CHRONOTOXICITY AS RELATED TO CHRONOBIOLOGY

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SUMMARY

This review focuses primarily on the complexities of chronotoxicity and chronopharmacology (time-of-day effects on the metabolism of environmental chemicals and therapeutic agents as related to chronobiology). The nature of the melatonin signal may modify the function of the hepatic endoplasmic reticulum resulting in variations in the metabolism of xenobiotic chemicals. Concepts are explored for modification of exposure limits and/or Threshold Limit Values (TLVs) of industrial chemicals in risk assessment and health effects of workers on rotating shifts. The TLVs of chemicals may be changed during work shift schedules to minimize adverse health effects among workers.

1. INTRODUCTION

The late 1950s marked an era of investigations that helped to lay the foundation for an understanding of the fundamental role of melatonin in influencing the hypothalamic-pituitary axis. Early observations included: 1) the impact of a photoperiodic environment on the pineal gland function /1-3/; 2) the endocrine capabilities of the pineal gland secretion, N-acetyl-5-methoxytryptamine or melatonin, /4,5/; 3) the influence of the pineal gland on the reproductive function of photoperiodically-dependent rodents /1,6/; 4) the regulatory role of the sympathetic nervous system in the biosynthetic and endocrine activities of the pineal gland /7/; 5) the time-of-day effect on the efficacy and/or toxicity of pharmacotherapeutics as related to melatonin /8/. However, there existed some controversy regarding the modulatory effects of melatonin on the neuroendocrine-reproductive axis /9/. These controversial findings were not affirmed by other workers using hamsters with subcutaneously-placed melatonin pellets /10/. The changes in physiological parameters following melatonin administration during specific circadian phases provided convincing evidence for the role of pineal gland influence on the neuroendocrine axis. The intricacies of the pineal gland and its related functions in systems physiology have been reviewed /11-13/.

2. CHRONOPHARMACOLOGY AS RELATED TO CHRONOBIOLOGY

The general concepts of chronobiology or circadian rhythmicity and the effects it can have upon the concentration of body constituents in plasma and urine are well known /14,15/. Circadian principles have found applications in many areas of biology. However, the full extent of its importance in other areas of biology are less known. Marks *et al.* /16/ and Lewis *et al.* /12/ have reviewed the clinical and toxic responses of pharmacotherapeutics based on the time-of-day effect. If the adage that "pharmacotherapeutics is regulated toxicity" is accepted as true, chronopharmacology can be said to have had its beginnings more than 50 years ago when Agren and co-workers /17/ showed that mice, used to assay insulin for therapeutic use, were more resistant to insulin-induced convulsions if the insulin was given in the evening rather than in the morning. In order to produce the same percentages of convulsions in the mice, twice as much insulin was required when insulin was given in the evening as at noon. Some 20 years later, Carlson and Serin /18/ serendipitously observed that time-of-day of administration was a factor determining the toxicity of nikethamide in rodents. In 1960, Halberg /19/ showed a remarkable difference in the susceptibility of mice to the endotoxin of *E. coli* depending upon the time of day it was administered. A dose that would kill 95% of animals when given in the early afternoon would barely kill 10% if given at midnight.

The term chronopharmacology was coined in the early 1960s and its first application to clinical medicine was in the timing of steroid therapy to decrease both its real and potential toxicity, on the one hand, and to increase its efficacy on the other. DiRaimondo and Forsham /20/ suggested that the adrenocorticoid suppression by exogenous steroids could probably be minimized by administrating in the morning between 08:00 h and 10:00 h rather than in divided doses, or at night. Several clinical studies have since been published adding weight to these suggestions /21,22/. More dramatic, though numerically far less important, is the clinical application of these principles to the treatment of adrenogenital virilism by steroids, following the observation by Nichols and co-workers /23/ that a small dose of dexamethasone given at midnight to healthy human subjects was far more effective in suppressing endogenous adrenosteroid production than similar, or much larger, doses given in the morning.

Children with adrenogenital virilism who were seemingly resistant to conventional therapy were successfully treated merely by altering the time of steroid (dexamethasone) administration from twice daily, in the morning and early evening, to as near midnight as practicable, and monitoring progress not only clinically but also by endogenous plasma adrenosteroid measurements /16/. The diurnal fluctuations of several factors, such as absorption, distribution, metabolism and excretion, may influence circadian variation of drug response /15/. Circadian rhythm of brain sensitivity to drugs may also play a role in determining the response to CNS drugs.

3. CIRCADIAN VARIATION IN DRUG DISPOSITION

Rhythmic variation in drug disposition and action has been described for a variety of agents including salicylates, indomethacin, acetaminophen, theophylline, phenytoin, nortryptiline, erythromycin, cisplatin, potassium, and corticosteroids /24/. A major factor responsible for circadian variation in drug concentration is alteration in drug absorption, in relation to posture and meals consumed. Such variations may be accentuated by increasing the use of sustained release formulations of various drugs. In children a significant reduction in absorption of sustained release theophylline occurs when administered in the evening. This nocturnal malabsorption results in lower than anticipated serum theophylline concentrations during the night-time hours, particularly at the time when nocturnal asthma symptoms are likely to be maximal /25/. As our knowledge of circadian variation in drug absorption improves, it is likely that dosage will be tailored to the time of day. It is already apparent that pharmacokinetic studies, especially with sustained release formulations, should be carefully controlled for the hour of administration, relationship to meals, and posture of subjects. Ideally, studies with such products should cover a 24-hour period after drug administration if misleading conclusions are to be avoided.

4. TOXICITY AND CIRCADIAN OSCILLATION

The concentration of a drug in blood is determined by a number of variables, each of which may show circadian oscillation. Many drug

metabolizing enzymes, for example, have been shown to vary in amount and activity depending on time of the day /26,27/. Numerous studies have been reported in the literature describing both the therapeutic effects and/or toxicity of various xenobiotics, depending on the time of day. Hecht *et al.* /28/ reported on a series of pharmacological effects dependent on the brightness of the environment. Nelson and Halberg /29/ investigated circadian rhythm in sleep response of mice injected with different doses of pentobarbital. Significant differences were found at different times of the day in the length of sleep as well as in the plasma level of pentobarbital. Substantial evidence that drug responses have a circadian rhythm in rodents has been established for phenobarbital sodium, amphetamine /30/, and diazepam /31/. Similar variations have been demonstrated in the toxicity of some cytotoxic drugs, notably cytosine arabinoside /32/, adriamycin /33/ and cisplastin /34/. The toxicity and cure rate of cytotoxic drug combinations when used to treat L1210 murine leukemia have been shown to exhibit a circadian rhythmicity /35/. There are indications that the clinical response to adriamycin and cisplastin in patients with advanced ovarian carcinoma can be improved if the drugs are administered at specific circadian phases /16/. Similarly, survival rates of patients with a variety of solid tumors treated by chronochemotherapy with methotrexate or 5-fluorouracil followed by vinblastine and cyclophosphamide, were improved by administering at specific time periods /36/.

The urinary excretion of drugs, particularly those that are susceptible to changes in urinary pH /37/, is often markedly different at different times of the day /38/. So too is the absorption of drugs /39/, and even their binding to plasma proteins /40/. The latter may have pharmacodynamic effects not only through its direct effect on blood concentrations, but also by influencing drug availability at the end-organ receptors. Nonetheless, there appears to be a gap in the knowledge which may clearly define the correlates of circadian regulation of drug metabolism and/or pharmacokinetics in both animals and humans.

Perhaps it is not altogether surprising that one of the first "toxicants" to be studied for circadian variation in pharmacokinetics was alcohol /41/, long amenable to measurement in body fluids and of immense toxicological and social interest. Early results indicated that alcohol was metabolized faster between 10:00 h and 20:00 h than

between 20:00 h and 10:00 h. Despite this and subsequent studies /42/, knowledge of the chronopharmacology of alcohol is still rudimentary and has certainly not yet entered into our clinical, or even our social, way of thinking. This is due in part to the difficulty of carrying out properly controlled experiments in humans and, above all, to the difficulty of distinguishing differences caused by endogenous rhythms due to genuine biological clocks from the passive response of the body to a wide variety of environmental factors (zeitgebers). In this context, therefore, it is relevant to note that in their early paper on the chronopharmacology of alcohol, Wilson and co-workers /41/ wrote:

> *"...a sixth subject was a nurse on night duty; in her case the rhythm (of rate of alcohol metabolism) was reversed on three separate studies, in spite of the fact that the experimental period was over her day off, when she chose to sleep at night. This last is only mentioned parenthetically but is interesting since diurnal rhythms largely are associated with the development of habit patterns of sleep rather than specific episodes..."*

clearly indicating, as early as 1956, that simple environmental explanations of chronopharmacological phenomena were unlikely to be correct. Nevertheless, the extreme complexity introduced into the whole topic of chronobiology by academic purists, coupled with the difficulty of extrapolating data obtained from the use of nocturnally active laboratory rodents, e.g. rats and mice, to diurnally active human beings, has undoubtedly served to deter all but the most dogged from venturing into this field, which promises as much hope for improvement in clinical drug usage in the future as therapeutic drug monitoring has produced in the past.

Sauerbier /43/ has reported the circadian modification of ethanol damage *in utero* to mice. The study evaluated the ethanol toxicity for fetal development at different circadian phases. Pregnant mice were given a single intraperitoneal ethanol injection on day 7, 8, or 10 of gestation at one of four circadian phases (07:00, 13:00, 19:00 or 01:00 h). The dams were killed on the day before term (day 18). Prenatal exposure to ethanol resulted in an increased number of resorptions, reduced fetal body weight, and produced an increased incidence of phenotypic abnormality. The severity of damage was related to the dose, the period of gestation, and particularly to the circadian phase at

the time of treatment. Ethanol had the greatest effect on the embryo of a mouse when adminstered at the mid-dark span. Consequently exposure to a single dose of ethanol at one time or another along the 24-h time scale during organogenesis has important implications for a substantially increased risk /44-46/. In a human study, ethanol (0.67 g/kg) was ingested orally at 07:00 h, 11:00 h and 23:00 h by six healthy male subjects. Self-rated inebriety was found to be greatest at post 23:00 h ingestion and lowest at 11:00 h /47/.

Studies in rats have shown that the pineal hormone, melatonin, accelerates entrainment of the circadian adrenocortical rhythm to the reversal of the light cycle, suggesting that the circadian rhythm in melatonin production might influence that of corticosterone /48/. Since melatonin production is suppressed by light /2/, maximum toxicity of phenylbutazone (PZ) at 06:00 h coincides not only with the nadir plasma corticosterone concentration, but also follows the dark-phase increase in melatonin secretion. Dhami and colleagues /49/ studied the pharmacokinetics of PZ and the effects of PZ administration to rats pretreated with melatonin. Male Wistar rats were pretreated with melatonin for 14 days to induce circadian dyschrony and later treated for six days with PZ. On the day subsequent to the last PZ injection blood samples were taken at 00:00, 06:00, 12:00 and 18:00 h and analyzed for hematocrit, WBC, urea and aspartate aminotransferase. Rats receiving melatonin showed statistically significant differences for all parameters at all time points except at 12:00 h. The results indicate that rats receiving melatonin show altered time-dependent sensitivity to the effects of PZ. In a separate study, rats were given PZ or melatonin intraperitoneally at 16:00 h for six days. On day 7, blood samples were collected to determine the circadian rhythm of plasma corticosterone. Melatonin treatment resulted in decreased corticosterone at all times and considerable dampening of the rhythm amplitude. PZ treatment lowered corticosterone at 06:00 h and 00:00 h with much greater amplitude. The half-life of elimination of intravenously administered PZ (100 μg/kg) was highest at 00:00 h (115 ±15 min) and lowest at 12:00 h (43 ± 14 min) light/dark (L:D 12:12, light on 06:00 h) (Table 1). These observations emphasize the necessity of considering changes in pharmacokinetics and pharmacodynamics of commonly used drugs when given to patients experiencing profound disturbances in their biological timing mechanisms.

TABLE 1

Circadian variation in pharmacokinetics of intravenous phenylbutazone in male rats

Time of dosage (h)	Elimination half-life (min)
08:00	87 ± 10
12:00	57 ± 7*
16:00	43 ± 5*
20:00	70 ± 12
00:00	115 ± 15

Values are expressed as mean ± SEM of four rats in each group.
Phenylbutazone injected i.v. 100 mg/kg body weight.
*Values differ significantly from 00:00 h group, $p < 0.05$.
Light/dark cycle 12L:12D, lights on 06:00 h.

Experiments were carried out with dexamethasone, methylprednisolone, prednisolone, methotrexate and exogenous corticosterone using melatonin /50-53/. The rats were dosed at 15:30h each day, for six weeks, with melatonin (80 μg/kg body weight) dissolved in corn oil (1 ml/kg body weight) prior to challenging with methotrexate. Control animals were given corn oil alone. Rats dosed with corn oil alone showed the familiar diurnal methotrexate toxicity pattern. This was considerably modified in rats pretreated with melatonin which, in addition to displaying the expected high mortality when methotrexate dosing was performed at 06:00 h, now showed a 100% mortality when dosed at 24:00 h and a 33% mortality when dosed at 18:00 h. Only those dosed with methotrexate at 12:00 h remained relatively unaffected. It has not yet been determined whether suppression of melatonin secretion decreases methotrexate toxicity, as might be predicted. In another study, Wistar albino rats were exposed to different intensities of light (0.1, 1.0, 10.0, 100.0 lux, sunlight simulating fluorescent lamps, vitalite) and maintained on a 12-h L:D cycle for eight weeks. Methotrexate was administered intravenously at 06:00 or 18:00 h (100 mg/kg body wt.). Serum methotrexate, cholesterol, glucose, creatinine, urea nitrogen and bilirubin were

determined. At 18:00 h significantly different serum methotrexate levels were observed for the 0.1 lux exposed group as compared to 100 lux group. The serum methotrexate concentration was elevated with increasing light intensity when the methotrexate was administered at 18:00 h, but not when administered at 06:00 hours (Figs. 1,2). Cholesterol was significantly decreased by methotrexate in the 100 lux group at 06:00 h and at 18:00 h, as compared to controls (Table 2). The serum blood urea nitrogen was significantly increased in the methotrexate treated group exposed to 100 lux at 06:00 h (Table 2). Serum creatinine was decreased in the 0.1 lux and 10 lux exposed animals treated with methotrexate at 18:00 h (Table 2). Serum glucose was increased in the 100 lux group, as compared to the 0.1 lux group, in both 06:00 h and 18:00 h methotrexate-treated animals (Table 2). The methotrexate pharmacokinetics showed circadian variation, and methotrexate toxicity was well correlated to ambient light intensity exposure /54/.

There is a plethora of medical literature extensively describing molecular, cellular, and organismic time-keeping mechanisms, and circadian rhythms, in cytokinetic, pharmacokinetic and pharmaco-dynamic parameters. All these studies demonstrate the rhythmic relationship between dose and effect that occurs during a 24 hour cycle period. The practice of administering pharmacological agents at certain phases of circadian rhythm may diminish side-effects while increasing maximal safe dose-intensity of diverse classes of drugs including chemotherapeutic agents. Chemotherapy in divided dosage is notorious for its severe side-effects which may be minimized if the drugs are administered at an appropriate circadian time. Some widely used pharmaceuticals are now reviewed.

Anthracycline

Levi and workers /55/ demonstrated a statistically significant circadian rhythm pattern in the tolerance of 4'-O-tetrahydro-pyranyl-adriamycin (THP) in 226 male B6D2F1 mice synchronized with LD 12:12. Four intravenous dosages (18, 25, 32, and 40 mg/kg) and six different dosing times (3, 7, 10, 14, 19 and 23 h after light onset) were compared. Body weight loss and leukopenia in the surviving animals depended on both the dose and time of injection. The overall survival rate varied between 83% (light-rest span) and

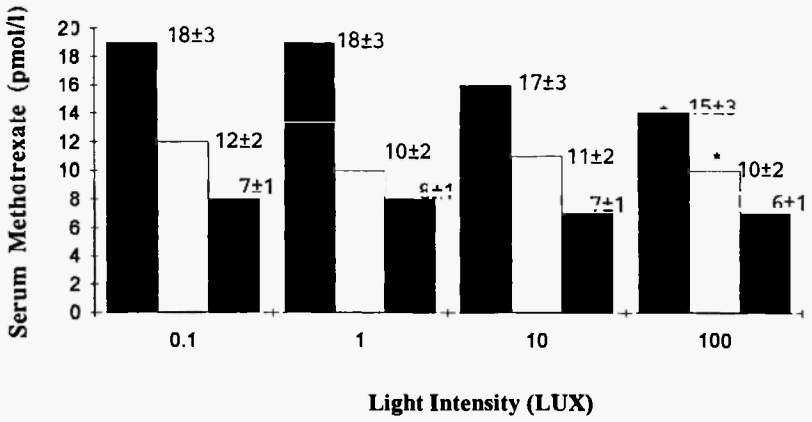

Fig. 1: **Elimination half-life of methotrexate in male rats dosed at 06.00 h.** Values are expressed as means ± SEM of four rats in each group. *Values differ significantly from 0.1 Lux control group ($p<0.05$) light/dark cycle 12L:12D, light on 06:00 h.

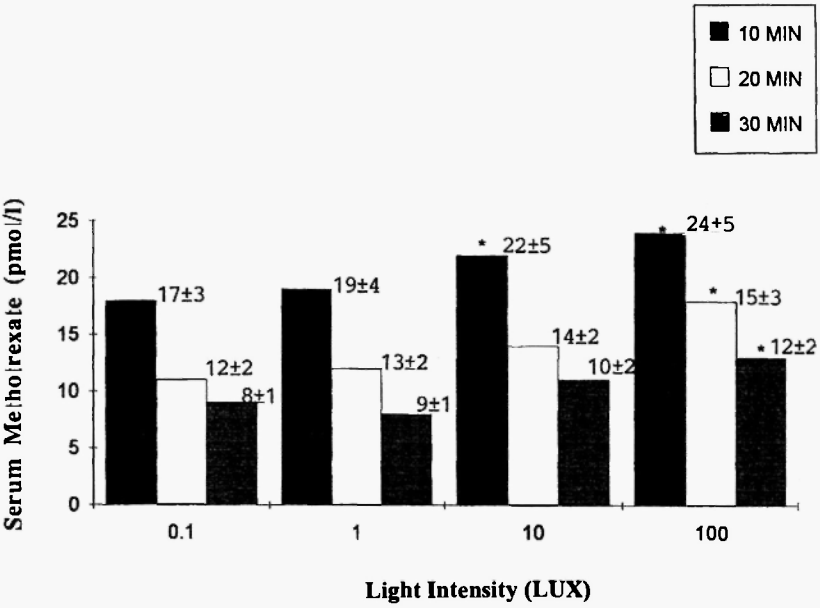

Fig. 2: **Elimination half-life of methotrexate in male rats dosed at 18.00 h.** Values are expressed as means ± SEM of four rats in each group. *Values differ significantly from 0.1 Lux control group ($p<0.05$) light/dark cycle 12L:12D, light on 06:00 h.

TABLE 2

Effect on blood biochemistry of methotrexate administered to rats at 06:00 h and 18:00 h and exposed to various light intensities

Light Intensity (Lux)	CHOLESTEROL (mg/dl)				UREA NITROGEN (mg/dl)				GLUCOSE (mg/dl)				CREATININE (mg/dl)			
Time of Dosage:	06.00h		18:00h		06:00h		18:00h		06:00h		18:00h		06:00h		18:00h	
	Control	MTX	Control	MTX	Control	MTX	Control	MTX	Control	MTX	Control	MTX	Control	MTX	Control	MTX
0.1	130±16	125±18	120±16	140±19	25±2	28±4	22±3	26±2	125±19	80±14	79±13	100±23	1.3±0.14	1.7±0.2	1.6±0.1	1.1±0.1*
1.0	126±19	108±16	132±21	95±13†	22±3	21±3	26±2	22±3	120±26	118±28	115±19	141±28	1.2±0.16	1.6±0.2	1.4±0.1	1.4±0.1†
10.0	139±14	110±16	130±22	96±20†	19±1	21±3	22±3	26±3	138±21	133±19	80±18	152±22*	0.8±0.1	1.0±0.1	1.8±0.2	1.1±0.1*
100.0	136±18	77±18*†	148±16	82±18*†	17±2	28±3*	21±2	17±2†	128±19	130±16†	131±16†	176±24†	1.5±0.1	1.3±0.1	1.7±0.2	1.3±0.2

*Values differ signicantly from corresponding control group ($p<0.05$). † Values differ significantly from 0.1 Lux group ($p<0.05$). MTX indicates methotrexate-treated group. (Light/dark cycle: 12L:12D, lights on at 06.00 h.)

56% (dark-activity span). Maximal body weight loss occurred 4-5 days after drug injection. Total leukocyte counts were reduced by approximately 100% in those mice injected in their late rest span as compared to those treated in the middle of their activity span. Minor cardiac lesions consisting of diffuse vacuolization and loss of muscular striation were observed in histologic sections from 3/32 hearts (16 controls, 16 treated). Thus lethal, hematologic and possible cardiac tolerance for THP were largely optimized by administering the drug to mice in their late rest span.

Antipyretics

There is considerable evidence that the circadian rise in body temperature may be attributed to an elevation in the thermoregulatory set point /56/. This rise in set point may be mediated by prostaglandins. Rats were injected with the prostaglandin synthesis inhibitors sodium salicylate, acetylsalicylic acid, and indomethacin at 17:00 h and at 09:00 h. Administration of these drugs had little effect on body temperature at night when temperature is normally in the rising or plateau phase of the cycle. Hence, it was concluded that prostaglandin synthesis is an important component of the circadian rise in body temperature in rat. In addition, data indicated that a cryogenic factor exists which opposes the night-time prostaglandin-mediated rise in body temperature.

Cimetidine

The effects of single doses of cimetidine, 800, 1200, and 1600 mg, given at 23:00 h, or 800 and 1600 mg at 18:00 h, have been studied in patients with duodenal ulcer disease in symptomatic remission, and compared with cimetidine 400 mg/kg (08:00 h and 23:00 h) and ranitidine 300 mg (given at 1800 h) respectively. A dose-related reduction in intragastric acidity was seen. All single nocturnal (23:00 h) doses of cimetidine produced anacidity overnight. This was not achieved with dosing at 18:00 h although the duration of inhibition of gastric acidity was longer. Inhibition of overnight acid and pepsin outputs were similarly dose- and timing-related, but inhibition of peptic activity was much less after dosing at 18:00 h. Cimetidine 1600 mg and ranitidine 300 mg were similar in their effects /57/.

Corticosterone

Circadian release of corticosterone was reported by Ehlers and co-workers /58/. The effects of corticotropin-releasing factor (CRF) and growth hormone-releasing factor (GRF) on electroencephalographic (EEG) and behavioral signs of sleep and wakefullness following their intracerebroventricular administration was investigated in adult male rats. Visual scoring of EEG records as well as spectral analysis revealed that CRF (0.0015-0.015 nmol) produced decreases in slow-wave sleep, concomitant with significant decreases in spectral power in lower frequencies (1-6 Hz) and increases in high frequencies (32-64 Hz). In contrast, GRF (2.0 nmol) produced increased EEG and behavioral signs of slow-wave sleep associated with significant increases in spectral power in the low frequencies (1-2 Hz) and decreases in high frequencies (32-64 Hz). I.c.v. administration of GRF was also found to produce decreases in locomotion when adminstered during the active part of the rat circadian cycle. These EEG and behavioral findings seen following CRF and GRF are consistent with the behaviors frequently correlated with the known circadian timing of the release of corticosteroids and growth hormone during the sleep-waking cycle in rat and human. As described above, circadian rhythms of corticosterone have been implicated in the toxicity of certain pharmaceuticals.

Indomethacin

Studies by Clench and co-workers /39/ showed a circadian rhythm of both peak height and time to peak in the bioavailibility of indomethacin in heathly male and female subjects. The data indicate that indomethacin reaches the highest peak more rapidly when taken at 07:00 h or 11:00 h than when taken at 15:00 h, 19:00 h or 23:00 h. The pattern exhibited by indomethacin (with highest plasma concentration peak, the shortest time to peak, and the fastest disappearance rate, when the drug is given in the morning) resembles the chronopharmacokinetics of ethanol and theophylline. These workers recommend a night-time administration of indomethacin for maximum efficacy in patients with rheumatoid arthritis.

Ketamine

Data from Winters and colleagues /59/ on ketamine-induced analgesia and catalepsy in rats suggested that it is factors other than dose which modified the difference in the latency of the flick response (a measure of analgesia), and the duration of the loss of the righting reflex (a measure of catalepsy). Untreated female rats showed a longer latency than most males in their response to a noxious stimulus at midnight, but not at noon. Females also showed a longer loss of righting reflex response to ketamine than did males, whether at noon or at midnight; the loss of righting at night was augumented in both. Although females showed analgesia after administration of ketamine at doses smaller than those which induced catatonia, males showed no analgesia without catatonia, and comparable loss of the righting reflex occurred at doses much larger than for females. There was a 3-fold increase in the latency of the tail flick reponse and loss of righting reflex during the winter, as compared with summer, for the females treated with ketamine; males showed a similar variation in the loss of righting reflex.

Lithium

Lithium, a widely used substance for treatment of manic-depressive illness, has been reported to alter the phase relationship of a variety of circadian rhythms which have been implicated in the etiology of depression and manic-depressive disorder. Although its mechanism of action is not understood, the therapeutic action of lithium has been related to its ability to alter circadian rhythms and/or act as a chronobiotic. Chronic lithium administration to rats resulted in lithium levels comparable to the human therapeutic range. These lithium levels affected a broad range of biological variables by significantly modifying their circadian pattern of variation, notably during the dark period of an alternating light/dark (12L:12D) schedule. These included water intake, body weight, retina weight and melatonin levels of pineal, serum, retina and hypothalamus. Retinal lithium levels were significantly higher than serum lithium levels, and furthermore, lithium decreased the melatonin levels. It was suggested that lithium may exert its therapeutic effects by influencing melatonin levels at several locations

along the retinal-hypothalamic-pineal pathway, resulting in a modulation of the potential cue value of this physiolgical stimulus for synchronization of circadian rhythms. Such an effect of lithium could have important chronobiological implications for circadian rhythms which use light and dark as a phase cue. Furthermore, dietary potassium supplementation has been shown to improve the therapeutic index of lithium treatment as well as to ameliorate the side-effects of lithium treatment for mania and depression /60/.

Methyl-prednisolone

The first drug to be studied chronopharmacokinetically was methyl-prednisolone, the timing of the administration of which had already been shown /35/ to alter dramatically both the cure rate and the drug-induced mortality of leukemic mice treated with a combination of cytosine-arabinoside, cyclophosphamide and vincristine. The results revealed highly significant differences in the plasma half-life of methyl-prednisolone in rats, depending on the time of dosing. The time of maximum half-life in rats (18:00 h) corresponded closely with the time found /51/ to be associated with the minimum cure rate of leukemia and minimum drug-associated mortality in the mouse, another nocturnal rodent. The relevance of these observations to man awaits further study.

Prednisolone

In a series of experiments, using volunteer humans, the subjects showed highly significant differences in plasma half-life ($t_{1/2}$) and other pharmacokinetic parameters of prednisolone, depending upon the clock time of administration /53/. Here, as in the rats, maximum half-life ($t_{1/2}$) was observed after dosing at 18:00 h.

Propylthiouracil

The thyroid neuroendocrine axis has been implicated in the control of circadian rhythmicity. Morin *et al.* /61/ examined the ability of the thyroid hormone-inhibiting agent, propylthiouracil (PTU), to modulate phase and period of the hamster wheel-running circadian rhythm. The circadian period (tau) of blind male hamsters was lengthened by approximately 0.2 h when they were fed a diet containing 0.6% PTU.

Removal of the PTU reversed the change in tau. Pinealectomy did not alter the response to PTU. Blind ovariectomized female hamsters showed changes in tau during and after PTU treatment that were similar to those of males. Males were also tested with 0.3, 0.6, or 1.2% PTU diets, and a dose-response relationship was established. Under 14:10 light-dark (L:D, 14:10) conditions, the phase of activity onset relative to lights off (psi) was not affected by 0.6% PTU. In LD 6:18, the mean psi was 16.7 h, but this shortened to 13.3 h during PTU, returning to 16.0 h after PTU removal. In intact males under LD 14:10, the three PTU diet concentrations failed to differentially suppress thyroxine and triiodothyronine levels. Food intake and body weight were differentially reduced by the PTU treatments. A 0.5% quinine hydrochloride diet also reduced food intake and body weight but did not change tau. The inconsistency in response suggested that PTU may affect circadian rhythmicity independent of its action on the thyroid neuroendocrine axis /61/.

Sedative-hypnotic drugs

Twenty-four hour LD_{50} values of secobarbital, pentobarbital and phenobarbital, in male Swiss-Webster mice weighing 30 g each were determined /62/. The animals were adapted for three weeks in environmental lighting of 12:12 (L:D) periods. Each mouse was injected intraperitoneally at 6-h time intervals with either phenobarbital or chloral hydrate for toxicity analysis. Secobarbital, pentobarbital and hexobarbital were injected at 3-h time intervals. Peak toxicity was reached at 06:00 h with all drugs except chloral hydrate which was least toxic at 06:00 h. These results suggest a circadian periodicity in the toxicity of sedative hypnotics. The circadian fluctuations in the metabolism of these compounds may be responsible for toxicity and lethal effects.

5. ENVIRONMENTAL CHEMICALS AND CIRCADIAN VARIATION

Ozone

Rats and guinea-pigs were exposed to 0.8 mg ozone (approximately 0.4 ppm) for 12 hours during daytime or night-time or

continuously for 24 hours. Night-time ozone exposure of rats resulted in larger increases of protein, albumin, and inflammatory cells in broncho-alveolar lavage fluid as compared to daytime ozone exposure. Single daytime or night-time ozone exposure of guinea-pigs showed similar differences in the parameters but without a daytime/night-time difference. Night-time and continuous ozone exposure of rats for 3 days resulted in comparable increases in lung antioxidant enzyme activities, both of which differed from daytime. Continuous ozone exposure of guinea pigs for 3 days caused greater increases in lung tissue parameters as compared to night-time exposure. The results suggested a correlation between ozone-induced acute pulmonary biochemical and inflammatory responses and the level of physical and respiratory activity. For rats, effects from continuous ozone exposure appear to be controlled by the night-time, physically active period /63/.

TCDD

Studies were initiated by Jones and co-workers /64/ to determine whether 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects circadian rhythms of prolactin (PRL), corticosterone, thyroxine (T_4), and triiodothyronine (T_3) in male Sprague-Dawley rats. In addition, the effect of PRL to induce ornithine decarboxylase (ODC) was determined. The earliest effect detected following TCDD administration was a significant decrease in the serum PRL concentration compared with that of pair-fed controls within 4 h ($P< 0.05$). This was followed by a significant decrease in serum T_4 by 6 h ($P<0.05$). By 8 h the serum peak of corticosterone was shifted to 2 h later in the TCDD-treated rats. The serum PRL concentration 7 days after TCDD administration was significantly higher ($P<0.05$) in TCDD-treated animals than in pair-fed controls. The elevation of ODC activity in response to PRL, 2 days after TCDD, was decreased in the order of thymus > adrenal > spleen > heart > kidney > liver. By 7 days, liver ODC activity in response to PRL was only 12% of that detected in pair-fed controls. Liver ODC activity in response to dexamethasone and aminophylline was decreased to 25 and 22% of pair-fed controls, respectively, by 7 days after TCDD administration. However, in kidney of TCDD-treated rats, there was an increased ODC response to aminophylline reaching 191% of pair-fed controls

by day 7. These results suggest that the ability of TCDD to alter receptor coupling, or the receptor number, for diverse hormones may play a role in TCDD toxicity. However, alterations in the serum PRL levels may play a critical role in the toxicity of TCDD by a time-dependent alteration in multiorgan biosynthetic responses to hormones and growth factors. It is not yet clear whether these alterations are due to modified hypothalamic/pituitary secretions or direct cellular mechanisms or both.

Toluene

Harabuchi and colleagues /65/ reported circadian variation in the acute toxicity of toluene in rats exposed to 2000 or 4000 ppm both in the dark (active phase in animals) and the light (the inactive phase) for 4 hours. The performance decrements of rats were greater in the light phase than in the dark phase in all time zones of exposure. The animals recovered faster, almost to pre-exposure performance, in the dark phase than in the light phase. The concentrations of toluene in blood and brain were higher in the light phase than those in the dark phase at four hours after exposure to 2000 ppm toluene, or two hours after exposure to 4000 ppm toluene. The data suggested that there was a significant difference in the circadian susceptibility of exposure to toluene. This intolerance may be caused by circadian differences in the pharmacokinetics of toluene in the light and dark phases.

6. SIGNIFICANCE OF CIRCADIAN RHYTHMICITY IN SHIFT WORK

In the current era of concern for the health and safety of the worker, and environmental and toxic substance awareness, it has become necessary to establish limits of exposure to potentially hazardous substances in the workplace. NIOSH and other agencies have established the "threshold limit values" (TLVs) for exposures to a number of substances. It should be noted that the TLV recommendations are mostly based on exposure during the normal working day. There is ample evidence that the operations of aircrews round the clock, and on transmeridian routes, is rhythmic and demonstrates circadian (about 24 hours) variability /66-68/. It is also known that the metabolism and toxicity of various substances show circadian variation. Hence, the TLVs established for day shift workers

may not be valid for night shift or rotating shift workers. The safety evaluation of industrial and environmental chemicals is of fundamental importance in ensuring high standards of occupational and public health.

What should be clear is that shift work cannot just be abolished because it might be detrimental for workers, even if the evidence for such ill effects were incontrovertible, which it certainly is not. The asbestos, lead and coal industries are well recognized as carrying risks of disease and death among workers, but the continuing need for these materials implies that solutions have to be found to the problems of worker exposure. Chronic exposure to toluene diisocyanate in the polyurethane foam manufacturing industry, and polyvinylchloride compounding workers, caused significantly higher diurnal variation in peak expiratory flow rates than in unexposed controls /69,70/. This is no less valid for shift working in general. There are two aspects of shift working which need to be considered in discussing optimal systems, namely, the shift rotation itself, and appropriate preventive health measures.

Management frequently gives little consideration to the implications of introducing shift work, nor do they experiment enough with different systems. The rigid adherence to three shifts at 0600, 1400 and 2200 h is detrimental in many ways. Many physiologists feel that, in addition to selecting suitable persons for shift work, more attention might be paid to changes in the timing of the shifts: for example 0400-1200-2000 h or 0800-1600-0000 h. These rotations might lessen the social unacceptability of the morning or night shifts. Such schemes can result in 48 h off every week for 3 weeks and 72 h off every fourth week. As night work seems to cause the most problems, the hours worked at night might be reduced, or each night on could be followed by 24 h off. Several physiologists subscribe to the view that if sufficient volunteers could be found to work permanent nights (and apparently this is feasible in many instances) then the other workers could rotate on rapidly alternating morning/ afternoon shifts. A wider use of flexible timing and a greater participation of workers in shift practice decisions could alleviate many of the current difficulties.

7. SAFE EXPOSURE LIMITS OF CHEMICALS OR THRESHOLD LIMIT VALUES (TLVS)

Whenever novel work schedules are utilized, uncertainty exists concerning the effects of exposure to chemical agents. The modified occupational limits, which are calculable from methods suggested in the literature, estimate the required decrease in the TLV to provide protection for exposed workers. However, these reduced limits are no guarantee that an adequate safety margin will have been achieved. Therefore, we suggest that close medical surveillance be maintained for a reasonable period until it is confirmed that the modified limits do indeed protect the workers. The TLV must be reduced by an amount to take into account not only the increased hours of exposure per day but also the decreased hours of recovery. This will be expressed as the product of each factor calculated for a given workday:

Reduction of TLV = effect of increased exposure × effect of decreased exposure-free time

TLV reduction factor (RF) = 8 / h × 24-h / 16
(h = hours worked per day)

The RF value should be applied (a) to TLVs expressed as a time-weighted average with respect to the mean and permissible excursion, and (b) to TLVs which have a C (ceiling) notation except where the C notation is based solely on sensory irritation. In this case the irritation response threshold is not likely to be altered downward by an increase in number of hours worked, and modification of the TLV is not needed.

The proposal by Brief and Scala /71/ to adjust the TLVs according to work schedules is very much in order, particularly for substances with a small TLV safety factor, and when there is a large departure from the seven- to eight-hour work schedule. Certainly, some such stopgap procedure is needed in the absence of any toxicologic information that has been tailored to the particular work schedule; and even more so, when it is realized that the development of toxicologic criteria for all the permutations and combinations of work schedules for the 600 or so industrial substances with TLVs is an absolute impossibility.

At the same time, it should be recognized that the general formula approach is a first approximation only, and guarantees no exact

solution. Brief and Scala /71/ have recognized this, and have pointed out the necessity for cautious application of the formulae with appropriate medical surveillance. With such surveillance, information will accumulate that, in turn, will permit the development of firmly based TLVs.

A limited amount of toxicologic information does exist for a few industrial substances for the extreme case of continuous (24 h/day, round-the-clock) exposures. This information unquestionably supports the need for TLV reduction for increased exposure periods; some substances that are well tolerated under TLV conditions suddenly become lethal or near lethal when exposure at the TLV is continuous. In addition, the data clearly show that not all substances behave in this manner, and that the TLV for some is satisfactory without adjustment even for continuous exposure. In these instances, the use of the reduction factor will obviously add an increased safety factor.

A poorly studied, but important, factor mediating the well-being of the shift worker is the role of circadian rhythmicity in the body's ability to metabolize toxic chemicals and drugs. The time of day that drugs are given can have profound effects upon their pharmacokinetics (the way they are distributed and metabolized) and pharmacodynamics (biological effects). Similarly, the effects of other drugs and chemicals found in the workplace may have greater effects on the worker at night as opposed to the day shift. The overall effect would be modified detoxification/bioactivation and metabolic mechanisms which may modulate the toxicity index.

8. NEUROHYPOPHYSEAL CONTROL OF HEPATIC DRUG METABOLISM AS RELATED TO MELATONIN

It has been recognized that many biological processes exhibit daytime-dependent peak efficiency rhythmicities. It is logical, then, to expect rhythmicities in the response of a chemical or drug interacting with a biological system. Light and dark phases influence the melatonin release from the pineal gland: light inhibits melatonin secretion while darkness stimulates it. It has been shown that melatonin plays a regulatory role in growth hormone secretion by blocking serotonergic transmission /72/. Growth hormone levels as represented by the total area under the plasma growth hormone curve

(daytime 169.4 ± 8.4 vs night-time 74.9 ± 11.8 ng/h/ml) have been reported /73/. Therapeutic doses of melatonin inhibit growth hormone secretion /74-76/. It is generally accepted that growth hormone levels would increase during the light phase and decrease during the dark phase. Elevated levels of growth hormone cause lipolysis from fat depots which in turn increases fatty acid concentration in the blood. As a result, fat accumulation occurs in the liver. Scott and Potter /77/ have demonstrated circadian rhythms in the oxidation of CH_3COO- to CO_2 and in its incorporation into lipids of different rat tissues. The changes in lipogenesis in the course of the day were investigated by Kimura *et al.* /78/. There is clear evidence of the existence of circadian variations in the free fatty acid content of the liver microsomes. They may be correlated to the feeding of the animals, since the greatest increase is produced during the first part of the dark period, in which they feed actively. The peak observed at 16:00 h is probably related to the nonesterified fatty acids released by the adipose tissue during the light period of rest and fasting. These nonesterified fatty acids may in turn serve as precursors for the *de novo* synthesis of polyunsaturated and monounsaturated fatty acids (polyenoic acids) in the endoplasmic reticulum of the hepatocytes. Hence, melatonin may ultimately impact upon membrane fluidity, affecting the functions of the endoplasmic reticulum.

It has also been shown that fatty acid desaturation of the endoplasmic reticulum membrane is modified by circadian changes /79/ and other physiological and non-physiological factors /80,81/. The endoplasmic reticulum represents a lipid-protein macromolecule, the functioning of which depends on lipid-protein interactions evoking conformational changes in enzymic activity, thereby altering the kinetic properties of the membranous enzymes. A recent study by Dhami and colleagues /82/ indicated that changes in the membrane phospholipid fatty acids are intricately involved with the enzymic activities in the hepatic endoplasmic reticulum of the rat. The linoleic acid content fluctuates under different conditions and correlates with an increased ratio of saturated to unsaturated fatty acids in phosphatidycholine and phosphatidylethanolamine fractions of the endoplasmic reticulum /83/. Furthermore, these changes also correspond with modifications in the aminopyrine N-demethylase and coumarin 3-hydroxylase activities /84/. We hypothesize that the neurohypophyseal axis, as influenced by melatonin, may play a modulatory role

in the drug metabolizing activity of the organism by a complex mechanism.

Since the ratio of saturated to unsaturated fatty acids is an important factor that determines fluidity or microviscosity of liver microsomes, which in turn can influence the interaction between components of the drug metabolizing enzymes /84/, any circadian fluctuation in the saturation index may influence the metabolic activity of the endoplasmic reticulum. Greinert and coworkers /85/ proposed a model in which cytochrome P450 forms disc-shaped rotamers immersed in the bilayer membrane to a depth which is varied by substrate-induced and redox state-dependent conformational changes. It has been suggested that a uniaxial rotation of cytochrome P450 about the normal to the membrane is correlated with the microviscosity of the membrane. Vertical mobility of proteins in biological membranes, influenced by temperature changes, has been demonstrated by studies of X-ray low-angle scattering /86/. Alteration of protein-lipid interaction induced by a change in protein conformation may trigger the shift /87/. A vertical motion could, hypothetically, be the mechanism by which microsomal oxygenation is regulated. The site is the contact for transfer of electrons between cytochrome P450 reductase or cytochrome b_5 and cytochrome P450 which necessitates a local encounter of their prosthetic groups. For their proper alignment, mobility of proteins in the plane of the membrane and normal to the membrane is proposed. Optical alignment to NADPH cytochrome P450 reductase may be achieved by the downward movement induced by substrate binding, and result in an increased electron-transfer rate. An upward shift, bringing cytochrome P450 closer to cytochrome b_5, would promote transfer of the second electron. However, none of these models or studies reveal the significance of the binding of the immobilized fraction of cytochrome P450 which may be as large as 54%. The inductive actions of xenobiotics may, indeed, regulate the microsomal oxidases through this proposed rotation and mobility; speculatively, the decreased ratio of saturated to unsaturated fatty acids in the heptic endoplasmic reticulum may facilitate the contact between cytochrome P450 and its reductase for transfer of electrons. On the other hand, an increased ratio of saturated to unsaturated fatty acids may restrict this mobility and rotation, thereby affecting drug metabolism. A 24-h cycle of alternating light and darkness is well known to synchronize circadian rhythms in mammals; many hepatic

cellular processes including the activities of certain microsomal drug metabolizing enzymes have been reported to show circadian variation /27/. There may also be reduced drug binding towards the end of the light cycle due to the occupation of the relevant binding sites by increased levels of endogenous 11-hydroxysteroids causing inhibition of microsomal drug metabolizing enzyme activity. Hence, the circadian alterations of the fatty acids in the endoplasmic membranes may increase or decrease the rotation and mobility of proteins for electron transfer which may modulate the metabolism of xenobiotics in a 24-h cycle.

9. ISSUES AND CONSIDERATIONS IN CHRONOTOXICITY

Although there is extensive literature on the circadian aspects of shifts in the light:dark cycle, i.e. desynchronization, no animal model has been examined matching pharmacokinetic and toxicity analysis in relation to photoperiods or changes in ambient light intensities. The neglect of pharmacokinetics was due as much to lack of suitable methods of repeated sampling and for measuring chemicals and drugs in biological fluids as to anything else. Even the effect of time of administration of drugs upon their pharmcokinetic parameters in animals is much less understood than time of administration upon their pharmacodynamics (which is itself still at an early stage of development). There is little information on environmental chemicals as related to circadian rhythmicity. The combination of these concepts will have a bearing on the calculation of risk assessment and management. We may argue that application of uncertainty factors in the calculation of risk assessment may account for all other variables including time of day effect. However, we cannot ignore this important physiological phenomenon which has been shown to have a tremendous impact on the efficacy or toxicity of xenobiotics. By the same token, TLVs (threshold limit values) of chemicals in workplaces may adversely affect the health of workers should the work schedules be changed to other than 8 h/day or 40 h/week based on the standard eight hour day shift time structure. Therefore, TLVs of chemicals may have to be altered during various work shifts. Further doubt is cast on the utility and scientific validity of ED_{50} or LD_{50} tests, unless these tests are performed at various stated circadian stages and the

application of these results is considered in the time-structure related sense. In view of the inter-relationship between circadian rhythmicity and toxicity, the common practice of using numerical values for TLVs, BLVs, LD_{50}, ED_{50} or LC_{50} or Maximum Acceptable Levels for environmental chemicals may perhaps be too simplistic.

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