a wide distribution. In Krebs cycle, succinate dehydrogenase catalyses the reversible oxidation of succinate to fumarate (Harper, 1990). In mitochondria, succinate dehydrogenase (SDH) activity is greater than the other enzymes of Krebs cycle. The enzyme isocitrate dehydrogenase (ICDH) catalyses the dehydrogenation of isocitrate to α-ketoglutarate. Glucose-6-Phosphate dehydrogenase (G-6-PDH) is an important enzyme of the HMP shunt which is an alternative pathway for glycolysis. Glutamate dehydrogenase (GDH) catalyses the reversible oxidative deamination of glutamate to α-ketoglutarate and ammonia and is known to occur in the tissues of various animals including human beings (Kochakian et al., 1959; George and Talesara, 1962; Katz and Kalow, 1965; Turner and Manchester, 1970; Prameelamma and Swami, 1975). Aldolase cleaves fructose 1,6-diphosphate into two triose phosphates. The activity of this enzyme plays an important role in the continuation of glycolysis at the level of fructose diphosphate. Breakdown of hexoses into trioses favours not only the operation of glycolysis but also synthesis of glycerol (Lehninger, 1978). These are all essential for catalytic activity of an animal.

The phosphorylase ‘a’ is an active form in the absence of allosteric activator AMP while phosphorylase ‘b’ is an inactive form in the absence of AMP (Fischer et al., 1971; Gross and Mayer, 1974). Glycogen phosphorylase catalyses the conversion of glycogen to glucose-1-phosphate and plays a central role in the regulation of glycogen metabolism. Aspartate aminotransferase and Alanine aminotransferase are the most studied aminotransferases catalysing the exchange of aminogroups between glutamate and aspartate (Braunstein, 1973) and between glutamate and alanine. Aspartate involved in the synthesis of protein, urea, uric acid, purines, pyrimidines, aspartagine and oxaloacetate.
Aminotransferases are concerned with intermediate metabolism of amino acids and the feeding of amino acids into carbohydrate and lipid metabolism.

Studies on the enzyme activity in relation to dial variations in amphibians are lacking. Hence the present study was undertaken to observe the possible variations in the enzyme activity in relation to dial variations at 4 hour intervals of 24hr day in the toad, *Bufo melanostictus*.

**Oxygen equilibrium curves:**

**Haemoglobin:**

Oxygen capacity of blood containing haemoglobin is higher than that of the blood containing haemocyanin (Prosser, 1973). Biochemically haemoglobin is made up of heme and globin. The red colour is due to the presence of heme an iron porphyrin compound. Dr. Perutze described the three dimensional structure of the haemoglobin for which he was awarded nobel prize. The molecular weight of haemoglobin in corpuscle is 6800. Each 100 ml of blood of normal man contains about 16 gms of haemoglobin and in the normal women 14 gms. It is a complex protein

\[
\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2
\]

Vertebrate blood carries 20 volumes of oxygen. Metabolic regulation depends upon blood pigments. Haemoglobin reacts or unites with oxygen to form oxyhaemoglobin. One haemoglobin molecule contains 4 atoms of iron. The formation of oxyhaemoglobin is dependent upon the tension of oxygen in the blood at any time.

Among the respiratory pigments, the haemoglobin is the most common and the best known in animals. Porphyrins take an active biological role in the formation of pigments. A largely formed pigment in the biological kingdom is the chlorophyl in the plants and the haemoglobin in the animals.
Oxygen equilibrium curves are important in order to understand the functional properties of different respiratory pigments. These curves are obtained by determining the amount of oxygen which combines with blood when exposed to various oxygen pressures. The oxygen equilibrium curves of the blood of arthropods and molluscs have been investigated (Redmond, 1955; 1968; 1971; Manwell, 1960; Wolfevamp and Waterman, 1960; Padmanabha Naidu, 1966b; Jones, 1972; Prosser, 1973; Krupanidhi, 1978; Venugopala Reddy, 1978; Mangum, 1980; Pramoda Kumari, 1985; Sambasiva Naidu., 1993; Aruna Kumar, 1994).

One of the physical characteristic features of the haemoglobin is the "absorption spectrum". The visible range of the spectrum and the ultra violet are the two main regions in the absorption of light. The intensity and position of various absorption bands of the oxyhaemoglobin depends on the nature of the protein, prevalence of the iron and nature of any group combined with heme (Lee and Smith, 1965).

The oxygen equilibrium curve indicates the relationship between the oxygen tension and the percentage saturation of the haemoglobin. There is an extensive literature on the oxygen equilibrium curves.

The oxygen equilibrium curves can be described by the Hill's (1913) well known equation as shown below:

\[ Y = \frac{100(p/p_{50})^n}{1 + (P/P_{50})^n} \]

where 'Y' is the per cent saturation of the haemoglobin; 'P' is the partial pressure of oxygen; 'P_{50}' is the partial pressure of oxygen at 50% of the pigment saturation; and 'n' is the slope of the haemoglobin molecule (Atkinson, 1966) and is also known as Hill's approximation (Manwell, 1960). The slope of the curve at the half saturation point where 'Y' is equal to 50% gives 'n' value. The 'n' values for the oxygen equilibrium curves at 50 per cent saturation can be obtained by plotting
log \( P \) against log \( Y/(100-Y) \), the slope of the resulting straight line equals \( n \) (Jones, 1972). The affinity of the haemoglobin for oxygen is usually expressed in terms of partial pressure of oxygen (PO\(_2\)) at which half the haemoglobin molecules exist in oxygenated state. This is known as \( P_{50} \). \( P_{50} \) may be used as a convenient index to know the position of the oxygen equilibrium curve. When the \( P_{50} \) value was low, the equilibrium curve is found towards the left and in such a case the pigment is set to have more affinity towards oxygen. On the otherhand, if the \( P_{50} \) is high as in the case of some active animals, the oxygen equilibrium curves turns towards the right, and the pigment is said to have less affinity for oxygen (Prosser and Brown, 1961; Redmond, 1968; Prosser, 1973). Thus \( P_{50} \) of the haemoglobin varies from animal to animal and is characteristic of each species.

The position of the oxygen equilibrium curve is influenced by several physical and chemical factors. However, some of the studies have thrown light on the effect of temperature on the oxygen equilibrium curves of the blood of fish (Riggs, 1970; Johansen, 1970; Johansen and Weber, 1976; Bunn and Riggs, 1979; Powers et al., 1979). Normally, an increase in temperature decreases the affinity of the pigment for oxygen, shifting of the oxygen equilibrium curves to the right. On the other hand, decrease in temperature shifts the Oxygen equilibrium curves (OEC) to the left. Haemoglobin possess exceptional capacity to react with a variety of dissolved substances such as inorganic ions and organic phosphates. The influence of various salts on the oxygen equilibrium curves of haemoglobin has long been recognised (Benesch et al., 1969; Antonini and Brunori, 1971; Antonini et al., 1972). It has been stated by these authors that the affinity of the pigment for oxygen decreases with increase in salt concentration.

The allosteric influences of \( H^+ \), \( CO_2 \) and organic phosphates with oxygen binding by haemoglobin are now firmly established for the blood of mammals (Garby et al., 1972; Benesch and Benesch, 1974; Duhm,
1976; Breepoel et al., 1981), birds (eg. Meyer et al., 1978; Weingarten et al., 1978; Lapennas and Reeves, 1983), reptiles (Grigg and Cairnercross, 1980; Lapennas and Lutz, 1982), fish (Weber and Lykkeboe, 1978; Farmer, 1979; Jensen and Weber, 1982), frog (Wells and Weber, 1985). Pesticide impact on the fish haemoglobin has been studied by Rangaswamy (1984). Diurnal variations have been noticed in the function of the fish blood (Lykkeboe and Weber, 1978). However, not much is known about the dial variation in the transport functions of the blood in amphibia, despite their great variation in the degree of emancipation from the aquatic environment. Hence, in the present investigation, an attempt was made to study the physiological functions of the blood pigment, haemoglobin in the toad, Bufo melanostictus in relation to the dial variations.