DAY NIGHT VARIATION IN PHAGOCYTOSIS AND SUPEROXIDE PRODUCTION BY LEUCOCYTES IN FRESH WATER SNAKE, NATRIX PISCATOR

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KEY WORDS
Diurnal variation
Phagocytosis
Superoxide anion
Leucocyte
Snake

Received on: 2
Accepted on: 1

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ABSTRACT

Aim of the present study was to understand the diurnal variation in phagocytosis and superoxide production by blood leucocytes in the fresh water snake, Natrix piscator. Leucocyte phagocytosis and superoxide production are important constituents of innate immune response and form the first line of host defense. Yeast (Saccharomyces cerevisiae) cells were used as target cell to study phagocytosis. Oxidative burst activity was measured by reduction of a tetrazolium dye. Snakes were sacrificed at mid day and mid night. Blood was obtained through cardiac puncture, and leucocytes were separated. Equal amount of blood and yeast cells were incubated for 30 minutes, and smear of mixture was prepared on a clean glass slide. Slides were stained and observed in oil immersion. Percent phagocytosis was significantly (p<0.05) higher (60.75 ± 1.89) during mid day as compared to mid night (50.75 ± 1.18). Phagocytic index showed non significant increase during mid night (2.44 ± 0.20) when compared to mid day (2.21 ± 0.25). Superoxide production was found to be significantly higher during night (0.357 ± 0.02) as compared to day (0.255 ± 0.02). It is suggested that diurnal variation is a part of immune system circadian oscillation.

INTRODUCTION

Immune system shows two but interrelated types of response: acquired and innate immune responses. The acquired immune response evolved in early vertebrates and allow for a stronger immune response as well as immunological memory. The innate immune response act as an initial defense mechanism against microbial growth shortly after infection occurs (Merchant et al., 2003). The innate immune response of reptiles has been addressed in literature (Koppenheffer, 1987; Freedberg et al., 2008). Some reports are also available on seasonal variation in cell-mediated innate immune responses in reptiles (Munoz and Fuente, 2001). Phagocytosis is also an important constituent of innate immune system and critical for the survival of organisms. The cell-mediated innate immune responses in reptiles has been addressed in literature, with reference to phagocytosis and cytotoxic response of splenic macrophages (Mondal and Rai, 1999a, b, 2001, 2002a, b), mixed leucocyte reaction and lymphocyte proliferation (Farag and El Ridi, 1985, 1986; Munoz et al., 2000; Cray et al., 2001; Work et al., 2001; Munoz and Fuente, 2003; Bumham et al., 2005; Keller et al., 2005, 2006). There are a few reports on day night variation in phagocytic activity in mammals and birds (Barriga et al., 2001; Berger and Slapnickova, 2003; Hruscu, 2004). With regard to ectothermic vertebrates, reports are confined to fishes only (Esteban et al., 2006; Roy et al., 2008). Respiratory burst function resulting in the release of reactive oxygen species (ROS) such as superoxide anion (O2-) from neutrophils is one of the key mechanisms of the innate immune systems. Nitroblue tetrazolium (NBT) is a yellow, water-soluble dye that can be reduced by accepting electrons in the presence of free oxygen radicals to form a blue-black water-insoluble compound known as formazan (Baehner et al., 1976). Thus, the NBT reaction indirectly reflects the ROS generating activity in the cytoplasm of cells. Reptiles represent an important phylogenetic group being ancestor of both birds and mammals. The objective of the present study was to explore the day night variation in phagocytosis and oxidative burst activity of leucocytes in an ophidian model, Natrix piscator.

MATERIALS AND METHODS

Animals
Fresh water snakes, weighing 80-120g, were obtained from a local supplier who collected these animals in the suburbs of Varanasi (28°18’N; 83°11’E). Animals were housed in vivarium (wood and wire net cages; size 50x30x30cm) conta ring earthen bowl filled with water. Snakes were fed on small fishes once a week. Cages were cleaned, and bowl water was changed next day following feeding. Animals were acclimatized to the laboratory conditions for two weeks and experiments were performed. The guideline of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Ministry of Statistics and Programme Implementation, Government of India, were followed in maintenance and sacrifice of animals.

Chemicals
Culture medium (RPMI-1640), L-glutamine, gentamycin, fetal
bovine serum (FBS), and other chemicals were purchased from Himed Laboratories Pvt. Ltd. (India). The culture medium was supplemented with 1 µL mL⁻¹ gentamycin, 10 µL mL⁻¹ of 200 mM L-glutamine, 10 µL mL⁻¹ anti-ant (Gibco) and 5% FBS and referred to as complete culture medium.

**Experiment**

Animals were divided into two groups of four animals each (n = 4); one group was sacrificed at 12h mid day and other group at 12h mid night. Animals were weighed, anaesthetized and blood was collected in a heparinized syringe through cardiac puncture. Blood was processed for phagocytosis and NBT assay.

**Blood phagocytosis**

For phagocytic assay, the yeast cells were used as target cell. The yeast cell suspension was prepared by mixing 20ng of commercial baker's yeast (Saccharomyces cerevisiae) in 10mL of 0.2 M PBS. The suspension was kept at 30°C for 15min. The cells were washed three times in PBS and finally suspended in complete culture medium to get a concentration of 1x10⁶ cells mL⁻¹. Equal amount (20 µL) of blood and yeast cell suspension was mixed and incubated for 30 minutes at room temperature. Smeared was prepared on a clean glass slide, air dried, fixed in methanol, stained with Giemsa, and examined under oil immersion. For each slide, a total of 100 neutrophils were examined randomly without any predetermined sequence. The phagocytic index was determined by calculating the average number of yeast cells engulfed by single neutrophil. The percent phagocytosis was calculated by dividing the number of neutrophils showing phagocytosis by 100.

**NBT assay**

Peripheral Blood Leucocytes (PBL) were collected from the buffy coat (the layer of PBLs between the plasma and RBCs) using a slow spin technique as described by Keller et al. (2005). The tubes were centrifuged at 500 rpm (42 x g) for 25 min at 8°C. The PBLs were collected by gently swirling the buffy coat into the plasma and transferring the cells into a new tube. Following centrifugation at 1200 rpm for 10 min, the plasma was removed and the cell pellet was gently resuspended in 1mL of culture medium. NBT assay was performed following the methods of Berger and Slapnickova (2003). Leucocytes were counted and adjusted to 2x10⁶ cells mL⁻¹ in complete RPMI. Cell viability was checked through trypan blue exclusion test, which exceeded 95%. 50 µL of leucocytes (10⁶ cells) was mixed with 50 µL of RPMI containing NBT (1 mg mL⁻¹) and well culture plate in triplicates. One well with culture medium without cells served as blank. Plates were then incubated in CO₂ atmosphere at 37°C for 2h, centrifuged at 700 x g, washed with PBS and fixed in 70% methanol. 20 µL of 0.1% triton X-100 was mixed in each well. The formazan crystals were dissolved by mixing 120 µL KOH (2 M) and 140 µL DMSO in each well. Optical density was measured at 630 nm with the help of ELISA plate reader (Thermo Multiscan).

**Statistical analysis**

Data are presented as mean ± SEM. Means were compared, and statistical difference between means was determined by Student's t-test.

**RESULTS**

Leucocytes obtained from snake showed day night variations in phagocytic activity. Percent phagocytosis was significantly (p<0.05) higher at mid night and phagocytic index was insignificantly higher at mid night (Fig. 1). Superoxide production, as judged by NBT reduction assay, was found to be significantly (p < 0.05) higher during night time as compared to day (Fig. 2).

**DISCUSSION**

Daily rhythms in immune parameters have been documented for most species of mammals and birds studied to date. Most of these investigations, however, have focused on rhythmicity in the number of circulating immune cells and splenic lymphocytes (Haus et al., 1983; Nelson et al., 2002; Pelegri et al., 2003; Oishi et al., 2006); while few studies have examined changes in functional activity of immune cells. Diurnal rhythms in human bone marrow were first demonstrated in the work of Aardal with Laerum (1983; and Mauer (1965). Leucocytes and its subtypes, in human, vary in a circadian pattern: some show increase in daytime; while others, at night (Haus et al., 1983, Suzuki et al., 1997). There is now considerable evidence that magnitude of the immune

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**Figure 1: Day night variation in neutrophil phagocytosis in fresh water snake Natrix piscator**

**Figure 2: Day night variation in NBT reduction in fresh water snake Natrix piscator**
response varies with time of the day. The present study in fresh water snake, N. p. piscator, demonstrates the day night rhythmicity in leucocyte phagocytosis and superoxide production. The phagocytosis was higher during dark phase than light phase. This is in agreement to that in most of the endothermic vertebrates in which phagocytic activity by polymorphonuclear granulocytes remained elevated during the dark phase, though the precise timing of acrophase varies in different animals, when the hormone melatonin secretion is high (Hriscu et al., 2002-2003; Hriscu, 2004; Melchart et al., 1992). Surprisingly, there are other studies in mice in which the phagocytosis is reported to be high during the light phase, for example, the maximum engulfment of carbon particles by reticuloendothelial cells in CBA mice occur during the second half of the light span (Szabo et al., 1978), while phagocytes collected from different tissues of C57BL/6 mice showed peak phagocytic activity in the first half of the light span (Kryszynski and Fischer, 1981; Hayashi et al., 2007). The inconsistent results pertaining to the circadian pattern of phagocytic activity are reported in humans also. The polymorphonuclear cells in one of the studies were unresponsive to the LD cycle (Bongrand et al., 1988), while the same cells exhibited diurnal periodicity with peak phagocytosis at midnight in the other study (Melchart et al., 1992). In ectothermic vertebrates, the knowledge is rudimentary and confined to reports in which diurnal rhythmality of humoral innate immune functions is described in fishes, gillhead seabream, and sea bass (Esteban et al., 2006). The peak complement activity in both fishes is reported during the light phase. Immune responses seem dependent on species, strain of animals, and type of immune cells and their specific functions. Neutrophil phagocytosis and oxidative burst activity in reptiles were studied by Froese et al. (2005). Other work related to reptiles is confined to the study of effect of sex steroids on splenic macrophage phagocytic activity (Mondal and Rai, 1999 a, b, 2002a, b).

The innate immune activity of blood cells attains maximal value during day time. In analyzing the influence exerted by the light regimen upon innate immune functions of blood leucocytes, two distinct aspects have to be considered: the circadian structure of the rhythms and the level of the assessed functions. There are several indirect and also direct indicators that melatonin, secreted exclusively at night, would play a role in the immune function. In vitro studies employing pharmacological doses of melatonin (5-10µM) revealed a dose-dependent activation of phagocytic function (Rodriguez et al., 1999). However, such doses are far above the physiologically available range. We may speak, more plausibly, about role of melatonin on innate immune response of N. p. piscator after further experimentation involving in vitro and in vivo melatonin administration and accessing immune parameters. In summary, these data indicate a clear cut variation in innate immune function of blood leucocytes in N. p. piscator. The findings of this study may in part explain the variations by demonstrating changes in innate immune activity of leucocytes to encounter and process antigen.

ACKNOWLEDGEMENT

Financial Grant (SR/90/AS-15/2005) from DST, New Delhi, Govt. of India to RS is gratefully acknowledged.

REFERENCES


DIURNAL VARIATION IN PERIPHERAL BLOOD LEUCOCYTE COUNT IN THE FRESH WATER SNAKE, *NATRIX PISCATOR*

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ABSTRACT: Present study aims at diurnal variation in leucocyte in the fresh-water snake, *Natrix piscator*. Leucocytes are important immune cells and variation in their number is directly related to the immune status of an organism. Snakes were sacrificed during mid day and mid night. Blood was obtained through cardiac puncture and processed for total leucocyte count and differential leucocyte count. Total leucocyte count was significantly higher (p<0.05) during mid night. Thin smear of blood was prepared on a clean glass slide, dried, fixed, stained with Giemsa and scored microscopically in oil immersion to study differential leucocyte count. Eosinophils and neutrophils were significantly higher during mid night. It is evident from this study that peripheral blood leucocytes show diurnal variation in the fresh-water snake, *Natrix piscator*.

Key words: Snake, Leucocytes, Diurnal variation, Natrix piscator.

INTRODUCTION

It is known that most behavioral and physiological functions, including immune function, are expressed rhythmically across day and night. Most of these rhythms appear to be endogenous (Scheping et al., 1968), but are generally synchronized with light: dark cycle. These daily changes enable organisms to adapt daily environmental changes such light, temperature etc. Peripheral blood leucocytes are the primary immune cells. Leucocyte number and its responses are the important immune parameters that vary on the physical conditions of the host. Diurnal variation in hematology parameters of a number of mammals has also been studied. Diurnal variations in selected hematological values in the central and peripheral blood circulation of rabbit was studied by Agnieszka and Moneczewska (1975). So far, non mammalian vertebrate species are concerned; some information is available on fishes and birds (Shaw,1933; Lagler et al.,1962 and Glick,1960). The earliest papers that deal with changes in leucocyte counts during day were written by Sapha (1900), Sabin et al. (1927), Dominus (1931) and Halberg & Visacker (1950). More studies are required to throw light on reptilian leucocyte as reptiles represent the important phylogenetic group. Hence this study was undertaken to explore the diurnal variation in leucocytes count in water snake, *Natrix piscator*.

MATERIAL AND METHODS

**Animal:** Male fresh-water snakes, weighing 50-120 g, were obtained from a local supplier who collected these animals from the suburbs of Varanasi (25°18'N; 83°11'E). Animals were housed in vivarium (wood and wire net cages, size 50x30x30 cm). Snakes were fed on small fish once a week. Cages were cleaned, and bowl water was changed next day following feeding. Animals were acclimatized to the laboratory conditions for two weeks, and experiments were performed. The guideline of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), Ministry of Sciences and Programme Implementation, Government of India, were followed in maintenance and sacrifice of animals.

**Experiment:** Animals were divided into two groups of four animals each - one group was sacrificed at 12 hour mid day and other group at 12 hour mid night. Animals were weighed, anesthetized and blood was collected in a heparinized syringe through cardiac puncture. Blood was processed for total leucocyte count (TLC) and differential leucocyte count (DLC). For DLC, blood smear was prepared on a clean glass slide, dried in air, fixed in methanol and stained in Giemsa. Slides were observed in oil immersion microscopically without any predetermined sequence. For TLC, blood was diluted twenty times in Turk’s solution and leucocyte count was determined on a haemocytometer.

**Statistical analysis:** Data are presented as mean ± SEM. Means were compared, and statistical difference between means were determined by Student's t-test.

RESULTS AND DISCUSSION

Total leucocytes were significantly higher during night (Fig.1). Differential leucocyte count also showed day night variation. Neutrophils and eosinophils were significantly higher during mid night. Lymphocytes and monocytes did not show considerable variation (Fig.2).

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Diurnal rhythms in human bone marrow were first demonstrated in the work of Mauer (1965) and Aardal with Laerum (1983). There is now considerable evidence that magnitude of the immune response varies with the time of the day. The variability in peripheral blood cell count has been noticed. The present study on fresh-water snake N. plicatator was done for the first time in ecotermic vertebrates, demonstrates the day night variation in blood leucocytes. Total leucocyte count was higher during mid night. This result is in parallel with that of Tumbleson et al. (1972), who reported higher leucocyte count during night in swine. Differential leucocytes count were also significantly higher during mid night. Periodic changes in the number of leucocytes circulating in the peripheral blood might result from several factors. These include the distribution of circulating and marginal cell components of tissues and organs, influx from storage sites, cell proliferation release of de novo cells into the circulation, and cell destruction and removal (Haus and Smolansky, 1999) as well as endogenous and exogenous factors. The endogenous factor like endocrine hormone, that itself shows rhythmicity, may be responsible for variation in leukocyte count. Recently Berger (2004) has suggested that exogenous factor like visible light can penetrate the skin and may directly interact with circulatory lymphocytes. In summary, these data indicated a clear cut day night variation in leucocyte number of N. plicatator.

Fig. 1 Day night variation in Total Leucocyte count in the fresh water snake Natrix plicatator (*p<0.05).

Fig. 2 Day night variation in differential Leucocyte count in the fresh water snake Natrix plicatator (*p<0.05).

ACKNOWLEDGMENT

Financial Grant (SR/SO/AS-15/2005) from DST, New Delhi, Govt. of India to RS is gratefully acknowledged.

REFERENCES


Research Paper

EFFECT OF PHOTOPERIOD ON NITRITE PRODUCTION BY LEUCOCYTES IN FRESH WATER SNAKE, NATRIX PISCATOR

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Recent evidence suggests that immune function varies substantially on a seasonal basis. The primary cue to perceive seasonal change is change in photoperiod. The purpose of the present experiment was to study the role of photoperiodic manipulation on the nitric oxide production by peripheral blood leucocytes in the fresh-water snake, Natrix piscator. To study effect of photoperiod, animals were subjected to 24 hour continuous light and continuous dark for 30 days. Animals kept in natural day length served as control. At termination of experiments, animals were sacrificed, and blood was collected. Leucocytes were isolated and were incubated for 24 hours and nitric oxide production was measured by measuring the nitrite concentration. Nitrite production was significantly decreased to the cultures obtained from the animals kept under continuous light while nitrite concentration was increased in animals kept under continuous dark, when compared with the animals kept under natural day length. The possible role of increased melatonin synthesis in dark was suggested to increase the nitric oxide production.

Keywords: Nitric oxide, Photoperiodic manipulation, Snake, Leucocytes

INTRODUCTION

Seasonal phenomena may be imposed on animals by their environment and reflect an interaction between an individual's intrinsic seasonal clocks and the environment. Most formal studies on seasonality have focused on day lengths, i.e., photoperiod as the environmental cue used by animals to coordinate intrinsic seasonal rhythms with extrinsic seasonal environmental changes. Animals undergo seasonal changes in physiological state. Seasonal changes in photoperiod act as direct cues to predict the time of the year. Photoperiodic information is used to initiate or terminate specific seasonal adaptations (Nelson and Demas, 1997). Most studies on seasonality have focused on the role of photoperiod in providing temporal information for reproduction (Reiter, 1991). Environmental factors, such as day length, food availability, temperature, and social interaction, can have pronounced effects on immune function (Klein and Nelson, 1999). Peripheral blood leucocytes

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This article can be downloaded from http://www.ijpmbs.com/currenttissue.php
are primary immune cells and important elements of non specific immunity.

Effect of photoperiodic manipulation on immune parameters have been documented in non tropical rodent species including deer mice (*Peromyscus maniculatus*) (Champney and McMurray, 1991) and prairie voles (*Microtus ochrogaster*) (Nelson *et al.*, 1996), as well as Syrian (*Mesocricetus auratus*) (Brainard *et al.*, 1988) and Siberian (*Phodopus sungorus*) (Drazen *et al.*, 2002; Yellen *et al.*, 1999) hamsters. In some species (e.g. Siberian hamsters, prairie voles) specific immune responses are suppressed in short days. In contrast, individuals of other species (e.g. deer mice, Syrian hamsters) display enhanced immune functions in short winter-like day lengths compared with long summer-like days. Some of the earliest experimental studies implicating melatonin in changes in immune functions involved the functional suppression of melatonin concentration via experimental manipulation of the photoperiod. Effect of photoperiodic manipulation on immune parameters have also been documented in siberian hamsters (Bilbo and Nelson, 2004; Demas *et al.*, 2003).

Nitric Oxide (NO) produced endogenously from L-Arginine by nitric oxide synthetases. Nitric oxide is extremely unstable. It undergoes rapid oxidative degradation to nitrite (NO$_2^-$) and nitrate (NO$_3^-$), which can be spectrophotometrically determined. NO plays an important role in many physiological processes including vascular regulation, immune responses, and neural communication. Some reports are available on seasonal variation in cell-mediated innate immune responses in reptiles (Garcia and Fuente, 1991; Zapata *et al.*, 1992; Munoz *et al.*, 2000; Munoz and Fuente, 2001). Mondal and Rai (2000) have studied the effect of temperature on nitrite production by splenic macrophages in wall lizard, *Hemidactylus flaviviridis*. Study regarding photoperiodic manipulation and nitric oxide production is lacking in fresh-water snake. Hence, present study was undertaken to study the effect of photoperiodic manipulation on nitric oxide production by leucocytes in this species.

**MATERIALS AND METHODS**

**Animals**

Male fresh-water snakes, weighing 80-120g, were obtained from a local supplier who collected these animals in the suburbs of Varanasi (28° 18’N; 83° 1’E). Animals were brought to the unconditioned laboratory. Animals were housed in vivarium (wood and wire net cages; size 50 x 30 x 30 cm). Each cage had an earthen bowl (4L capacity) filled with water and accommodated 4-5 snakes. Snakes were fed on small fishes once a week. Cages were cleaned, and bowl water was changed next day following feeding. The guidelines of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), Ministry of Statistics and Programme Implementation, Government of India, were followed in maintenance and sacrifice of animals.

**Chemicals**

Culture medium (RPMI-1640), L-glutamine, gentamycin, fetal bovine serum (FBS), and other chemicals were purchased from Himedia Laboratories Pvt. Ltd. (India). The culture medium was supplemented with 1 μl ml$^{-1}$ gentamycin, 10 μl ml$^{-1}$ of 200 mM L-glutamine, 10 μl ml$^{-1}$ anti-anti (Gibco) and 5% FBS and referred to as complete culture medium.

**Experiment**

Animals were divided into three groups of five
animals each. Group one animals were maintained in natural light dark cycle (10L:14D) and served as control, group two animals, in continuous light (24L), and group three animals, in continuous dark (24D) for 30 days. Animals were sacrificed at the termination of experiment and blood was drawn in heparinized tubes through cardiac puncture and stored at 4°C.

**Total Leucocyte Count (TLC)**

TLC was performed using Neubauer chamber and routine hematological methods.

**Isolation of Peripheral Blood Leucocyte**

Peripheral Blood Leucocytes (PBL) were collected from the buffy coat (the layer of PBLs between the plasma and RBCs) using a slow spin technique as described by Keller et al. (2005). The tubes were centrifuged at 42 x g for 25 min at 8°C. The PBLs were collected by gently swirling the buffy coat into the plasma and transferring the cells into a new tube. Following centrifugation at 200 x g for 10 min, the plasma was removed and the cell pellet was gently resuspended in 1 ml of complete culture medium. Leucocytes were counted and adjusted to 1x10⁶ cells ml⁻¹ in complete RPMI. Cell viability was checked through trypan blue exclusion test, which exceeded 95%.

**Nitrite Assay**

Nitrite assay was performed following the method of Ding et al. (1988). In brief, 100 µl of leucocytes (10⁶ cells) or standard NaNO₂ was added to 96 well, culture plate in triplicate and incubated for 24 hours at 25°C. Plate was then centrifuged at 200 x g for 10 minutes. 50µl of supernatant and 50 µl of Griess Reagent (25 µl of 1% sulphanilamide prepared in 3 N HCl and 25 µl of 0.1% N-naphthyl ethylenediamine prepared in distilled water) was mixed in microplate and after 10 minutes, absorbance was measured at 540 nm with the help of ELISA plate reader (Thermo). Different concentrations of sodium nitrite were used to obtain the standard curve.

Nitrite concentration (µM) was calculated for each sample. Data are presented as mean ± SEM. Means were compared, and statistical difference between means was determined by Student’s t-test.

**RESULTS AND DISCUSSION**

Total leucocyte count was significantly decreased in animals kept under continuous dark when compared to control (Figure 1). Nitric oxide production, as measured by nitrite concentration, was significantly decreased (p < 0.05) to the cultures obtained from the animals kept under continuous light. In contrast there was significant enhancement in nitric oxide production by leucocytes obtained from animals kept under continuous dark, when compared with animals kept under natural day length (Figure 2).

In the present study, leucocyte number was significantly decreased in the snakes kept under continuous dark. This is in contrast to that reported by Bilbo et al. (2003) and Prendergast et al. (2002); they have reported increased total leucocyte number in short day mammalian species. This may be due to species specific differences. In birds, Kliger et al. (2000) have reported increased heterophil number but decreased lymphocytes in chickens exposed to continuous light. Reactive nitrogen intermediates (RNI), produced by leucocytes and other cell types in response to IFN-α, or IFN-α plus tumour necrosis factor-a (TNF-a) and interleukin-1 (IL-1), have been shown to play an important role in killing of pathogens (Flesch and Kaufmann, 1991). In the present study nitric oxide production...
Figure 1: Effect of Photoperiod on Total Leucocyte Count in the Fresh-Water Snake, *Natrix piscator*

![Graph showing the effect of photoperiod on total leucocyte count.]

Notes: DD - Complete dark; LL - Complete light, *p < 0.05

Figure 2: Effect of Photoperiod onNitric Oxide Production by Peripheral Blood Leucocytes in the Fresh-Water Snake, *Natrix piscator*

![Graph showing the effect of photoperiod on nitric oxide production.]

Notes: DD - Complete dark; LL - Complete light, *p < 0.05

was significantly reduced in the animals kept under continuous light which might be result of reduced synthesis of melatonin in light – i.e., physiological pinealectomy. In analyzing the influence exerted by the light regimen upon innate immune functions of blood leucocyte, two distinct aspects have to be considered: the circadian structure of the rhythms and the level of the assessed functions. Mondal and Rai (2000), have shown that lower temperature suppresses macrophage phagocytosis and nitrite release by splenic macrophages in wall lizard. Sex steroids also play an important role in suppression of nitrite release. There are several indirect and also direct indicators that melatonin, secreted exclusively at night, would play a role in the immune function. *In vitro* studies employing pharmacological doses of melatonin (5-100 µM) revealed a dose-dependent activation of phagocytic function (Rodriguez *et al.*, 1989).
However, such doses are far above the physiologically available range. In summary, the result of the present study shows that continuous light exposure to fresh-water snake reduces nitric oxide production, while continuous dark increases nitric oxide by peripheral blood leucocytes.

CONCLUSION
This study is helpful in understanding the comparative immunology of the reptiles, a phylogenetically important group being ectothermic amniotes. The present work, for the first time, elucidates the role of photoperiod on nitric oxide production in *N. piscator*. In brief, findings of this study indicates that continuous dark enhances innate immune function in fresh-water snake.

ACKNOWLEDGMENT
Financial Grant (SR/ SO/ AS–15/ 2005) from DST, New Delhi, Govt. of India to RS is gratefully acknowledged.

REFERENCES


9. Garcia S and De la Fuente M (1991),


