Chronobiology: The Dimension of Time in Biology and Medicine

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Chronobiology that includes study of biological rhythms (chronos - time; bios - life; logos - science) is a multidisciplinary branch of science. Biological rhythms are pervasive. They are exhibited by all eukaryotes and by at least one group of prokaryotes, the cyanobacteria. Further, all levels of biological integration, such as ecosystem, population, group, individual, organ-system, organ, tissue, cell and subcellular structure exhibit rhythms with diverse frequencies. The periods of most of the documented biological rhythms match with that of any one of the geophysical cycles present in the nature. Any biological variation with an approximately 24-hour period is called a circadian (circa - about, approximately; dies - day or 24 hours) rhythm. It persists even in the absence of environmental time cues like day and night cycle and maintains its periodicity with a t (tau) very close to 24 hours. Thus circadian rhythms are endogenous. The basic circadian system is believed to consist of at least three important components: (1) photoreceptor (2) pacemaker (3) outputs. Molecular bases of cyanobacterial and eukaryotic circadian clocks have been partly understood with respect to their period length, sustainability, and relationship with light. The underlying molecular mechanisms of these clocks seem to have extraordinary degree of evolutionary conservation and appear to be a universal feature of the clocks in models as divergent as algae, fungi, fruit flies, mice and humans. The latest discovery of the hPer2 gene and its homology to *Drosophila* and mouse genes would definitely help in having important insights into the human circadian system. This review outlines the concepts and current developments in the field of chronobiology; the status of chronobiology in India; and discusses the possibility of effectively applying chronobiological principles in the optimization of treatments in the clinics and in the management of problems in shift workers.

Key Words: Circadian rhythm, Prokaryotes, Ultradian, Infradian, Circannual rhythm, PRC, Temperature compensation, Photic & nonphotic entrainment, Pacemaker, Photoreceptor, Clock genes, Chronotherapy, Rhythm desynchronization, Sleep disorder, Shift-work optimization. Chronotypes, Chronoclinic

Introduction
The movements of the planets drive several periodic environmental changes on Earth. The rotation of the Earth on its axis causes the most pervasive natural phenomenon, the day and night. There are many other periodic events in nature, namely the seasons, lunar cycle, tidal cycle, temperature cycle and the like, of which the cycle of day and night is the most dominant and predictable event. The planet Earth exhibited these natural periodic events at the time when organisms evolved on its surface. Therefore, it is not surprising to find or anticipate oscillations, essentially similar in frequency to one of the geophysical counterparts, in many overt behavioral, physiological and biochemical processes in microorganisms, plants, animals and humans (Bünning 1958, 1960, 1973, Halberg 1960, 1969, Pittendrigh 1960, 1981, Palmer 1976, Pati 1982, Bell-Pedersen 1998, Johnson & Golden 1999, Johnson, 2001).

This review is dedicated to the memory of my friend, Prof. R. Subbaraj, the past president of the Indian Society for Chronobiology, who left us at a young age.

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What is Chronobiology?
Chronobiology is a relatively new branch of science and deals with the study of biological rhythms and their underlying mechanisms. All levels of biological integration: ecosystem, population, group, individual, organ-system, organ, tissue, cell and subcellular structure exhibit rhythms with different frequencies (Bünning 1958, 1960, 1973, Halberg 1960, Pittendrigh 1960, Bell-Pedersen 1998, Johnson & Golden 1999, Johnson 2001). Among other major subspecialities, chronobiology includes: chronophysiology, chronopathology and chronopharmacology. In addition, chronopharmacology includes chronotoxicology and chronotherapy in its premise. Also, the study of problems associated with transcontinental travels (jet lag) and shift work falls in the domain of chronobiology.

Chronophysics
It focuses on temporal aspects of various physiological processes, namely of nervous, endocrine and metabolic systems and their interactions with the environment.

Chronopathology
It describes alterations in biological temporal characteristics of organisms attributed to pathological state, such as psychoses, cancers, endocrinopathies, ulcers, and blood pressure disease.

Chronopharmacology
It evaluates harmful/undesired effects of drugs, poisons, pollutants and other agents as function of timing of their administration (chronotoxicology). It also includes chronotherapy that concerns cure or prevention of disease with proper regard to timing of administration of various types of therapeutics. Reinberg et al. (1975) coined many other terms which are pertinent to chronopharmacology and chronotherapy. These terms are discussed later in an exclusive section devoted to chronotherapy.

Historical Outline
In various species of plants, leaves are held more or less horizontally during the day and assume an upward or downward position at night. The credit for observing biological rhythm for the first time goes to a Greek philosopher cum an army officer, Androsthenes in the corps of Alexander the great (350 B.C.), who during the later’s march on India witnessed and made a note in his dairy that the leaves of Tamarindus indica exhibit sleep movement (Thapliyal 1980a). Similar observations were also made by the astronomer J. J. de Mairan in 1729. He observed that the rhythm in leaf movements persisted when plants were transferred from daily light/dark cycles to continuous darkness. He related this phenomenon to some kind of biological timing mechanism. In 1751 Linnaeus constructed a floral clock after carefully studying the time of opening and closing of petals of various flowers belonging to different species. A Genevan plant physiologist, de Candolle (1832) documented for the first time that the leaf movements of Mimosa pudica persist in constant conditions with a period of about 22-23 hr, differing from 24 hr.

Later, Wilhelm Pfeffer conducted an experiment on Flemingia congesta in 1914 and objectively documented circadian rhythm in leaf movements (Pfeffer 1915).

J. C. Bose was the first Indian who performed elegant experiments and demonstrated biological rhythms in many plant species (figure 1). He demonstrated diurnal variation of excitability in Mimosa (Bose 1913), daily opening and closing of the leaflets in Cassia alata (Bose 1927) and diurnal

![Figure 1 Diurnal movement of the leaflet of Cassia alata. The leaflets remain tightly closed during the night. But from early morning onwards they begin to open and remain widely spread out throughout the day. (Modified from Bose 1927)]
rhythms in petal movements in water lily, *Nymphaea* and *Jhinga, Luffa acutangula* (Bose 1927). He demonstrated the phenomenon of entrainment of rhythms in various kinds of plant movements to alteration of light and darkness. Further, although Bose was not aware of it, some of the plants he was studying were actually expressing endogenous rhythms.

Sanctorius was the first to demonstrate biological rhythms in humans. In 1657, he constructed a huge balance with a tray upon which a completely furnished room was set. He lived on his balance tray for several consecutive months and recorded readings on the balance scale. He later made repeated self-measurements of physiological variables as function of time to document bioperiodic phenomena. This procedure of self-measurement is now termed autorhythmometry. This term was coined first by Halberg and his colleagues (Halberg et al. 1977a). Sanctorius reported a monthly rhythm both in his body weight and in the turbidity of his urine. Later Seguin & Lavoisier (1797) reported a circadian rhythm in the body weight of the healthy human male. They emphasized that a subject who does not exhibit a circadian rhythm in his body weight should be suspected to be ill.

Schweig in 1843 and Vogel in 1854 discovered within-day variations in the excretion of urine. In 1845, Zehetmeier observed the change in the timing of circadian urinary volume rhythms in patients with heart disease, designating it nycturia (Jores 1975). J. Davy (1845) was the first to report the existence of both circadian and circannual rhythms in his own body core temperature. Jürgensen in 1873 objectively described what we now call circadian variations of human body temperature.

In the real sense circadian rhythm research started in 1930s when Erwin Büning and his colleagues discovered free running rhythm in leaf movement of the common bean *Phaseolus vulgaris* (Bünning & Stern 1930, Büning et al. 1930, Büning 1931, 1935). He observed that the leaf movements of beans oscillated in constant darkness with a period of about 25.4 hr and he was also the first to demonstrate that exposure to light/dark cycle of 24 hr could entrain the 25.4 hr free-running rhythm of *Phaseolus* leaf movement (reviewed by Sweeney 1987). This was the period when von Frisch (1950) and von Frisch & Lindauer (1954) observed the remarkable time sense in bees and Kramer (1950) demonstrated that starlings used the sun as a compass to migrate. Hoffmann (1960) further demonstrated that the clock persisted in constant dim light and thus is endogenous to the birds. Chronobiology witnessed major breakthroughs in the mid-twentieth century (1950-60). The temperature compensatory behavior of circadian clocks was demonstrated by Pittendrigh (1954). By then Büning and Pittendrigh together discovered all three important diagnostic features of circadian clocks which are valid even today. During this period chronobiology was accepted as an important quantitative biological science (Pittendrigh 1960). Now, it is well known that the biological rhythms have far-reaching implications. Undoubtedly, it is going to remain in the fore front of biomedical research in this century.

### Types of Rhythms

Rhythms, depending upon the periods they exhibit, are mainly classified into three types, viz., ultradian, circadian and infradian. Circadian rhythm relates to any biological variations with a frequency of 1 cycle in 24 ± 4 hr; circa (approximately) and dies (24 hr). Ultradian rhythm exhibits a frequency (τ < 20 hr) higher than that of the circadian, whereas, infradian rhythm relates to certain biological variations that shows a frequency (τ > 28 hr) lower than that of the circadian (Halberg et al. 1977a). One may come across many other terms describing rhythms in organisms, namely circatidal rhythm or tidal rhythm, diurnal rhythm, diel rhythm, circalunar rhythm or lunar rhythm or circasemisemal rhythm or circasynodic rhythm in chronobiological literature. However, there is no reason to become confused by these terms. The tidal rhythm falls in the ultradian category. The lunar rhythm is synonymous with circatrigintan rhythm. Among all rhythms described above circadian rhythm is the most studied one. In this review, therefore, due emphasis shall be laid on this rhythm only. The term *circadian* was coined by Halberg (1959) to replace the formerly used terms such as daily, diurnal, diel, 24-hour, and nycthemeral.

### Circadian Rhythm

Organisms often exhibit rhythmic behavior in association with daily alterations of light and darkness. Interestingly, many of the rhythmic
responses to day and night continue even when organisms are kept under artificially made constant light (LL) or continuous dark (DD) environment. While in some organisms these rhythmic behaviors persist under constant conditions at least for a period of time, in others they persist indefinitely. Thus, by definition biological rhythms that continue to persist with a period of about a day with all obvious environmental factors constant are called circadian rhythms.

**Circadian Rhythm in Prokaryotes**

It was first proposed by Halberg & Conner (1961) that *Escherichia coli* exhibits rhythmic behavior with a $\tau = 24$ hr. Later Sturtevant reported circadian variation in *Klebsiella* (1973a) and *E. coli* (1973b). However, in these studies the diagnostic features of the circadian rhythm were not tested except that the oscillation in the growth rate of *Klebsiella* sp. was described to be $\sim 24$ hr. Therefore it was generally believed, until Stal & Krumbein (1985) reported circadian clock-controlled metabolic activities in *Oscillatoria* sp., that probably prokaryotes do not possess a circadian machinery (Johnson et al. 1996). The doctrine that prokaryotes are too simple to express circadian rhythm was so strong that articles written even in 1990s do not mention anything about circadian rhythms in prokaryotes (Chandrashekar 1994, 1997). Now circadian rhythms have been demonstrated in a number of cyanobacterial species, namely *Synechococcus* sp. strain PCC 7942 (Kondo et al. 1993), and the genera *Synechocystis* (Aoki et al. 1995, 1997), *Cyanotheca* (Schneegurt et al. 1994, 1997) and *Trichodesmium* (Ronneberg & Carpenter 1993, Capone et al. 1997, Ronneberg & Deng 1997, Chen et al. 1998). However, till this day according to Johnson & Golden (1999) "there is no persuasive evidence that circadian clocks reside in prokaryotes other than cyanobacteria".

**Properties of Circadian Rhythm**

All circadian rhythms have following properties in common (Thomas & Vince-Prue 1997): (1) The rhythms oscillate with a period length close to, but seldom equal to 24 hr, when organisms are kept under constant conditions of light, temperature and other possible geophysical factors, which could provide information about time to the organisms. Under these conditions the rhythm is said to be free-running and its period is called free-running period (figure 2); (2) The period of the free-running rhythm is temperature compensated within the physiological range. It has a $Q_{10}$ close to 1, with observed values ranging from 0.8 to 1.3; (3) The rhythms are entrained to 24 hr cycles of environmental time cues, most commonly the light/dark transition at dawn and dusk. They may also get entrained to periodicity in other geophysical factors, namely temperature and humidity.

Interestingly, the circadian rhythms may also differ from one another in a number of ways: (1) One rhythm may be more sensitive to light, while others are less; (2) The patterns of the phase response curves (PRCs) may vary; (3) Some rhythms are reentrained quickly, while others may take several days before achieving a stable reentrainment; (4) Some rhythms damp quickly, while others persist for several cycles in a constant environment; (5) When rhythms persist in continuous light, the characteristics of the rhythm

![Figure 2](image-url) Activity records showing idealized circadian rhythms immediately after a transfer from light/dark cycle (LD) to constant darkness. In the LD cycle the rhythm is entrained. The activity (dark line) occurring only during the dark period. In DD the rhythm free-runs and the onset of activity occurring later (upper panel) or earlier (lower panel) each day. The successive 24-hr records are arranged one above the other.
(period and PRC) may vary with the colour and/or intensity of the background illumination (Thomas & Vince-Prue 1997).

**Influence of Light**

The most powerful time cue for circadian rhythms is the day/night cycle. The transitions from dark to light and from light to dark act as important temporal signals. These signals are perceived and processed by the organisms every day so as to keep their circadian clock(s) tuned to the natural day-night cycle. In nature, depending upon the season the length of the day (or of the night) keeps on increasing or decreasing gradually, therefore, at any given place, the timings of sunrise or sunset keep on changing, although in a predictable fashion. Thus, time lag between any phase of organism's bodily rhythm and dawn or dusk (phase angle) is likely to alter significantly unless adequate corrections are made to keep the rhythms in a state of entrainment (Dann & Aschoff 1975).

The sensitivity of an organism to light does not remain constant all along a 24-hr day-night cycle. The sensitivity to light keeps on changing periodically and the sensitivity estimated as the magnitude of phase shift in a rhythm depends on the phase of the circadian clock at which the stimulus is perceived. Phase response curves (PRCs) are plots of these phase-dependent responsiveness of circadian clocks to light. PRCs can also be constructed for other time cues like temperature and chemical stimuli. The responses to a given stimulus in one species could be strong, whereas in another it may be weak irrespective of the strength (both quality and quantity) of the stimulus. On the basis of this behavior the PRCs are classified under two different categories (figure 3): (1) Type-0 (strong responses), (2) Type-1 (weak responses). Phase response curves to single light pulses have been described for several organisms. The pattern of PRC is species specific (Johnson 1990, 1992).

However, in a given species one may obtain a family of curves depending upon the strength of the stimulus (both duration and intensity). It is important to mention here that various phases of circadian oscillation are expressed in circadian time (CT), i.e., subjective day from CT0-12 and subjective night from CT12-24. For example, in a diurnal animal with steady state entrainment to LD 12:12 activity begins at CT0, whereas in a nocturnal animal onset of activity coincides with the CT12. In this condition both CT and ZT (zeitgeber time) scales coincide. However, when the photoperiod or the zeitgeber period is changed or under constant darkness or continuous illumination CT-point may come to lie at quite a different ZT-point. The term CT is used for the sake of convention and convenience. Thus CT12 can be defined as the phase in the freerun which extrapolates back to the last dusk (i.e., lights off of the last seen LD 12:12 cycle prior to release into constant conditions - DD or LL).

In many animal species, circadian rhythms in locomotory activity persist in continuous light (LL) as well as in constant darkness (DD), but with their $\tau$ altered. This phenomenon appears to be universal specially among the vertebrates. This generality is known as *Aschoff's rule*. This rule states that $\tau$ lengthens with an increase in light intensity or

![Figure 3 Schematic phase response curves (PRCs) showing Winfree's (1971) Type-0 curve (A) and Type-1 curve (B).](image-url)
transfer from DD to LL for dark active animals (i.e., \( \tau_{DD} < \tau_{LL} \), nocturnal) but shortens for light active animals (i.e., \( \tau_{DD} > \tau_{LL} \), diurnal).

In higher vertebrates, the ratio of activity time (\( \alpha \)) to rest time (\( \rho \)) in each daily cycle and the overall amount of activity increase with light intensity in diurnal species, but decrease with light intensity in nocturnal species. This phenomenon is called \textbf{Circadian rule}.

The quality of the light has been known to influence circadian rhythm parameters in various organisms (Pittendrigh 1960). This is more pronounced in plants. In the bean, \textit{Phaseolus coccineus}, for example, \( \tau \) for leaf movement is longer (28.1 hr) in LL red light (610-690 nm) and shorter (24.7 hr) in LL far red (690-850 nm). The threshold light value for the promotion and inhibition of flowering has been worked out for a number of short-day and long-day plant species (Thomas & Vince-Prue 1997). Also, there are number of studies that document the influence of light irradiance and wavelength on circadian clock parameters of various animal species. For example, the free running period in Djungarian hamsters has been shown to change as function of light irradiance (Klante & Steinlechner 1994). Circadian rhythm in locomotor activity of an Indian mouse, \textit{Mus booduga} entrains to RD (red light:darkness) cycles only when the imposed light intensity remains at about or above 150 mW/cm\(^2\) (Viswanathan & Chandrashekaran 1985).

\textbf{Influence of Nonphotic Stimuli}

\textbf{Temperature}

The temperature is another important time cue of circadian rhythms. In nature, organisms experience temperature cycles with peaks coinciding with the early part of the afternoon and troughs in the late dark phase (close to dawn). However, it has been well established that free-running period of a circadian rhythm is temperature compensated (figure 4). Normally, a number of rate processes within an organism are very sensitive to temperature. The rates of those processes double or triple with every 10°C rise in temperature. The \( Q_{10} \) for any such process is said to be 2 or 3 if rate increases 2-fold or 3-fold, respectively, for every 10°C increase in temperature. Any system is said to be temperature-compensated if its \( Q_{10} \) value is 1. The \( Q_{10} \) for free-running period of circadian rhythms vary between 0.8 and 1.3. The amplitude of temperature cycle is another important factor that influences circadian rhythms. The amplitude of temperature cycle as low as 1°C may synchronize circadian rhythms in a number of plant species and ectothermic vertebrates, whereas high amplitude thermal cycles, for example, 15°C-30°C, fail to influence activity rhythms of various rodents. The phenomenon of temperature compensation for the rhythm of gene expression in cyanobacterium, \textit{Synechococcus} has been studied. The \( Q_{10} \) for the free-running period is almost the same in the range of 25-36°C (Kondo et al. 1993).

\textbf{Other Nonphotic Stimuli}

Results of the experiments performed by Halberg and his colleagues in 1950s (Halberg & Visscher 1952, Halberg et al. 1953, 1954) provided an indication that the stimuli other than the light/dark cycle may possess abilities to entrain circadian physiological rhythms. Subsequent studies demonstrated that periodic meal-feeding can synchronize several variables and may even override the influence of the LD cycle (Pauly et al. 1975, Apfelbaum et al. 1976, Philippens et al. 1977, Pradhan et al. 1989, Mistlberger 1994, Mrosovsky 1996). Sharma et al. (2000b) also suggested that restricted feeding cycle can modify the expression of light-entrainable pacemaker (LEP) in field mouse, \textit{Mus booduga}. Later it was discovered that not only meal-scheduling (Halberg et al. 1954) but
many other nonphotic stimuli, such as novelty, activity, motivation and arousal (Janik & Mrosovsky 1992, Bobzynska & Mrosovsky 1994), cortisol (Sumova et al. 1994), melatonin (Hastings et al. 1992), dark pulse/benzodiazepines (Subbaraj & Chandrashekaran 1975, Van Reeth & Turek 1989) presence of females (Aschoff & Goetz 1988), presence/absence of mother (Viswanathan & Chandrashekaran 1985), song cycle (Gwinner 1966), noise (Lohmann & Enright 1967, Rees 1989), and social activity (Crowley & Bovet 1980, Regal & Connolly 1980, Marimuthu et al. 1981) can also entrain and phase shift circadian rhythms. Sometimes phase-shifts induced by nonphotic stimuli can be more than or as large as those are to light pulses (Mrosovsky 1996). The most remarkable effects of nonphotic entrainment of circadian rhythm have been reported in hamsters (Mrosovsky 1996). A single bout of running activity in the middle of their usual sleep phase (subjective day) has been demonstrated to induce a phase shift of 2-4 hr. Recently it has been demonstrated that nonphotic resetting of the mammalian clock is associated with the acute suppression of the putative clock genes in the SCN (Maywood et al. 1999). This is obviously an interesting area of inquiry specially because in nature both photic and nonphotic stimuli exist together and are expected to interact with each other while inducing phase shifts. At present we have practically no knowledge on the nature of their interaction.

Organization of the Circadian System

Circadian rhythms are endogenous. They are both an organismal and a cellular phenomenon (Johnson et al. 1998). Light/dark cycle works as one of the strongest entrainers of these rhythms, although other periodic cycles can also entrain circadian rhythms. They free-run in the absence of a light/dark cycle. This suggests that there is a pacemaker(s) which generates circadian rhythms in various physiological, biochemical and behavioral variables. Thus a basic circadian system has necessarily three important components, such as photoreceptors, pacemaker(s), and observable rhythmic outputs (figure 5). The entraining pathways transduce information between the photoreceptive elements and the pacemaker(s). The coupling pathways are also necessary as a link (the efferent pathways) between the pacemaker(s) and the multiple effector systems. The effector outputs are the overt rhythms that allow us to study the properties of the central clockwork (pacemaker). There may be multiple photoreceptors, multiple clocks and many overt rhythms (figure 6). These components may interact with each other in a variety of ways. A possible operation of feedback of the clock onto the photoreceptive pathway has been strongly suspected (figure 6).

In most of the animals it was found that the eyes possess the primary photoreceptors. They (except mammals) also have an ability to perceive extraoptic light signals. However, in 1998 it was demonstrated that circadian rhythm in body temperature and melatonin concentration in humans could be phase shifted by light pulses presented to the popliteal (behind the knee) region (Campbell & Murphy 1998). The presence of blood-borne photoreceptors has also been suspected to play a key role in the extraocular photoperception. However, later three papers appeared that ruled out Campbell & Murphy’s hypothesis. Eastman et al. (2000) reported failure of extraocular light to facilitate circadian rhythm reentrainment in humans. Yamazaki et al. (1999) and Meijer et al. (1999) did not find any evidence in favour of the hypothesis that extraocular phototransduction is capable of inducing phase shifts of the locomotor activity rhythm or of suppressing pineal melatonin synthesis in hamsters.
Hunt for the Anatomical Loci of the Clocks
In the past, several attempts have been made to locate the endogenous circadian clock in the animal body by performing lesion experiments with the presumption that the destruction of the pacemaker would result in the disappearance or desynchronization of the overt rhythms. Such attempts have been made in diverse animal models, such as cockroaches, crickets, moths, crayfish, crabs, molluscs, reptiles, amphibians, fish, birds and mammals (Kawamura & Ibuka 1978).

In most of the animals the master clock is located in the central nervous system. The circadian clocks in cockroach and cricket have been shown to be localized in the optic lobe (Kawamura & Ibuka 1978). The master circadian clock of mammals resides in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Reppert & Weaver 2001). It has been documented that SCN has a number of single-cell circadian oscillators and in entrained state these oscillators regulate overt circadian rhythms through the generation of coordinated circadian outputs.

In lower vertebrates, experimental evidence suggested that the pineal and retina contain endogenous pacemakers (Herzog & Block 1999). In higher plants, populations of pulvinar and guard cell protoplasts have been shown to function as endogenous clocks (Johnson et al. 1998). The retinal clocks have also been demonstrated in mammals (Tosini & Menaker 1996, Herzog & Block 1999). Now there is evidence to presume that probably all mammalian cells may contain a circadian clock (Whitmore et al. 1998). Further, Plautz et al. (1997) demonstrated expression of circadian rhythms in almost all tissues except ovary in *Drosophila*. These rhythms persisted in vitro, most importantly, were also entrainable by light (Plautz et al. 1997).

Molecular Components of Circadian Clock
The discovery of X-chromosome-linked period (per) mutations in *Drosophila* by Konopka & Benzer (1971) has laid the foundation of molecular chronobiology. The period gene appeared to possess three mutant
alleles, namely long period gene \((\text{per}^l)\), short period gene \((\text{per}^s)\) and an abolish-period gene \((\text{per}^a)\) mutants (Takahashi 1993). The \(\text{per}^l\) and \(\text{per}^s\) mutants were found to have lengthened \((\tau = 29\ \text{hr})\) and shortened \((\tau = 19\ \text{hr})\) circadian rhythms, respectively. The \(\text{per}^a\) mutants did not exhibit circadian rhythms in locomotor activity. The importance of \(\text{per}\) gene in the circadian clock of \textit{Drosophila} has led to a series of interesting studies aimed at understanding clock genes in a range of other systems, such as cyanobacteria, green algae, fungi, higher plants and mammals. These studies conducted in the last couple of decades have partly unravelled the molecular basis of circadian clocks (Devlin & Kay 2001, Johnson 2001, Loros & Dunlap 2001, Reppert & Weaver 2001, Williams & Sehgal 2001).

The \(\text{per}\) gene has sequence homology with the \textit{single-minded} \((\text{sim})\) gene, ARNT (human aryl hydrocarbon receptor nuclear translocator) gene in the \textit{Drosophila} genome. All these genes share a common PAS (PER, ARNAT, SIM) protein domain (Takahashi 1993). This domain has been implicated in the protein-protein interaction. PER protein binds with the protein product of another clock gene \textit{tim} \((\text{timeless})\). Both \text{PER} and \text{TIM} have been shown to be involved in the circadian systems of \textit{Drosophila}.

Recent developments in the field of molecular chronobiology revealed two common features among clocks in all organisms (Devlin & Kay 2001). Firstly, all clocks belonging to diverse systems are intimately associated with their photoreceptors. Secondly, the presence of PAS domain as one of the critical components of all clocks made up of a transcriptional feedback loop. The clocks may differ from one another in two different ways: (1) the molecular mechanisms of photoreception may be different in different systems and (2) the highly conserved transcriptional-translational-feedback loop of the clocks in microorganisms, plants, insects, and mammals may consist of different components and may require different types of molecular interaction between these components to generate the feedback loop (Devlin & Kay 2001).

\textbf{Input Component}

Light is a dominant time cue of circadian clocks. Till this day a complete information regarding the mechanisms of circadian photosensitivity in different model systems are not available. However, one thing is becoming increasingly clear that in vertebrate systems the photoreceptors mediating circadian responses may be different from those of the visual system. The light input pathway and its molecular components have been partly understood in several organisms.

In \textit{Gonyaulax polyedra} two distinct light input pathways have been shown (figure 7). A combined red/blue-light sensitive pathway has been shown to induce phase delays during the subjective day whereas a different blue-light sensitive pathway causes phase advances during the subjective night. It was also discovered that the process of photosynthesis and flavoprotein have some modulatory role in the mechanism of photoperception through the involvement of red/blue-light sensitive and blue-light sensitive pathways, respectively (Devlin & Kay 2001). This conclusion has been drawn by performing experiments that used specific inhibitors for photosynthetic electron transport chain and flavoprotein.

In \textit{Neurospora crassa} the white-collar genes \textit{wc-1} and \textit{wc-2} are essential for the photoresponses (figure 8). It has been shown that \textit{wc-1} and \textit{wc-2} mutants do not exhibit rhythmic clock-controlled conidiation after a light-to-dark transfer (Russo 1988). Interestingly in \textit{N. crassa} these genes are responsible for both photo and thermo responses as \textit{wc-1} and \textit{wc-2} mutants do not show entrainment to either light or temperature. WC-1 and WC-2 have been shown to bear sequence similarity to the GATA family of transcription factors found in fungi and vertebrates.

![Figure 7 Light input to the clock in Gonyaulax polyedra. Schematic diagram of light input pathways to the clock in \textit{G. polyedra}. A combined red- and blue-light-sensitive pathway shows a requirement for photosynthesis, whereas a second blue-light-sensitive pathway shows a requirement for flavoprotein. (After Devlin & Kay 2001, With permission, from The Annual Review of Physiology, Volume 63, ©2001, by Annual Reviews).](image-url)
Both contain a PAS domain common to a number of higher plant photoreceptors and to components of the clock itself in insects and mammals (Bell-Pedersen 1998).

Cryptochrome, a light-absorbing protein was first discovered in Arabidopsis thaliana and are present in two forms, CRY1 and CRY2 (Devlin & Kay 1999, 2001). It has been shown recently that these proteins are also present in fruit flies and mice (figures 9, 10). However, in Drosophila melanogaster a single cryptochrome dCRY has been demonstrated (Stanewsky et al. 1998) whereas in mice two cryptochromes, mCRY-1 and mCRY-2 are present (Vitaterna et al. 1999). It has been suspected that in Drosophila apart from dCRY some other cryptochromes may be involved in the process of photoperception, since the rhythm of PER and TIM expression within lateral neuron cells remains entrainable by light in cry\textsuperscript{b} mutants, which lacks cryptochrome. Now there is evidence to suggest that mCRYs are probably functioning as both photoreceptors and clock components. It has been recently demonstrated that mutant mice lacking mCRY1 and mCRY2 are arrhythmic in constant conditions (van der Horst et al. 1999). However, it has been suspected that in mice photoreceptors other than the cryptochromes may be involved in the mechanism of circadian photoreception (Devlin & Kay 2001). The mechanism of circadian photoreception requires further elucidation in order to answer many other interesting and relevant questions.

**Clock Component**

The fungus N. crassa and the fruitfly D. melanogaster are two such model systems that have provided significant information about the molecular component of the circadian clocks (Devlin & Kay 2001, Loros & Dunlap 2001, Williams & Sehgal 2001). As has been described earlier some of the components of the clock and the generalized scheme of a molecular feedback loop appear to be conserved in all organisms (Bell-Pedersen 1998, Devlin & Kay 2001).

A clock component must fulfil three important conditions: (1) The activity or the amount of the

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**Figure 8** Light input to the clock in Neurospora crassa. The *FREQUENCY* (*FRQ*) gene forms a negative, autoregulatory transcriptional-feedback loop that is essential for light entrainment of the N. crassa circadian clock. The white-collar proteins WC1 and WC2 (wc1 and wc2 in the figure) act as positive regulators of *FRQ* transcription, with WC1 essential for light induction of *FRQ* leading to phase resetting. (After Devlin & Kay 2001, With permission, from The Annual Review of Physiology, Volume 63, ©2001, by Annual Reviews).

**Figure 9** Light input to the circadian clock in Drosophila melanogaster. The blue-light photoreceptor cryptochrome (cry) binds to the timeless protein (tim) in a light-dependent manner. This resets the phase of the clock by removing the PER-TIM-mediated repression of the CLK-CYC transcriptional-activation complex. (After Devlin & Kay 2001, With permission, from The Annual Review of Physiology, Volume 63, ©2001, by Annual Reviews).
component should oscillate; (2) Complete loss of the component must lead to arrhythmicity; and (3) A phase shift in the clock should follow a transient change in the level of the component (Block et al. 1993, Somers & Kay 1998).

In *N. crassa* the *frq* gene is a rhythmically expressed critical clock component and plays a role in the autoregulatory feedback loop (Loros & Dunlap 2001). A complete cycle of this loop takes about 24 hours and involves repression and activation of the *frq* transcript (Merrow et al. 1997, Bell-Pedersen 1998). The amount of *frq* transcript begins to rise at dawn and thereafter *frq* mRNA reaches its peak (around noon) and FRQ protein begins to accumulate (Garceau et al. 1997). Then FRQ protein enters the nucleus and starts to repress the levels of *frq* mRNA. For the rest part of the day and early evening FRQ protein continues to keep *frq* switched off. The cycle restarts when FRQ falls below a critical level that can no longer repress *frq* along with its activation by positive factors encoded by *wc-1* and *wc-2* (Crossthwaite et al. 1997). It has been shown that mutations in *wc-1* completely block light induction of *frq* and mutations in *wc-2* allow only a transient *frq* photoresponse. Further, expression of *frq* observed in *wc-2* mutant strains is not sufficient for circadian oscillations of *frq*. These findings suggest that while both *wc-1* and *wc-2* are the critical clock components, *wc-1* is a component that provides a link between the photoreceptor and the clock (Talora et al. 1999, Devlin & Kay 2001, Loros & Dunlap 2001).

The underlying molecular mechanism of circadian rhythms in pupal eclosion and adult locomotor activity in *D. melanogaster* is again a Neurospora-like 24 hr transcriptional/translational based feedback loop (figure 11). In *Drosophila*, transcription of the period (*per*) and timeless (*tim*) genes begins late in the day induced by CLOCK-CYCLE hetero-dimer, and both genes peak in accumulation just after dusk (Sehgal et al. 1994, 1995, Darlington et al. 1998, Devlin & Kay 2001). Both *PER* and *TIM* proteins display an approximate 6 hr lag in peak levels relative to peak in their mRNA levels (Lee et al. 1996). Then stabilization and heteromerization of PER and TIM take place in the absence of light. The PER-TIM Complex then enters into the nucleus around midnight and inhibits further transcription of *per* and *tim* by repressing the activity of a positively acting transcriptional - activation complex made up of CLOCK and CYCLE. Then the cycle repeats following a decline in the levels of *PER* and *TIM* just before dawn (Sehgal et al. 1995, Darlington et al. 1998).

The involvement of some additional protein in the regulation of feedback loop has been demonstrated. A protein called DOUBLETIME (DBT) acts on this loop by way of phosphorylating and destabilizing PER and thus delaying the speed of the cycle. This helps in maintaining the period of the rhythms approximately at 24 hr. Of late the role of another protein VRILLE (VRI) has been demonstrated to be involved in the *Drosophila* clock (Blau & Young 1999).

In mice there are three *PER* genes (m*PER1*, m*PER2*, m*PER3*), two *CRY* genes (m*CRY1*, m*CRY2*) and *TIM*, *CLK*, and *CYC* (also called BMAL1). These components interact with each other and generate two interlocking feedback loops (Devlin & Kay 2001). The first vertebrate clock gene, CLOCK, has been cloned and characterized by Takahashi and his colleagues (Antoch et al. 1997, King et al. 1997). The Clock mutants have been shown to exhibit period lengthening and eventual arrhythmicity in constant darkness (Vitaterna et al. 1994). The CLOCK protein has a PAS-domain motif. The *PER* and *CRY* genes
are the important constituents of a transcriptional-feedback loop in mice (figure 12). They negatively feedback on their own transcription. Homo- and heterodimers such as CRY-CRY and CRY-PER enter the nucleus and repress the activity of CLK-CYC. There is evidence to suggest that the CLK-CYC transcriptional complex is affected largely by the CRY proteins (Reppert & Weaver 2001). In another interlocking loop PER2 has been shown to act as a positive activator of CYC transcription, whereas CRY has been demonstrated to stabilize PER2. However, the role of mTIM in the regulation of the mammalian circadian clock has not yet been understood clearly, although it has been shown that the mammalian CRYs are able to dimerize with the mPER and mTIM proteins.

The clock genes have also been studied in cyanobacteria (Johnson 2001) and A. thaliana (Devlin & Kay 2001). Three genes, KaiA, KaiB, and KaiC have been identified in cyanobacteria that specifically affect circadian phenotypes. These genes are adjacent to each other on the chromosome like a cluster. The products of this clock gene clusters KAIA, KAIB, and KAIC appear to interact with each other and are the components of an autoregulatory feedback loop.

![Figure 12: The mechanism of the circadian clock in mice.](image)

The PER and CRY genes in mice form a transcriptional-feedback loop that negatively feeds back on their own transcription. Mice possess three PER genes and two CRY genes (per and cry in figure). CRY-PER and CRY-CRY homo and heterodimers reenter the nucleus and repress the activity of a CLK-CYC (clk and cye in figure) transcriptional-activation complex. In a second, interlocking loop, PER2 acts as a positive activator of CYC transcription, whereas CRY stabilizes PER2. (After Devlin & Kay 2001, With permission, from The Annual Review of Physiology, Volume 63, ©2001, by Annual Reviews).

In Arabidopsis thaliana both the red-light-activated phytochromes (phy) and the blue-light-activated cryptochromes (cry) act as photoreceptors that mediate light input to the clock. Further it has been suggested that cry1 acts as a signal transduction component downstream of phyA in light input to the clock (Devlin & Kay 2001). Available evidence favours TOC1 as a genuine molecular component of the Arabidopsis clock (Somers & Kay 1998).

Recently it was discovered that a mutation in a gene, hPer2 is responsible for familial advanced sleep-phase syndrome (FASPS) in members of a Utah family (Toh et al. 2001). These authors further found out that this gene is homologous to Drosophila and mouse genes that when mutated were known to speed up circadian rhythm. It was further demonstrated that the mutation disrupted phosphorylation of the hPER2 protein by an enzyme, casein kinase epsilon (Toh et al. 2001).

The recent upsurge in the techniques in molecular biology may eventually help us in answering some of the basic questions related to the enigmatic circadian clock and its evolutionary significance.

**Evolutionary Significance of Circadian Rhythms**

When did circadian clocks evolve? Nobody knows when or how these clocks evolved, although several models have been proposed (Edmunds 1988). Surprisingly, we also do not have much experimental support in favour of the reproductive fitness of circadian rhythms. The circadian basis of photoperiodic time measurement in seasonal breeders advocates in support of the adaptive value of circadian clocks. The study by Ouyang et al. (1998) suggested for the first time that the fitness of an organism is likely to be higher if there is a resonance between the endogenous clock and the environmental cycle (figure 13). Their suggestion was based upon competition experiments involving asxual cyanobacteria. A mixture of different strains were grown together in competition and changes in the composition of the population as a function of time were monitored. It was revealed that the strain whose endogenous free-running period most closely matched that of the imposed LD cycle was able to out-compete others with a nonoptimal period. It has been demonstrated that a circadian phenotype having a free-running period of about 30 hr out-compete its
Figure 13 Competition of circadian strains in different light/dark cycles. A. Different strains of cyanobacteria were mixed together in batch cultures and grown in competition under different light/dark cycles. Every 8 days, the cultures were diluted with fresh medium. At various times during the competition, aliquots were plated as single colonies, and the luminescence rhythms of individual colonies were monitored to determine the frequency distribution of the different circadian phenotypes. B. The strain whose endogenous free-running period most closely matched that of the environmental light/dark cycle was able to out-compete strains with a non-optimal period. In constant light (non-selective conditions), all the strains were able to maintain their initial fraction in the population. C. Phase of a luminescence rhythm for wild-type (wt) and the mutant exhibiting a 30-hr period (C28a) on a light/dark cycle of 12 hr light/12 hr dark (left) versus 15 hr light/15 hr dark (right). (After Johnson

wild-type counterpart when grown on a LD 15:15 hr cycle. However, the wild-type strain was found to be superior to the tau (τ = 30 hr) phenotype strain when grown on LD 12:12 hr cycle. Further, recently the adaptive significance of circadian rhythm in a Drosophila parasitoids has been investigated (Fleury et al. 2000). However, the reproductive fitness under competition in natural conditions for circadian clocks in any organism has not yet been measured systematically (Johnson et al. 1998). It has been speculated that probably the circadian clocks evolved first in the cyanobacteria that appeared very early in the fossil record about 3.8 billion years ago.

Chronobiology In India: Past and Present
In the context of chronobiology Sir J. C. Bose’s Plant autographs and their revelations is a landmark volume published by The Macmillan Company, New York in 1927 as it contains three exclusive chapters dealing with information on rhythms in various species of Indian plants. In the preface to this volume he wrote: “I have been able to make the dumb plant the most eloquent chronicler of its inner life and experiences by making it write down its own history”. He was indeed successful in demonstrating measurable plant rhythms. He first reported short term (1 min) lateral leaflet rhythms and endodermal
rhythms of the terminal leaves in Indian telegraph plant *Desmodium gyrans*. He was also the first who objectively documented the phenomenon of persistence of the endodimural leaf movements in continuous light and constant darkness. Today we would call such rhythms as being *ultradian* (1-min rhythm in lateral leaflets) and *circadian* (24-hr rhythm in terminal leaves). But unfortunately a phase of quiescence prevailed between 1927 and 1954. There was practically no contribution during this period from Indians which can be classified and included in the domain of biological rhythm research (chronobiology). The interesting investigations of Bose probably could not stimulate others to undertake research in the area of biological rhythms. But the earliest works of Thapliyal (1954) and Chandrashekaran (1965) subsequently attracted many young Indian biologists to pursue research work in chronobiology. Eventually their pioneering work led to the emergence of two distinct schools that contributed significantly to the progress of chronobiology in India. In this section, important investigations carried out by Indian scientists working in India have been reviewed. Efforts have been made to avoid omissions of important contributions. If at all there are any they are certainly not deliberate and could only be attributed to reviewer’s ignorance.

S. Visalakshi worked in a project sponsored by National Institute of Science Technology and Development Studies (NISTADS), New Delhi, and compiled research contributions of Indian scientists in the field of chronobiology during the period from 1972 to 1985. It revealed that most of the papers in this field fall either under circadian rhythms or photoperiodism or circannual rhythms (Visalakshi 1986). Further most of the studies have been more often on phenomenological aspects of biological rhythms. Even on this day chronobiological work of Indian scientists are mostly phenomenological and there has not been even a single paper on molecular components of biological clock.

**Circadian Rhythm**


**Photic & Nonphotic Entrainment**

The entrainment of circadian rhythm is produced by rhythmic environmental input and also sometimes by behavioural rhythms. In this area of chronobiological research, Indian biologists have made significant contributions. Subbaraj & Chandrashekaran (1981) demonstrated for the first time mirror-imaging phase response curves in a vertebrate system. The entrainment characteristics of circadian rhythm have been examined extensively in mammalian system in relation to light intensity, spectrum and duration of pulses (Sharma et al. 1999a, 2000c, Sharma & Chandrashekaran 2000). It was discovered that the shortest light flashes of 0.5 ms have an ability to reset the circadian clock (Joshi & Chandrashekaran 1983, 1985). This group working on the leaf-nosed cave bat, *Hipposideros speoris* demonstrated that the day light dimmer than sunlight (0.0001 – 0.0006 lux) entrains the circadian rhythm. Sharma et al. (1998) demonstrated in *Mus booduga* that the PRC constructed for UV-A is similar to that obtained for daylight. Recently Sharma et al. (2000c) reported that the phase shifts evoked by UV-A increases nonlinearly with irradiance. In another recent study Sharma et al. (2000a) reported that the transient cycles that are usually noticed after a phase shift do not mirror the state of the circadian pacemaker.

The phenomenon of nonphotic entrainment of circadian rhythm has been examined by Indian biologists in a number of animal models. The earliest report in this area was published by Subbaraj & Chandrashekaran in 1978. They demonstrated that pulses of darkness have an ability to phase-shift circadian rhythm in an insectivorous bat. Subsequently series of papers were published by Chandrashekaran, Subbaraj, Marimuthu and their colleagues in which phase shifting and entraining ability of social *entrainer* (Marimuthu et al. 1978, 1981, Marimuthu & Chandrashekaran 1983a), presence/
absence cycle of mother (Viswanathan & Chandrashekar 1985, 1987, 1988), melatonin (Singaravel et al. 1996, 1998, Sharma et al. 1999a,b) and restricted feeding cycle (Sharma et al. 2000b) have been examined. The papers that reported maternal and social entrainment of circadian rhythms in mice and bats, respectively, stand out from others.

**Maternal Entrainment**

Neurophysiological studies in infant rodents suggest that maternal coordination plays an important role in setting the phase of the circadian clock of developing pre- and postnatal pups (Viswanathan & Chandrashekar 1984). Recently Bishnupuri & Haldar (1999) suggested that maternal photoperiodic exposures may alter the neonatal growth, pineal function and sexual development in Indian palm squirrel *Funambulus pennantii*. However, physical presence and absence of mother do not carry any photoperiodic information. Mother's presence may lead to enhanced activity in pups whereas activity may decline in her absence. Viswanathan & Chandrashekar (1985) performed experiments to explore if presence/absence (PA) cycles of mother could entrain circadian rhythm in locomotor activity of the pups of nocturnal mouse *Mus booduga*. The findings were in the affirmative. They demonstrated that PA cycles can act as an effective zeitgeber (figure 14). Subsequently they reported that LL abolishes the maternal entrainment and worked out the limits to this maternal entrainment (Viswanathan & Chandrashekar 1988).

**Social Entrainment**

It is well known that most birds and social mammals leave and return to their roosting sites *en masse*. This socially synchronized behaviour might have been evolved as a strategy to confuse predators (Marimuthu 1984). A colony of microchiropteran bats of the species *Hipposideros speoris* that inhabits a Madurai cave exhibits exodus flying-out activity at the time of sunset. This behaviour precedes by an arousal activity during the pre-dusk hours in which bats sample light intensity near the mouth of the cave to ascertain that the outside is sufficiently dark and conducive for emergence (Marimuthu 1984). The question arises: does each bat sample light? Marimuthu et al. (1978, 1981) and Marimuthu & Chandrashekar (1983a) answered this question by performing a series of elegant experiments. They discovered that free-flying members of bat colony transmit the message of dusk time to their conspecifics roosting deep inside the cave. This was experimentally confirmed by studying the flight activity circadian rhythm in a group of captive bats and in a solitary bat in a solitary cave (figure 15). The circadian rhythm free ran only in the solitary bat, but not in the captive bats that lived with other free flying conspecifics. Further, they demonstrated that this social synchronization is species specific and is abolished by the imposition of continuous light (Marimuthu & Chandrashekar 1983b). The conjecture that this phenomenon of social synchronization takes place through the mediation of flight noise or pheromones or ultrasound emanating from free-flying members.

It is indeed surprising that both maternal and social synchronization of locomotor activity rhythm
in captive bats and murine neonates are abolished by LL. Could it be that the cues leading to these entrainments modulate the same set of photoentrainable components of the clock?

**Circadian Rhythm in Cavernicoloes (Troglobites?)**

Hypogean animals provide an excellent opportunity for the understanding of the evolutionary processes of adaptation to subterranean life. Study of biological rhythms in these animals are interesting as they live under perpetual darkness and at near constant temperature and humidity. Circadian rhythms in distribution, swimming or locomotor activity and melatonin synthesis in the pineal organ have already been documented for hypogean fishes (see Pati 2001). While most authors have not compared their findings on biological rhythms of hypogean fishes with the epigean ones, others have not adopted adequate methodology to characterize endogenous biological rhythms. Pati and his colleagues at Raipur published a number of papers in which they documented 24-hr rhythms in burying behaviour (Biswas 1990, Biswas et al. 1990a), phototactic behaviour (Pradhan et al. 1989, Biswas 1994), colour change behaviour (Pradhan & Biswas 1994) and in a number of biochemical constituents of different tissues in *Nemacheilus evezardi* (Pradhan 1984). Recently Pati (2001) demonstrated a strong circadian bimodal rhythm in locomotor activity of the LD-acclimated hypogean loach *N. evezardi*. The rhythm was characterized by the presence of two peaks, one at

![Figure 15 Schematic actograms show activity/rest pattern of a bat living with free flying conspecifics (a). The same is shown for a solitary bat in a solitary cave without any conspecifics (b). The pattern of activity/rest cycle exhibits social synchronization. (Modified from Marimuthu & Chandrashekar 1983a).](image)

![Figure 16 Activity records in double plot showing locomotor activity rhythm of a hypogean (top) and an epigean fish *Nemacheilus evezardi* maintained firstly under LD cycles and then DD. The hypogean fish exhibits a bimodality under LD and a weak circadian component (τ = 21.71 hr) under DD. (From Pati 2001).](image)
the time of lights-off and the other at the lights-on (figure 16). It was further noticed that this bimodality is covert in case of its epigean ancestors acclimated to LD. The entrainability of locomotor activity rhythm of *N. evezardi* to LD 12:12 hr photoperiod is comparable with the findings reported by others for a cave catfish (Trajano & Menna-Barreto 1996, 2000). Also recently Koilraj et al. (2000) demonstrated entrainment of locomotor activity of a cave-dwelling millipede to LD 12:12 hr cycle. The findings of Pati (2001) strongly suggest that *N. evezardi* possesses a faint endogenous circadian component under DD. This observation and that of Trajano & Menna-Barreto (1996, 2000) and Koilraj et al. (2000) corroborate the hypothesis that circadian rhythmicity is mainly selected in the epigean environment by ecological factors such as daily alteration of light and darkness. It is usually presumed that true caves lack reliable seasonal time cues and also 24-hr zeitgebers (Twente 1955, Koilraj et al. 2000). However, Pati (2001) discovered a reliable rhythm in the rate of seepage in the truly subterranean Kotumsar cave in the Kanger Valley National Park, Jagdalpur, Bastar, India (figure 17). He simulated the seepage/no seepage cycle in the laboratory and found that such a cycle has an ability to entrain the locomotor activity rhythm in *N. evezardi* (unpublished). In view of this it seems mandatory that other caves should be reexamined to rule out the possibility of existence of any such nonphotic zeitgebers. There is a strong likelihood that bat-inhabited caves would certainly possess one-especially a predictable rhythm in the rate of bat droppings which may indirectly transmit time of the day information to the hypogean organisms.

**Human Circadian Rhythm**

In an isolation facility Chandrashekaran et al. (1991) examined the relationship between the circadian rhythm in physiological processes and the menstrual rhythm. They reported internal desynchronization of sleep-wake (SW) rhythm from that of the body temperature. The body temperature rhythm freeran with a period of about 25.1 hr whereas SW rhythm exhibited a circadianian (τ = 45.9 hr) rhythm. They reported that the menstrual cycle of the female subject was normal with two episodes of onset of menses occurring 28 calendar days apart. They concluded that in social and temporal isolation the menstrual cycle of the female subject may not be coupled to the SW circadian rhythm. However, this conclusion was based on only 47 women-day data and the experiment was carried out on a single female human subject. Chandrashekaran et al. (1991) however did not cite an earlier study in which a single human female spent nearly 130 days alone in a cave 30 feet below ground (Guinness 1990). The subject's four months in the cave seemed to her like only two. She exhibited SW rhythm with a period of about 35 hr and stopped having menstrual period. In light of these findings the proposition of Chandrashekaran et al. (1991) appears to be equivocal. Could it be that the 2nd episode of menses observed in the female subject of their study was already programmed before her entry into the isolation chamber? This however remains to be tested. Chandrashekaran et al. (1991) subsequently reported a direct correlation between the circadian SW rhythm and time estimation (TE) in humans under social isolation, thereby confirming an entirely original correlation reported earlier by Aschoff (1985). Chandrashekaran et al. (1997) reported yet another interesting finding that during internal desynchronization, wake duration exhibits a positive correlation with the subsequent sleep.

Chandrawanshi 2001). However, here the focus is on shift workers. It was rigorously documented that the phenomenon of internal desynchronization of various physiological rhythms is common among Indian shift workers (see shift work section in this review for details). Pati & Gupta (1994) reported that the ability of man to estimate short-time (10 sec) interval oscillates with a period of 24 hr and that in shift workers this rhythm undergoes disruption. Further, they observed an interesting association between TE ability and body temperature rhythm. The TE in an individual was nearer to accuracy only when its body temperature was at peak.

**Biological Rhythms: Pineal & Melatonin**

The pineal complex is a part of the epithalamus of the vertebrate brain (Vigh & Vigh-Teichmann 1999). This complex in vertebrates plays an important role in measuring light in order to adapt functions of the organism to circadian and circannual changes of the environment. Indian biologists have been contributing significantly in this field of research (see reviews Shedpure & Pati 1995a, Kumar 1996, Haldar 1999). The annual pineal and testicular cycles have been worked out in a number of vertebrate systems (Haldar & Thapliyal 1977, Haldar & Pandey 1989, Haldar & Ghosh 1990). Haldar et al. (1992) and Haldar & Dubey (1996) reported a diurnal rhythm in melatonin circulation and that pineal/melatonin may influence thyroid function in Indian palm squirrel *Funambulus pennanti*. Recently Guchhait & Haldar (2000) demonstrated a time- and reproductive phase-dependent effects of exogenous melatonin on the ovary of a nocturnal bird *Athene brama*. In another recent paper Haldar et al. (2001) reported a relationship between the annual rhythms in melatonin and immune system status in *Funambulus pennanti*. In a recent paper Jagota et al. (1999) demonstrated that pineal rhythms can be entrained by LD cycles in congenitally anophthalmic mutant rats. They concluded that this may happen only through the involvement of a nonvisual system of light perception. However, this aspect of extraocular light perception in mammals is still debatable (discussed earlier in this review).

A recent study by Varghese and Pati (1997a) provided the first demonstration of a direct pineal influence on thermal tolerance and its temporal organization. They demonstrated that pinealectomy in *Clarias batrachus* abolishes the normal circadian cycle in critical thermal maximum (CTM) and dampens its amplitude considerably (figure 18). An increase in thermotolerance was also observed in pinealectomized *C. batrachus* (See review Varghese & Pati 1996, Varghese & Pati 1997a,b). These observations indicate that pineal plays a vital role in thermoregulation and in modulating a mechanism that controls the endogenous thermoregulatory clocks. However, the extent and nature of the control remains obscure.

Joshi and his colleagues also worked extensively on skipper frog *Rana cyanophlyctis*, Indian desert gerbil *Meriones hurrianae* and male albino rat to assess the effects of melatonin, LL, DD and different spectra of light on reproductive parameters (Joshi et al. 1994, Sinhasane & Joshi 1997a,b, 1998, Udaykumar & Joshi 1997, Joshi & Udaykumar 1998). Joshi & Udaykumar (2000) observed that ocular melatonin seems to play an important role in the regulation of reproduction in *Rana cyanophlyctis* and that extraretinal perception of colored light occurs in this species (Joshi & Udaykumar 1998).

**Photoperiod and Gonadal Response**

Many photoperiodic species need a critical daylength in order to induce a physiological (e.g. gonadal) response. Experimental manipulations of photoperiod in various ways have revealed that all photoperiodic

![Figure 18 Daily cycles of critical thermal maximum (CTM) in sham-pinealectomized (Sham-Px) and pinealectomized (Px) Clarias batrachus. (From Varghese & Pati 1997a)](image-url)
species possess a photoinducible phase. A long day is registered when this phase is illuminated by the photophase of the daily LD cycle. Although this photoperiodic response system appears simple it is indeed complex and involves a photoreceptor that interprets light input, a clock that measures light signal and a neurosecretory system that translates light signal into endocrine secretions. Chandola et al. (1976) were the first Indians to provide evidence that the underlying basis of gonadal response in the tropical weaver bird *Ploceus philippinus* is an endogenous circadian clock. They worked out the photoinducible phase for this species. However, in this field of research later Kumar and his colleagues have contributed significantly. Working on a number of bird species Kumar (1988, 1997), Kumar & Tiwari (1989), Kumar & Kumar (1991, 1992, 1993a,b, 1995), Kumar et al. (1996) have rigorously demonstrated that a circadian photoperiodic clock is involved in the processes of induction and termination of seasonal reproduction. Further Kumar & Rani (1996, 1999) and Rani & Kumar (2000) reported that the avian photoperiodic clock responds differently to different wavelengths (Colours & spectra) and intensities of light and that these effects are phase dependent. Kumar (1996) demonstrated further that melatonin may not be involved in the circadian system that regulates photoperiodic gonadal responses in birds.

Circannual Rhythm

The circannual rhythms appear to be paramount for seasonally breeding animals. This makes provision for fine temporal tuning of the components that are responsible for the initiation of reproductive process within a specified and species-specific time window, its sustenance and termination. An annual reproductive rhythm thus represents a chain of distinct physiologic events, namely recrudescence of the gonad, ovulation or spermatiation, spawning or breeding, and regression of the gonad. Circannual rhythms have been studied extensively in a number of Indian vertebrate species.

Birds

Thapliyal and his colleagues worked on about 30 wild and semidomestic species of birds and demonstrated predictability in the annual sexual and body weight cycles of these species (Thapliyal 1954, 1961, 1967, 1968, see review Thapliyal 1978, 1981, Thapliyal & Gupta 1989). Although it was A. B. Mishra who first initiated experimental investigation of reproductive cycles of Indian birds at Varanasi in 1940s, his findings remained largely unnoticed as grey literature. Pandha & Thapliyal (1964) and Thapliyal & Pandha (1965) demonstrated for the first time the importance of thyroid in the regulation of gonadal activity in spotted munia, *Urolochus (= Lonchura) punctulata*. This was followed by a series of papers in which role of thyroid in annual breeding cycles of both sexes of adult and juvenile lal and spotted munia, black-headed munia, chest nut-bellied munia, weaver bird, red-vented bulbul, migratory redheaded bunting, common myna, rain quail and male house sparrow has been studied in detail (Thapliyal 1969, 1978, 1980b, 1981, Thapliyal & Chandola 1972, Chandola et al. 1973, Chandola & Thapliyal 1978, Lal 1982, Thapliyal & Gupta 1984, Lal & Thapliyal 1985a,b). It was demonstrated that in a number of Indian birds annual rhythm of thyroid activity runs inversely with the gonadal cycle while in others it runs parallel with the annual testicular cycle. It was further reported that thyroid hormones can dramatically modulate the photoperiodic responses of Indian birds. Mild hyperthyroidism or hypothyroidism depending upon the species has been shown to abolish the response of birds to stimulatory long photoperiod. Pathak & Chandola (1982) and later Lal & Thapliyal (1985a) demonstrated that thyroid plays a significant role even in the regulation of photorefractoriness in migratory redheaded bunting, *Emberiza bruniceps*. Initially the findings of Thapliyal and his colleagues that thyroid plays a critical role in annual reproductive cycles were viewed with scepticism by the contemporary peers in the west. However, Thapliyal’s hypothesis is no longer equivocal. Now it has been widely accepted that the thyroid is indeed important for the regulation of annual seasonal reproductive cycle not only in birds but also in other vertebrates including mammals (Boulakoud & Goldsmith 1991, Webster et al. 1991, Dahl et al. 1994, Wilson & Reinert 1996, 1999, 2000, Vigui et al. 1999).

On the basis of extensive investigations performed on Indian birds Thapliyal & Gupta (1989) proposed a model showing external and internal factors involved in the regulation of annual gonad development cycle of Indian birds (figure 19). It was shown that three external factors, namely
photoperiod, rainfall and temperature may modulate the output of an endogenous clock that regulates the annual rhythm of reproduction. The involvement of thyroid, adrenal, pineal and gonadal hormones in the regulation of annual gonadal cycle of Indian birds has been strongly suggested. A large number of papers have been published by Thapliyal’s group and others in support of this model (see Reviews by Thapliyal 1978, 1981, Chandola & Bhatt 1982, Pathak & Chandola 1983, 1984, Pavgi & Chandola 1983, see review Thapliyal & Gupta 1989, Chaturvedi & Suresh 1990, Halder & Ghosh 1990, Singh & Chaturvedi 1995).

Lower Vertebrates

Annual reproductive cycles gauged by morphometric and/or histomorphometric and/or radioimmunoassay data on gonadal state have been studied by Indian biologists in a number of reptilian (see Review Sarkar & Shivanandappa 1989), amphibian (see Review Saidapur 1989), and piscine species (see Reviews by Malhotra et al. 1989, Neelakantan et al. 1989, Pati & Shedpure 1999). Recently Shedpure & Pati (1994, 1995b) and Pati & Shedpure (1999) have demonstrated that not only gonadal activity but also number of other physiological variables exhibit significant annual rhythms. It has been further emphasized that many physiological and behavioural variables may also exhibit multiple low frequency rhythms simultaneously in addition to circannual rhythms (Biswas et al. 1990b, Shedpure & Pati 1994, Varghese & Pati 1997b, Lal et al. 1999, Maheshwari et al. 1999, Acharia et al. 2000). It was demonstrated recently that in C. batrachus many physiological variables showing annual periodicity exhibited their peaks coinciding with the peak of gonadal activity. Furthermore, those variables that did not show 12–mo period, but exhibited 6-mo or 3-mo periodicity showed coincidence for at least one of their peaks with the peak of the annual gonadal cycle (Shedpure & Pati 1994, Pati & Shedpure 1999). It may well be that successful breeding in animals may require a synchronization of rhythms in several physiologically important variables having diverse frequencies to build an appropriate internal milieu for the accomplishment of successful breeding (figure 20).
Are all reported circannual rhythms endogenous? This has not yet been answered except couple of papers that documented free running circannual rhythms in a fish (Sundararaj & Sehgal 1970), birds (Bhatt et al. 1986, Gwinner 1986, 1987, 1990) and in golden-mantled ground squirrel (Pengelley & Fisher 1963). These findings do suggest endogeneity. However, till this day very little is known about the molecular mechanisms involved in the generation and control of circannual rhythm.

**Applied Chronobiology**

Although symptoms of a number of chronic disorders exhibit 24-hr rhythms, Indian medical researchers and practitioners have been accepting chronobiology rather slowly (see Chronotherapy section in this review). Apart from the spectacular clinical findings of Deka (1975) and subsequent reports on clinical practice of chronoradiotherapy in cancer patients (Deka et al. 1976, Gupta et al. 1987, Gupta 1996, Singh et al. 1998) there are not many reports dealing with the application of chronobiologic principles in the clinics.

The circadian variations in urinary electrolyte excretion have been reported in hypertensive patients following administration of diuretics at different time of the day (Yegnanarayan et al. 1989, 1992, 1993). The time dependent efficacy of clonidine and alpha methyl dopa in lowering blood pressure was documented (Yegnanarayan 1988, Yegnanarayan & Balwani 1994). In a recent report Yegnanarayan et al. (1999) observed that bed time administration of phenytoin in patients of grand mal-epilepsy may lead to faster absorption of the drug and less side effects as compared with drug administration in the morning.

There are also couple of papers in which circadian pattern in the onset of manifestations of ischemic heart disease has been demonstrated (Dharmadhikari et al. 1998, Mehta et al. 1998, Singh et al. 1999). The pathophyslogic bases for the increased number of cardiac events in the early morning hours may be due to hemodynamics factors. It seems that gradually Indian practitioners are becoming increasingly aware of the importance of chronotherapy.

**Ecophysiological Significance of Biological Clocks**

Recently there have been a number attempts to understand the ecological significance of circadian clock. By applying techniques and approaches of modern evolutionary genetics Sheeba et al. (1999) reported that preadult fitness components of *D. melanogaster* maintained in LL for over 600 generations are not adversely affected when assayed under LL as compared to those of under LD or DD. However, they were skeptical about the effect of LL on the components of adult fitness. The question arises: is LL with constant temperature a selection regime? Sheeba et al. (2000) further suggested that to understand the effects of different light regimes on adult fitness in *Drosophila* it is necessary to study their effects on the physiological and behavioural processes that accompany reproduction. Recently Joshi & Gore (1999) reported that characteristics of eclosion rhythm in *D. ananassae* strains originating between 8° – 34°N are latitude dependent. They observed that among these strains the phase angle difference (ψ) and period (τ) in constant darkness vary by about 3 hours and amplitude of the rhythmicity by about 10%. These differences in the characteristics of circadian rhythm in eclosion among different strains of *D. ananassae* could be attributed to selection under fluctuating light intensity, temperature and humidity at different latitudes. In a recent study Pati and his colleagues (unpublished) observed that in a perpetually dark cave a population of moth slows down its vertical flight activity during daily emergence and return flight of a resident population of bats (figure 21). Thus the moths
optimize the chances of their own survival by temporally organizing their daily flight activity in such a way that there is the least possibility of them being detected and preyed upon by the out-flying or in-flying bats. Currently studies are being continued to understand the fitness components of moth’s rhythm in detail.

Chronotherapy
Chronotherapy is a clinical strategy that harmonizes medical treatment with the patient’s biological rhythms to have the maximum desired and the least undesired effects. It is based on the idea that the organisms respond differently to the same treatment modality or dose, directed against a specified ailment or disease, when administered at different points across any time scale, such as day, week, month and year. In other words, organisms, including human beings, exhibit rhythms of varying frequencies in susceptibility or sensitivity to a drug or any other kinds of treatment. Depending upon the types of treatment, chronotherapy could be termed as (a) Chronomonotherapy, with a single drug; (b) Chronopolytherapy or group chronotherapy, with multiple drugs; and (c) Chronoradiotherapy, with radiation (Halberg et al. 1977a).

Indian physicians had the knowledge of time and its significance in the physiology and treatment of human diseases as early as 2500 B.C. (Thapliyal 1980a, Sinha 1981). However, rigorous scientific analysis of this concept started only in the middle of 20th century. Pohle et al. (1961) for the first time documented that the dosing time of a carcinostatic drug may have a bearing on its antitumor activity. Since then chronobiologists have been reporting interesting time-related medical observations. However, chronobiology is still in the process of being accepted by the medical community. In recent years medical practitioners have started taking biological rhythms into account in diagnosing and treating diseases.

Concepts in Chronopharmacology
Chronopharmacology led to the development of the subdiscipline chronotherapy. It was later realized that there should be a set of concepts so that those would be followed universally by the modern chronopharmacologists. Reinberg et al. (1975) took the initiative and coined several important conceptual terminologies.

Chronesthesia and Chronergy
Chronesthesia, a chronopharmacologic counterpart of phase response curve (PRC), designates predictable rhythmic (e.g. circadian) changes in the susceptibility (or the sensitivity) of a target biosystem to an agent. The target biosystem may involve at the molecular level, subcellular systems or higher levels of biological organization, such as cells, tissues, organs and organ systems.

Chronergy designates time dependent effect(s) of a drug on the organism as a whole. It depends upon both the chronopharmacokinetics of a given drug and chronesthesia of involved target biosystems (Reinberg et al. 1975). Thus it involves both desired (chronoefficacy) and undesired (chronotolerance) effects of a drug on the whole organism.

Chronopharmacokinetics
Chronopharmacokinetics (or chronokinetics) describes rhythms in parameters like peak height (Cmax), time to peak (Tmax), area under the curve (AUC), and half-life, used to characterize pharmacokinetics of a substance.
Chronobiology

Chronoptimization
This is a state that characterizes a compromise between chronoefficacy and chronotolerance in terms of an appropriate time of medication along a time scale so as to enhance its desired effects and minimize undesired ones. The chronoptimization is the goal of chronotherapy.

Rhythm characteristics
The efficacy of a given treatment as a function of time of administration can also be described with respect to modulation of four basic characteristics of biological rhythms: (a) period; (b) time series average (mesor); (c) rhythm dependent variation limits (amplitude); and (d) peak time (acrophase).

Preclinical Chronopharmacologic Studies
Chronopharmacologic studies first begun with the advent of new carcinostatic drugs. It is well known that most of the anticancer agents, such as cisplatin, oxaliplatin, carboplatin, doxorubicin, 4'-O-tetrahydroprynlyadriamycin (THP), methotrexate, 5-fluorouracil, peptichemo, etoposide, vinblastine, vincristine, docetaxel, produce excessive host toxicity. A fundamental question arises: does the extent of host toxicity vary as a function of dosing time? Results from several studies were startling and revealed that host toxicity may be different depending upon the time of drug treatment on a specified time scale (Haus et al. 1972, Lévi et al. 1988). The survival (end point of drug toxicity) in healthy rodents varies considerably, sometimes 3-fold and even more as a function of dosing time of several anticancer agents (Lévi et al. 1988). Now it has been documented that the phase of murine chronotolerance is different for different drugs across a circadian time scale. The extent of toxicity and anticancer activity of over 30 anticancer agents that include anthracyclines, platinum analogs, alkylating agents, antimetabolites, spindle poisons, plant alkaloids and radiation have already been examined in mice or rats kept under controlled conditions of photoperiod and temperature (Lévi 1997). The anticancer effect of these drugs was found to be circadian phase dependent. Thus the time of drug administration is critical with regard to chronoptimization.

In hepatic tissue of rodents, circadian changes in DNA synthesis, RNA synthesis, RNA translational activity, mitotic index, weight, glycogen content and activity of several enzymes are well known (Hrushesky 1985, Hrushesky et al. 1992). The circadian rhythms in DNA synthesis in other tissues, such as stomach, duodenum, rectum and bone marrow, have also been documented in rodents (Burns 1981). These rhythms in all probability affect the drug pharmacokinetics leading to manifestation of circadian dependent chronergy. In addition, several drug-metabolizing enzymes in kidney also exhibit circadian rhythms in their activities. Physiological rhythms in cytokinetics, nucleic acid metabolism, immunological variables, drug metabolism, and hormones could serve as basis for time-dependent drug response of the organism (Hrushesky et al. 1992).

Chronopharmacology of Cyclosporine-A
The immune system is indispensable for the organism's life, because it helps to prevent diseases by microorganisms, foreign molecules and malignant transformed cells. Several steps are necessary to make the immune system able to eliminate foreign agents. Firstly a foreign substance is identified and subsequently it is destroyed. In between these two steps many other important steps such as recognition, activation, proliferation and differentiation are involved. All these steps are autoregulated and have been proposed in one of our reviews (Lévi et al. 1992) to be coordinated along the circadian time scale both in mice and man (figure 22).

Cyclosporine-A (CsA) also known as ciclosporin (Cs) is a powerful immunosuppressive agent that prevents rejection of both experimental and clinical allografts. It is used in kidney, liver and heart transplantation, and bone marrow allografts. It seems to block the activation of resting T-cells.

![Figure 22 Immune clock showing temporal coordination of different stages of immune defences. (Modified from Lévi et al. 1992)](image-url)
However, the renal toxicity of CiA constitutes its main dose-limiting side effects. The effects of the oral administration of different dosages of CiA have been examined on histological pictures of kidney in male B6D2F1 mice (Pati et al. 1986). It was observed that CiA is nephrotoxic (figure 23) and the extent of toxicity varies as a function of its ingestion on a circadian time scale (Pati et al. 1988). In another study attempts were made to determine the optimum time of oral administration of CiA by using 315 male B6D2F1 mice. Both toxicity and T-cell suppression were assessed (Pati et al. 1988). It was observed that a satisfactory compromise between the least renal toxicity (figure 24) and the maximum immunosuppression could be achieved when CiA is given orally near the transition of dark (activity) to light (rest). The time dependent immunomodulation observed in this study could be attributed to a circadian-adapted immune surveillance mechanism (Lévi et al. 1992).

Extrapolation of results from these and other preclinical chronopharmacological studies involving beta-receptor blocking drugs, anesthetics, nonsteroid anti-inflammatory drugs, anticoagulants, and hormones has laid the foundation of chronotherapy in the clinics.

Chronotherapy in Clinics

Currently chronotherapy is practiced in the clinics for a number of human diseases, notably cancer, allergy, asthma, arthritis, heart disorders, delayed and advanced sleep phase syndrome (DSPS and ASDS) and seasonal affective disorder (SAD).

Cancer

Cancer chronotherapy in humans is basically an extension of preclinical studies in animal models. Both toxicity and antitumor activity of commonly used drugs, such as doxorubicin (DOX), cis-diamminedichloroplatinum (Cisplatin), 5-Fluoro-2'-deoxyuridine (FUDR), 5-fluorouracil (5-FU), 4-tetrahydropyranyl-adriamycin (THP-ADM), etoposide, have been shown to be circadian stage dependent in human beings (Lévi 2000).

It has been demonstrated that 5-year survival time of ovarian cancer patients varies depending upon the dosing time of DOX/Cisplatin treatment. The patients receiving DOX at 06:00 hr and cisplatin 12 hr later exhibited 50% survival at 60 months (Hrushesky

Figure 23 Dose-dependent renal toxicity of CiA as assessed by histological score ranging from 0 (normal) to 3 (extensive necrosis) after five days of treatment. Results from Kruskal-Wallis non-parametric analysis of variance. (Modified from Pati et al. 1986).

Figure 24 Dosing-time dependent renal toxicity of CiA in mice, as gauged from the extent of microscopic renal lesions. Mice were killed 24 h after the 5th or 10th daily dose (100 mg/kg/day P.O.). Murine chronotolerance for CiA kidney lesions in a total of 105 male B6D2F1 mice receiving 100 mg/kg/day of CiA for 5 or 10 days; histologic lesions scored from 0 (normal) to 5 (extensive tubular necrosis). Their kidneys were removed and fixed in Bouin’s microformol solution for 24 h before being processed for histologic analysis. Hematoxylin-eosin stained slides were observed in random order by a histopathologist, who was unaware of treatment group. Tubular lesions were scored from 0 (no lesion) to 5 (necrotic spread throughout the whole cortex). Double readings performed several months apart yielded the same score in 60% slides, and differed by one point in 38% of them. Highest renal toxicity corresponded to CiA administration at 15 HALO. Partial recovery of renal lesions were observed following CiA treatment at 23 or at 3 HALO. HALO = Hours After Light Onset. The area shown within two lines defines the limits of CiA toxicity (Mean ± 1 SE) as a function of dosing time. (Based on data from Pati et al. 1988).
In the last decade, effectiveness of chronotherapy has been examined by conducting number of multicenter clinical trials in three different phases on 1275 patients with metastatic colorectal cancer (Lévi 1999). The therapy termed as group chronotherapy included two or three anticancer drugs and was found to be 3 to 4-fold more effective than the conventional regimens in terms of antitumor activity (Lévi 1999). The circadian rest-activity cycle has been considered as a marker rhythm and has been the basis for the current chronotherapy schedules. Again this idea stems from the preclinical research on rats and mice that emphasized the importance of phase relationship between rhythms in sensitivity to anticancer agents and rest-activity cycle. What is interesting is that malignant tumors and cancer bearing hosts may exhibit nearly normal or drastically altered circadian rhythms. The rhythm alterations have been described to depend upon several factors, namely tumor type, growth rate and level of differentiation (Mormont & Lévi, 1997). This, therefore clearly suggests that in chronotherapy we cannot imagine of a generalized approach rather it should be a case-based approach. Probably this is the reason why chronotherapy cannot be followed globally, specially in those regions that prominently lack supportive infrastructure.

Deka (1975) and Gupta & Deka (1975) reported that timing the radiation doses could accelerate the rate of remission of circumoral cancer. They discovered that the oral tumor exhibits a rhythm in its surface temperature. In their study radiation was given for five weeks. The patients with large tumors were given radiation either at the time of their peak tumor temperature or 4 or 8 hours before or after the peak time. It was discovered that the tumor regression rate is the maximum when the patients were given radiation at the time of their peak tumor temperature (figure-25). The results of this study were the main stimuli for the creation of a new subdiscipline, chronoradiotherapy (Halberg et al. 1977b). The work of Deka (1975) is most frequently cited in chronobiology literature. However, unfortunately there has been no follow up work to consolidate the principles of chronoradiotherapy.

Chronotherapy has been shown to be quite effective for childhood acute lymphoblastic leukemia, ovarian cancer, breast cancer, and many other types of cancer (Hrushesky et al. 1992, Lévi 1999, 2000). In summary, cancer induction, promotion, progression and also response to treatment are influenced by circadian rhythms. However, extension of cancer therapy to non-circadian based chronotherapy such as on time scales of low frequency rhythms is desirable.

**Cardiovascular diseases**

It is well known that various types of cardiovascular complications, such as stroke, myocardial infarction, sudden cardiac death, myocardial ischemia, and angina, occur more frequently during the early morning hours (White 1999, Munger & Kenney 2000). In the morning both blood pressure and heart rate rise dramatically. Further, a circadian variation in beta-2-adrenergic receptors (β-R) that counteract platelet aggregation on lymphocytes is well known. Low dose of aspirin, when taken soon after awakening or 3 hours thereafter, enhances activity of these receptors. But, the same amount of aspirin taken 6 or 9 or 12 hours after awakening produces very less or no effect on β-R (Cornélissen & Halberg 1994). It has been observed that an increased efficacy of antihypertensive drugs, such as propranolol, clonidine and α-methyldopa with lesser amount of dose can be achieved by employing chronotherapy in human patients. Munger & Kenney (2000) scanned the literature on circadian variation associated with cardiovascular diseases published from 1990 to March.
2000 and concluded that there has been an increased interest among the scientists and physicians in favor of application of chronotherapeutics to obtain desirable cardiovascular outcomes. Attempts have been made to formulate new chronotherapeutic agents with special release mechanisms and to administer traditional agents at appropriate times along the 24-hr time scale according to the patient’s rhythm.

Allergies and asthma
The symptoms of rhinorrhea, and seasonal or perennial allergic rhinitis exhibit circadian variations. The patients suffering from allergies confront with the worst symptoms in the morning especially during the initial hours after awakening from nocturnal sleep (Gervais & Reinberg 1987, Smolensky et al. 1991, 1995). These authors have demonstrated that the efficacy of antihistamine and antiinflammatory drugs can be enhanced if they are administrated at the approximate phase of circadian rhythm in symptom manifestation and intensity of allergies.

Asthma consensus report acknowledges that asthma is a nocturnal disease in as many as 70% of the afflicted patients (D’Alonzo et al. 1999). They experience attacks in the night. In untreated patients asthma attacks may occur 100 times more often at night than during the day (Martin & Banks-Schlegel 1998). A high evening dose of sustained-release theophylline or a single daily evening dose of theophylline has been shown to result in the best plasma theophylline disposition, clinical tolerance and effectiveness, and is superior to the conventional dosing pattern for nocturnal asthma. The bronchodilator uniphyl, a long-acting theophylline preparation has been shown to be more effective when taken once a day in the evening (around 18:00 to 19:00 hr). This treatment maintains an optimal blood level of theophylline during early morning thus considerably improving lung function during 03:00 and 05:00 hour. It has also been shown that for nasal inhalation of corticosteroids and cromoglicate, the best timing of administration is the activity span near the acrophase of bronchial patency. At that time the rate of absorption of the drug is optimal. Recently chronotherapy is being used routinely to manage nocturnal asthma (Pincus et al. 1995, Burioka & Sasaki 1996, Martin & Banks-Schlegel 1998, D’Alonzo et al. 1999, Kraft 1999, Burioka et al. 2000).

DSPS, ASPS and SAD
The term delayed sleep phase syndrome (DSPS) refers to a type of abnormality involving sleep-wake cycle (Wagner 1999). Takahashi et al. (2000) have suggested that a predisposition to DSPS includes biological, genetic, social and psychological factors and their interactions in various combinations. The term advanced sleep phase syndrome (ASPS) is exactly the opposite of DSPS, where sleep is advanced to the early hours of the night following an early rise. Although relatively rare, ASPS is common among elderly. The patients with seasonal affective disorder (SAD) suffer from a depressive mood, phase-delayed sleep, daytime hypersomnia, fatigue, and increased craving for carbohydrate-rich diets accompanying with weight gain (Lam & Levitan 2000). The prevalence of SAD is greater in northern latitudes and increases as the distance from the equator increases. It is indirectly related to decreasing hours of day length. SAD exacerbates in winter and could be attributed to the relationship among circulating levels of melatonin, body temperature and the sleep-wake cycle. The increased production of melatonin during winter may be a principal contributing factor to SAD (Lam & Levitan 2000).

Bright light and melatonin either singly or in combination have been used successfully to treat patients with DSPS (Skene et al. 1999, Harrer 2000, Hori et al. 2000, Kamei et al. 2000). The objective of the therapy is to restore synchronization between the body temperature rhythm and sleep-wake rhythm. By employing phototherapy the phase of the minimum body temperature is shifted to the latter portion of the sleep phase. Light also acts as a therapeutic agent for some patients suffering from SAD, if exposures are appropriately timed in accordance with the patients’ circadian rhythm (Gloth et al. 1999, Mazereol & Donaldson 1999, Lam & Levitan 2000). SAD is a severe recurrent winter depression that affects predominantly women, as nights grow longer. Patients with SAD respond favorably to the exposure to bright light (2500 lux) in the mornings. Light has also therapeutic potential for patients suffering from premenstrual syndrome (PMS).

Drug delivery system
Programmable-in-time, bedside, implantable and ambulatory pumps popularly called chronopumps
are now available in the market. The characteristics of different kinds of chronopumps have been described by Lévi et al. (1988). Drug can be delivered as multistep doses or as on-off timed boluses by using these pumps. With regard to infusion modes, several possibilities exist for employing chronotherapy in managing human diseases involving analgesics, carciinostatics, and antihypertensive drugs. These pumps help in timing medications or in modulating the drug infusion rate along the circadian time scale in patients (Von Roemeling et al. 1987, Hrushesky et al. 1992). Thus these pumps are used as tools to obtain reliable and desired chronotherapy.

Shift work

The term shift work defined as an arrangement of work hours that uses two or more teams (shifts) of workers in order to extend the hours of operation of the work environment, beyond that of the conventional office hours. The varieties of shift work include stable/permanently displaced work hours in which the work schedule used does not require a person to normally work more than one shift (including night work), rotating shift work in which an individual is normally required to work more than one shift, changing from one shift to another and unscheduled work hours. On call shift is also a special form of shift work, where in case of emergency the particular group of workers are called for their duties. The most widespread shift system is when production is organized in eight hour shifts, called morning, evening and night shifts (Knutsson 1989).

Shift work is a necessity in manufacturing, transportation, health care, security, and many other essential sectors that operate around the clock. Therefore, in these sectors groups or crews of workers succeed each other at the same work stations in shifts. Recent research has established that shift work disrupts biological rhythms, sleep and social life, and leads to a number of clinical and non-clinical problems (Pati et al. 2001). It retards human performance and increases the chance for the occurrence of major industrial accidents. This section presents some recent data attributing deleterious consequences of shift work to disruption of human biological rhythms. Can these disruptions be minimized? Can principles of chronobiology be applied to optimize human shift work? Attempts have been made here to address these important issues in the light of available information in the field of chronobiology relevant to shift work and its optimization.

Consequences of Shift Work

Disruption of Circadian Rhythms

Recent research has proved beyond doubt that rotational shift work affects human health and performance by disrupting biological rhythms and by causing numerous alterations in their behaviour and physiology. The phenomenon of internal desynchronization is of common place among shift workers (Rutenfranz 1982, Folkard et al. 1983, Reinberg et al. 1984, 1988, 1989, Motohashi 1990, Pati & Saini 1991, Gupta & Pati 1993, 1994a, Gupta et al. 1997). The desynchronization includes alterations in one or all of the important rhythm parameters, such as phase (peak), amplitude and 24-hour average. The term internal desynchronization was used by Aschoff & Wever (1981) to describe a process during which the period (τ) of a circadian rhythm may differ among variables in apparently healthy human subjects. "...the circadian system can split into components that run with different frequencies..." (Aschoff & Wever 1981). Internal desynchronization has been documented in shift workers with both short- (3 years) and long-duration (14 years) shift work experience (Reinberg et al. 1988). This clearly reveals that internal desynchronization of circadian rhythms does not depend upon the length of shift work experience. Motohashi (1990) as well as Pati & Saini (1991) and recently Chandrawanshi & Pati (2000) confirmed the phenomenon of internal desynchronization among intolerant shift workers dwelling respectively in Japan and India (figure 26).

Shift workers most often exhibit a phase shift in their bodily rhythms (Aschoff 1978, Wever 1979, Reinberg et al. 1984, Matsumoto & Morita 1987, Härma et al. 1990). However, the direction of phase shift may vary as a function of age (Matsumoto & Morita 1987, Härma et al. 1990). There is an interesting relationship between the magnitudes of phase shift and amplitude: the larger the phase shift the smaller is the amplitude. Reinberg & Smolensky (1992) and Reinberg et al. (1984, 1988) have substantiated the above findings.
Pati & Gupta (1994) examined the ability of shift workers to estimate time intervals of short duration (10 sec). A circadian rhythm in time estimates (TE) was documented in control subjects, but it was found to be disrupted in shift workers (figure 27).

The amplitudes of circadian rhythms in various variables of shift workers undergo changes as compared with those of the diurnal workers. Touitou et al. (1990) documented lower amplitude in serum cortisol rhythm and higher amplitude in melatonin rhythm among shift workers than in apparently healthy day working human subjects. They also reported alteration in the amplitudes of rhythms in prolactin and testosterone in shift workers with a fast rotating shift system. Recently, Chandrawanshi & Pati (2000) observed a decrease in circadian amplitudes of several rhythms, such as skin temperature, heart rate and peak expiratory flow rate in shift workers of a cement factory.

The 24-hr arithmetic averages of circadian rhythms in several physiological variables have been shown to undergo alterations in shift workers. Of the random number addition speed (RNAS) rhythm, circadian mesor (24-hour average of RNAS rhythm) increased in shift workers as compared to control subjects (Pati & Saini 1991, Gupta & Pati 1994a). This suggests that shift workers took longer time than their day working counterparts to perform the task comprising of additions of paired random numbers (figure 28). The 24-hr arithmetic averages of several physiological rhythms also undergo changes when a shift worker is shifted from one work schedule to another. Further, the magnitude of change may also depend upon the direction of schedule shift. Härmä et al. (1990) observed that the 24-hr arithmetic average of the oral temperature rhythm decreased slightly and that of the sleepiness rhythm increased highly significantly from morning to the second night shift in various age groups.

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Figure 27 Disruption of circadian rhythm of time estimation in shift worker. Power spectra of time estimation rhythm in a day worker (C #07) and a shift worker (SW #12). The prominent periods are expressed in hr. In day worker prominent τ is 24 hr, whereas τ differs from 24 hr in the shift worker irrespective of the shifts (N = Night shift; M = Morning shift; O = Off days). (Based on data from Pati & Gupta, 1994).

Figure 26 Day-to-day changes in acrophase of skin temperature (ST), heart rate (HR) and peak expiratory flow rate (PEFR) in shift workers SW #03 & SW #04. All three rhythms exhibit external desynchronization. (Based on Chandrawanshi & Pati 2000).
Consequences on Sleep

Scheduling of sleep timings is a major concern in the life of shift workers, particularly if their work schedule includes night work among others (Czeisler et al. 1982, Mahan et al. 1990). Tepas & Mahan (1989) have suggested that night shift workers suffer more often from insomnia like sleep disorder. This abnormality is characterized by difficulty in falling and staying asleep. The association between shift work and sleep disruption results in adverse medical and psychological consequences (Phillips et al. 1991). In many studies, a majority of shift workers admit to having experienced involuntary sleep on the night shift, whereas this is rare on day-oriented shifts (Kogi & Ohta 1975, Åkerstedt et al. 1983, Coleman & Dement 1986).

Several investigators have documented a significant circadian rhythm in subjective drowsiness/sleepiness in apparently healthy human subjects (Reinberg et al. 1989, Gupta & Pati 1994a,b). The drowsiness rhythm in these subjects exhibits a peak between 21.0 to 23.0 hr with a pronounced circadian period (figure 29). However, in case of shift workers, rhythm in drowsiness desynchronizes externally as well as internally (Reinberg et al. 1989, Gupta & Pati 1994a). Shift workers do have problems with sleep management specially because they attempt to have sleep at chronobiologically unsuitable time of the day. The problems include difficulty in initiating sleep and staying asleep. According to Czeisler et al. (1980) initiation of sleep is very difficult at the acrophase (maximum) of the body temperature rhythm and very easy at its nadir (minimum). Shift work disrupts the normal relation between rest/activity rhythm and the circadian regulation of bodily functions (Åkerstedt 1985). Among the most obvious effects of this disruption is disturbed sleep and increased sleepiness (Rutenfranz et al. 1981, Åkerstedt 1984). Åkerstedt (1992) reported sleepiness peak during the early morning in between 4.0 and 7.0 hr in night shift workers. A secondary peak has also been observed in sleepiness in the afternoon (Åkerstedt & Gillberg 1982).

The rotational shift workers report more fatigue than day workers (Åkerstedt 1988, Alfredsson et al. 1991). Usually, the fatigue is particularly widespread on the night shift, the least on the afternoon shift, and is intermediate on the morning shift (Gupta & Pati 1993). Kecklund et al. (1994) suggested that morning shifts (starting between 4.0 and 7.0 hr) is usually perceived as extremely fatigue inducing.

Social Problems

The shift workers have been shown to experience a number of psychological disturbances and family dysfunctions, as a result of which there is a serious impact on the family and social life (Åkerstedt 1990, Chang et al. 1993, Costa 1999). The irregular work
hours affect the whole family: the worker, his/her spouse and children. The displacement of the shift worker in time and space can result in domestic inconvenience both for the individual and spouse, as well as for other members of the family, to the extent that it could have detrimental effects on family relationships (Walker 1985). The difficulties in social life are mainly due to an inharmonious relationship between work schedules of shift workers and those of other day workers. Thus it is difficult for shift workers to participate in regular meetings and in other social events/activities, which are usually scheduled in the evening or on week ends (Carpentier & Cazamian 1977).

A recent study conducted by Pati and Chandrawanshi (2001) examined the effects of three-shift work schedules of shift workers on anxiety and mental health of their day active spouses and children. The levels of anxiety were found to be significantly higher in spouses and children of shift workers as compared with their counterparts sampled in the family of day workers. Also the status of mental health was significantly low among spouses of shift workers as compared with their day working counterparts (Pati & Chandrawanshi 2001). This indicates that disturbed daily schedules of shift workers may modulate anxiety and mental health in their spouses and children.

Clinical Problems
Shift work can lead to a host of problems attributed to the disturbances of the circadian system. It has been argued that shift work may result in significant morbidity. The long-term effects of shift work may induce coronary heart disease (CHD) and gastrointestinal diseases (see review Knutsson 1989).

Non-clinical problems
Performance
Poor sleep quantity (sleep deprivation) and quality have been considered as the key factors in modulating the performance of shift workers during the night shift (Freese & Harwich 1984, Siebenaler & McGovern 1991). Furthermore, in shift workers sleep deprivation and desynchronization of biologic rhythms lead to impaired physical performance (Smolensky et al. 1985, Costa 1999). Performance decrement has been reported in nurses during the night shift although there has been no sleep deprivation (Gupta & Pati 1993). Thus these findings negate the hypothesis that implicates sleep deprivation or sleep debt as one of the major reasons for performance decrement (Gupta 1992, Gupta & Pati 1993). Could it be that sleep during the habitual timing, but not the length of sleep is imperative for normal human performance?

The circadian rhythm and sleep wake cycle are mainly related to the psychophysiology of shift work. People working either in rotating shifts or in a static/shift system have to work during the night at the low phase of their circadian rhythm. This leads to severe sleepiness and reduced performance (Åkerstedt 1990). The results of the studies conducted by Gupta (1992) as well as Gupta and Pati (1994a) indicate that the shift rotation pattern is also important for normal performance. Studies on performance of shift workers working in three different type of rotational patterns revealed that 12-hr night shift system for 15 consecutive days was the worst one as compared to other two shift patterns, i.e., 12-hr night shift for 1 week and 8-hr weekly rotational shift system (Gupta 1992, Gupta & Pati 1994a).

Level of work performance efficiency on a night shift depends primarily upon: (1) the demands of task; (2) the type of shift system and hence potential for both short and long term adjustment; (3) individual differences between shift workers in the degree to which their rhythms adjust to night work, and (4) sleep deprivation (Folkard & Monk 1979, Phillips et al. 1991, Gupta & Pati 1994a).

On duty accidents
Several studies have documented that accidents and injuries are imputed to sleep deprivation and disruption of sleep-wakefulness rhythm that occur on account of shift work (Mitler et al. 1988, Costa 1999). A number of studies have demonstrated that the rate of serious accidents is higher at night than during the day (Glazer 1989, Folkard 1990, 1997, Gupta 1992, Åkerstedt 1995). Furthermore, it has been observed that despite considerable reduced traffic during night, single vehicle accidents occur past-midnight at a significantly higher rate (Hamelin 1981, 1987, Ouwerkerk 1987). Studies conducted on train drivers also revealed that they tend to overlook and/or issue more warning signals during the night shift. Various kinds of industrial injuries have also been
shown to be 2-3 times higher during the night shift as compared with the evening shift (Levin et al. 1985, Kreiger 1987, Novak et al. 1990).

The first “chronotoxic” industrial disaster occurred at Bhopal (Reinberg & Smolensky 1985). Another important point is that the circadian risk of accidents was used to be considered from the individual point of view before Bhopal and Chernobyl disasters. Now the question is: how to prevent population disasters with high-risk industries (e.g. nuclear power plant, oil refinery). The peak time of risk at night involves not only few given night workers but the population dwelling around the plant.

Shift optimization

Tolerance

Some shift workers tolerate shift work better than others (Folkard et al. 1983, Reinberg et al. 1989). On the basis of intensity of medical complications, it is possible to classify shift workers having good tolerance (with neither complaints nor medical problems), poor tolerance (with medical complaints) and very poor tolerance (severe clinical problems). Clinical intolerance to shift work was defined (Reinberg et al. 1988, Reinberg & Smolensky 1992) by the existence and intensity of a set of medical complaints: (1) Sleep alterations; (2) Persisting fatigue; (3) Changes in behaviour; (4) Digestive troubles; (5) The regular use of sleeping pills. Symptoms 1, 2 and 5 are present in any intolerant subject. Clinical intolerance to shift work appears to be independent of individual’s age and length of shift working experience (Reinberg et al. 1984, 1988). On the contrary, there are some other authors who believe that aging is associated with a decreased tolerance to shift work specially among male workers, critical age being on an average 40–50 years (Oginska et al. 1993, Tepas et al. 1993). There is a kind of ambiguity in using the term tolerance. “Clinical intolerance” relates to symptoms quoted above while “tolerance at large” involves also the incidence of diseases, which seem to occur more frequently in shift workers as compared to non-shift workers. Here the term tolerance has been used with reference to complaints or symptoms described above. It is extremely important to find out the levels of tolerance of shift workers. This has a bearing on the optimization of human shift work.

**Chronobiological Index**

It has been suggested that amplitude alteration could be taken as an index to assess individual worker’s shift work coping ability (Reinberg et al. 1984, 1988). The individuals with large circadian amplitude are more tolerant to shift work, since it helps the subjects to maintain their internal synchronization. It seems that persons who possess weak circadian time structure, i.e., a rhythm with low amplitude, are more prone to develop biological intolerance to shift work later in life. However, those with a strong (high-amplitude) time structure are the least prone (Smolensky & Reinberg 1990). Reinberg et al. (1988) reported large circadian amplitude of oral temperature, right- and left-hand grip strength and heart rate in good tolerant shift workers than with poor tolerant shift workers.

The shift work coping ability seems to be modulated by three important factors, namely, circadian, sleep and social factors (Reinberg et al. 1984, 1988, 1989, Folkard 1988, Monk 1988). Each of these factors consists of several other sub-factors (figure 30). The output of interactions of all or some of these factors seems to set the extent of complications in shift workers. It is also important to underline that in case of a given individual shift worker some but not all of these factors are relevant.

![Figure 30 Modulatory factors of coping ability to shift work. (Modified from Monk, 1988)](image-url)
simultaneously. The degree of relevance may depend upon the type of job and the genetic make up of the shift worker itself.

Chronotypes
There are basically two types of people in the population: (1) the lark people or the morning types and (2) the owl people or the evening types (Minors & Waterhouse 1989). There may be people who fall somewhere between these two extremes. These are called the chronotypes and could be identified in the local population by their peak phase of body temperature. Some workers have also designed specific inventories for identification of different chronotypes in the population (Östberg 1973, Gupta & Pati 1995). These variations may be attributed to contributions from multiple genes and environmental factors (Toh et al. 2001). It has been reported by them recently that a mutation in a gene called hPer2 is responsible for familial advanced sleep-phase syndrome (FASPS) in members of a Utah family. The affected family members sleep at around 7 p.m. and get up at around 2 a.m. Studies on this and related syndromes may help in having important insights into the human circadian timing machinery with potentially practical clinical benefits (Toh et al. 2001).

In this context circadian rhythm in body temperature assumes significance specially because it has been considered as a marker rhythm for several other rhythmic functions in humans (Reinberg et al. 1980, 1983, 1984, Motohashi et al. 1987). The body temperature rhythm has also been shown to vary as a function of morningness and eveningness. According to the time of going to bed and awakening time the owl people go to bed almost past midnight while the lark types go to sleep around 22.0 hr or even earlier. In contrast, as the day proceeds a subtle change slowly becomes evident in both types. The lark people show signs of fatigue first. Alert and sharp in the morning, they begin to slow down and ease up as sunset approaches. The owl persons on the other hand have a long way to go still.

A population study conducted earlier which included 582 subjects, those representing human population living in hot and dry tropical climatic conditions revealed that 75%, 16% and 9% have been found to be morning active, evening active and intermediate type individuals, respectively (Gupta & Pati 1995) (figure 31). These observations differ from the Gaussian distribution (10% MT, 10% ET and 80% either type) that is found in temperate climate, as shown by Horn and Östberg (1976) and others (Ashkenazi et al. 1997). It seems that it is unsafe to over generalize a local finding from a European country to the entire world. The acrophase timing of oral temperature rhythm in evening active individuals of the tropical population has been located in the late evening hours (around 19.4 ± 0.55 hr). In contrast, it has been observed that the morning active individuals have their peak about 4.9 hr earlier (14.5 ± 0.85 hr) than their evening active counterparts (Gupta & Pati 1995) (figure 32).

It has also been witnessed that the morning active subjects remain at their best between 8.6 - 10.7 hr with reference to the performance variable, random number addition speed, whereas, evening active

![Figure 31 Prevalence of morningness and eveningness in a human population. Based on information taken on questionnaires from 582 subjects, irrespective of age, sex and working habits, living in Chhattisgarh, India. MA = Morning active; EA = Evening active. (Based on data from Gupta & Pati 1995).](image)

![Figure 32 Acrophase map for oral temperature (OT) and random number addition speed (RNAS) in a group of morning active (MA) and evening active (EA) subjects. Each point represents the estimated maximum (acrophase, θ) of each group. The horizontal bar defines the 95% confidence limits of acrophase. OT peak occurred 4.9 hr earlier in MA subjects as compared with the EA subjects. Morning active subjects exhibited the best performance in between 8.6 and 10.7 hours whereas EA were at their best between 16.9 and 20.2 hours. (Based on data from Gupta & Pati 1994b, 1995).](image)
subjects remain at their best between 16.9 - 20.2 hr, approximately six hours later (figure 32).

**Frequency of Shift Rotation**

It has been unequivocally accepted that night shift alone or as a component of the 2-shift or 3-shift systems is not suitable for diurnally evolved humans (Folkard & Monk 1985, Waterhouse et al. 1992, Smith et al. 1998). Is it possible to get rid of the detrimental effects of night shift system? It is somewhat possible to reduce the effects of the night shift. The first thing that comes to our mind is the frequency of rotation of the system. The rotation could be either slow or fast. Several authors have examined this option. The quickly rotating shift system seems to find maximum favour. A fast rotation helps in minimizing sleep deprivation (Fischer et al. 1997), circadian rhythm disruption and improve social contacts (Knauth 1993, 1995), alertness and well-being (Williamson & Sanderson 1986, Phillips et al. 1991). Further, the shift workers working in rapidly rotating shift system have been shown to perform excellently while on memory loaded task (Monk & Embrey 1981). Rapid rotation (2 to 4 days) from a chronobiological point of view is advantageous with regard to the conventional weekly rotation. This has been demonstrated experimentally in field studies involving oil refinery operators (Chaumont et al. 1979, Foert & Benoit 1979, Vieux et al. 1979): (1) the transient desynchronization of the temporal organization is smaller with the rapid rotation than with the other, (2) the desynchronization of sleep patterns (EEG recordings = hypnogram) is smaller with the rapid rotation, and (3) as a result the recovery of a physiological temporal organization occurs more rapidly after the rapid rotation than the weekly rotation. But the rapid rotation does not solve the problem of nocturnal risk of accidents. Further, there are reports that do not support the quick rotation system. The main argument is that the extremely quick rotation would have reduced free time between shifts by several hours (Totterdell & Folkard 1990, Kurumatani et al. 1994) leading to substantial sleep loss. Then one should find out the threshold for free time between shifts that would not cause loss of sleep. However, there is no such study to support the above. Further the length of the shift itself is also very important from point of view of tolerance to shift work. It has been reported recently that the emergency medicine residents generally prefer and tolerate 8-hour and 10-hour shift lengths better as compared with 12-hour shift lengths (Steele et al. 2000). Nevertheless for the time being quickly rotating shift system appears to be better.

**Advancing or Delaying Shift System?**

Another important factor that deserves attention is the direction of the rotation of shift schedules. The direction of rotation may be either clockwise (forward rotation or delaying system) or counterclockwise (backward rotation or advancing system). Several authors have taken keen interest to examine the role of this factor in shift optimization. Although there are contradictory views as regards relative merits of advancing and delaying shift systems, clockwise rotation was noticed to be better tolerated by the shift workers than the one that follows counterclockwise pattern. A change from counterclockwise to clockwise rotation has been documented to improve production, well-being (Czeisler et al. 1982), sleep quality (Epstein et al. 1991, Barton & Folkard 1993), and reduce physical, social and psychological problems (Landén et al. 1981). Although there are limited evidences, clockwise rotation of work schedule (i.e., Morning-Evening-Night) seems to be the best universal pattern. The findings from jet lag research that the westward travel produces quicker resynchronization of human circadian rhythms as compared with the eastward travel support the above conjecture. Recently it has been advocated that advancing shifts may not be as harmful as early research indicated (Tucker et al. 2000). Further studies would definitely resolve this dilemma whether to accept advancing or delaying shift system.

**Switching Workers from Rotating Shift Duty to Day Duty**

A recent study conducted by Chandrawanshi & Pati (2000) shows that desynchronized circadian rhythms in shift workers returned to normal when they are allowed to behave as day workers with nocturnal sleep. Circadian time structure of shift workers was studied in two different spells. There was a lag of about 16 months between two spells. During this period the factory remained almost closed for nearly 8 months.
with moderate to low profile activities in the remaining months. At the time of the study in the first spell the shift workers had already experienced about 14 months of industrial slough. However, when the same shift workers were reexamined in the second spell after about 16 months they experienced about 30 months of cumulative industrial slumber that accompanied 8 months of near complete closure. However, during this period, shift workers were assigned shift duties, irrespective of the work load and activity of the factory. Despite on rotational shift duties, whenever there was no work load, they slept at their work places. In other words, they behaved reasonably like day workers with nocturnal sleep during the period between two spells of studies. Therefore, in the shift workers of the cement factory the once desynchronized rhythms in several variables became resynchronized. The process of resynchronization also accompanied an increase in the circadian amplitudes of these rhythms (Chandrawanshi & Pati 2000). figure 33 shows illustrative examples of spectral analysis that clearly support the above conclusion. Results indicate that in the 1st spell of study several variables, namely skin temperature, heart rate and peak expiratory flow rate had non-24 hr periods. However, in the 2nd spell after about 16 months all variables exhibited circadian periodicity (τ = 24 hr) when the shift workers had opportunity to have on job nocturnal sleep. These results support the findings reported by Reinberg et al. (1984) in that when a shift worker with poor tolerance was transferred from shift work to diurnal work, the desynchronized rhythm in oral temperature became resynchronized after about 1 year exhibiting prominent period equal to 24 hr in oral temperature rhythm.

**Nap**

Several authors have studied the effects of nap on alertness, performance, and sleep quality (Härnä et al. 1989, Batejat & Lagarde 1999, Hayashi et al. 1999, Takahashi et al. 1999). Brief naps during work may be helpful to some workers as this enhances alertness temporarily. It has been shown that short naps of 20 to 40 minutes can be beneficial as it may improve sleep quality, performance and mood (Härnä et al. 1989, Batejat & Lagarde 1999, Hayashi et al. 1999). In some cultures, particularly in Japan, night-shift naps are officially sanctioned (Kogi 1981).

**Exposure to Bright Light**

Furthermore, studies on the effects of simulated night work demonstrate that exposure to bright light during night can virtually eliminate circadian maladjustment among night workers (Bjorvatn et al. 1999). After four cycles of light treatment the endogenous circadian rhythms of body temperature, subjective alertness, cognitive performance, urine production and plasma cortisol secretion have been observed to be completely adjusted to the new schedule (Czeisler & Dijk 1995). In addition, exposure to bright light during the night shift has been reported to improve daytime sleep as compared to controls (Rutenfranz et al. 1981, Czeisler et al. 1990). NASA scientists in manned space flight have implemented this principle for the first time (Czeisler et al. 1991). NASA is now regularly using the bright light technology (therapy) on all space shuttle missions (Czeisler & Dijk 1995). Lithium (Engelmann 1973, Kripe et al. 1978, Johnsson et al. 1979) and to a certain extent other antidepressant drugs (Halberg 1963, Wirz-Justice et al. 1980a,b), used to control depression and manic-depressive illness, have
been shown to act on the period of circadian rhythms. The latest drug being used to reset rhythms is melatonin. Its potential use in circadian rhythm disorders has been investigated in field studies of jet lag and shift work and in simulated phase shifts (Redman et al. 1983, Arendt et al. 1995).

Can Shift Work be Abandoned?
A modern society probably cannot afford to abandon shift work, although it has been unequivocally established that it produces a series of acute and chronic effects on human beings. Therefore, there is an absolute need for an optimization of human shift work. Then question arises: how can the circadian rhythm desynchronization be minimized?

On the basis of studies done by our group and others it is suggested that each and every work places where shift work is mandatory, a chronoclinic should be established. Trained health care personnel of the chronoclinic should monitor intermittently (preferably every alternate year) the state of the biological clock (synchronized or desynchronized?) of each shift worker. Upon discovering rhythm desynchronization his/her transfer from shift work to day work for at least one year should be recommended to the employer/management. This would perhaps rule out the possibilities of ill effects of shift work that are expected to be impinged upon the workers. It has been proposed that while examining tolerance/intolerance of a shift worker to rotational shift work the levels of anxiety and mental health status of the individual under scrutiny should be taken into consideration. Sleep-wake disorder is another important variable that cannot be simply ignored while ascertaining intolerance to shift work. Appropriate chronotherapy, in addition, should also be administered into intolerant shift workers while they are being transferred from shift duty to day duty. A model has been proposed with a view to optimize shift work (figure 34). This model takes into account most of the important variables those are thought to have a bearing on the effective management of shift work.

All these countermeasures either individually or in combinations may improve the coping ability of shift workers thus minimizing the occupational health hazards and maximizing their performance. In addition, this would increase the productivity of the organization substantially for whom they are working.

Figure 34 Model suggesting optimization of human shift work. See text for details. (Modified from Pfitz et al. 2001).

Conclusions and Future Directions
Chronobiology has progressed tremendously in the last three decades. The molecular bases of cyanobacterial and eukaryotic circadian clocks have been partly understood with respect to their period length, sustainability and relationship with light. The recent advances in the techniques of molecular biology would help us further to understand the mechanisms of the clocks in a much better way in near future. However, our knowledge is fragmentary regarding relationship of the clocks with the nonphotic stimuli. In nature both photic and nonphotic stimuli exist together and they interact with each other while inducing phase shifts by way of acting upon the clocks. Some progress has been made to understand this relationship in hamsters. The
photic and nonphotic cues seem to have a common molecular target in the clock, although the signalling pathways appear to be different. This area of inquiry may have important bearing on the success and failure of modern chronotherapeutics. Further, in future circadian clocks would receive increased attention with reference to application of the principles of biological rhythms in chronotherapy, in the management of problems in shift workers, and in the treatment of human sleep disorders. In addition, attempts would also be made to elucidate the molecular mechanisms of nondiurnal-based clocks, namely ultradian and infradian clocks and their relationship with the circadian clocks.

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