this experiment. Phase shifts were computed according to the linear regression method[11]. Single light pulses did not induce phase advances or delays. This finding holds irrespective of whether the beginning or the end of the circadian increase of food intake is taken as a phase reference. In order to test for metabolic and pharmacological effects of Hypnorm, craniotomized rats were anaesthetized at various phases of their cycle but not exposed to light pulses. It was observed that Hypnorm did not alter the period or phase of the freerunning rhythm (figures 1,B, and 2). It is conceivable that the anaesthesia reduced the sensitivity of the putative hypothalamic photoreceptors in these light pulse experiments. This interpretation is not supported, however, by electrical recordings from directly illuminated unanaesthetized slices of hypothalamic tissue showing the absence of photoreceptors[12]. Moreover, in view of the high intensity of the pulses it is significant that no response was observed at all.

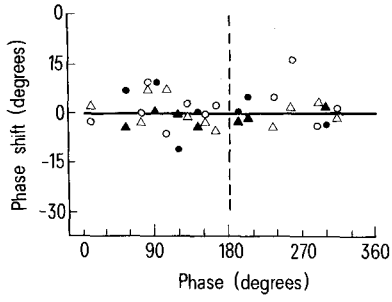


Fig. 2. Absence of light pulse induced phase shifts of the freerunning food intake rhythm of craniotomized rats. The period of each rhythm before the 1-h pulse was normalized to 360 degrees. Light pulses on just Hypnorm anaesthesia were delivered at different phases of the cycle. The phase shifts computed from linear regression analysis of eating onset times (circles) and times at which eating was terminated (triangles) are expressed in degress for light stimulated (open symbols) and control craniotomized rats (filled symbols). Phase 180° corresponds to the onset time of the circadian eating bout.

The present experiments show that increasing the photic energy penetrating into the exposed brain of blinded adult rats is ineffective in entraining the circadian food intake rhythm to daily light-dark cycles. Similar results were obtained in blinded animals without craniotomies. Our findings are in contrast with descriptions of extraretinal photoentrainment in neonatal rats[2,3] and the induction of photo-neuroendocrine reflexes by direct illumination of hypothalamic cells of the adult rat[4]. However, electrophysiological experiments showed the absence of such hypothalamic photoreceptors in adult rats[12]. Our observations suggest that extraretinal photoreceptors mediating entrainment of circadian rhythms in the rat, although present in immature animals, lose their functional significance at a later stage of life. This process may be correlated with the postnatal development of the retino-hypothalamic projection which is known to mediate entrainment in adult rats[2,13].

1 M. Menaker and H. Underwood, Photochem. Photobiol. *23*, 299 (1976).
2 B. Rusak and I. Zucker, Physiol. Rev. *59*, 449 (1979).
3 W.F. Ganong, M.D. Shepard, J.R. Wall, E.E. van Brunt and M.T. Clegg, Endocrinology *72*, 962 (1963).
4 R.D. Lisk and L.R. Kannwischer, Science *146*, 272 (1964).
5 M. Zweig, S.H. Snyder and J. Axelrod, Proc. natl Acad. Sci. USA *56*, 515 (1966).
6 D.E. Wolfe, in: Progress in Brain Research, vol. 10. Ed. J.A. Kappers and J.P. Schadé. Elsevier, Amsterdam 1965.
7 M.J. Baum, Physiol. Behav. *5*, 325 (1970).
8 W.J. Rietveld, F. ten Hoor, M. Kooij and W. Flory, Physiol. Behav. *21*, 615 (1978).
9 T. Pavlidis, in: Biological oscillators: their mathematical analysis. Academic Press, New York 1973.
10 K. Honma, F. Katabami and T. Hiroshige, Experientia *34*, 1602 (1978).
11 C.S. Pittendrigh and S. Daan, J. comp. Physiol. *106*, 223 (1976).
12 G.A. Groos, IRCS Med. Sci. *7*, 342 (1979).
13 C.A. Mason, N. Sparrow and D.W. Lincoln, Brain Res. *132*, 141 (1977).

Circadian variation of diazepam acute toxicity in mice

F.H. Ross, A.L. Sermons, J.O. Owasoyo[1] and C.A. Walker[2]

School of Pharmacy, Florida A & M University, Tallahassee (Florida 32307, USA), 4 March 1980

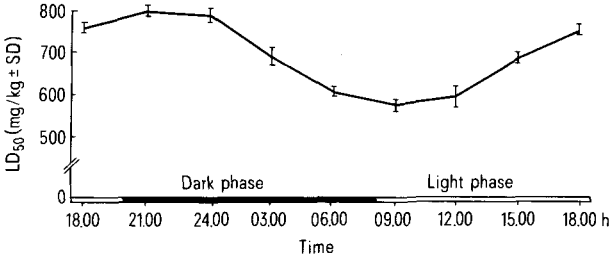
Summary. The LD_{50} of i.p. injected diazepam was determined every 4 h over a 24-h period in albino mice adapted to a 12-h dark/12-h light programmed illumination cycle. Results show that diazepam is more toxic during the light phase of the cycle than during the dark phase and demonstrate circadian variation in the toxicity of the compound in mice.

Several investigators have reported circadian variation in the toxicity and effectiveness of CNS drugs. It has been demonstrated that the so-called 'sleeping time' in mice after hexobarbital shows circadian variation[3]. Previous studies have shown that cholinergic drugs[4] as well as amphetamine[5] are more toxic during the dark phase of a light-dark cycle in rodents. It has also been found[6] that some adrenergic stimulants and blockers show opposite circadian patterns of toxicity in mice, in that while the adrenergic stimulants are more toxic during the dark phase of a light-dark illumination cycle, adrenergic blockers are, expectedly, more toxic during the light phase of the cycle. The circadian toxicity patterns of pentylenetetrazol, picrotoxin and sodium phenobarbital, and the ability of exogenous L-dopa, serotonin and gamma-amino-butyric acid (GABA) to alter those toxicity patterns have been reported[7]. While circadian variation in drug effectiveness and toxicity in mammals has not been fully explained, some investigators have suggested that these diurnal variations in CNS drug response and toxicity might be related to the endogenous brain levels of biogenic amines which have also been shown to vary diurnally[5,8-11]. The purpose of the present study was to investigate the circadian toxicity pattern of diazepam, a tranquilizer, in mice adapted to a programmed light-dark illumination cycle.

Methods. Male, Swiss-Webster mice weighing approximately 25 g each were used in this study. All animals were adapted, at a temperature of 23 ± 1 °C, to an environmental

chamber equipped to provide 250 foot candles of cool white flourescent light for a minimum of 3 weeks before use in this study. The mice were housed in clear plastic cages in groups of 8 animals. Feed and water were supplied ad libitum. The photoperiod was automatically-timed (12-h dark/12-h light) with the light period from 08.00 h to 20.00 h. Injections of diazepam in 0.9% saline (Hoffmann-La Roche) were made i.p. every 3 h over a 24-h period and LD_{50}-value (mg/kg) for each time was determined by the method of Litchfield and Wilcoxon[12]. For each hour 18 animals were used.

Results and discussion. Our data are presented in the figure. The maximum acute toxicity of diazepam occurred at 09.00 h during the light phase of the photoperiod while the minimum acute toxicity of the compound was at 21.00 h during the dark phase of the photoperiod. This study demonstrates for the first time the chronotoxicity of diazepam, a CNS depressant drug of the benzodiazepine group, in that the toxicity of the compound varies with the time of administration. The effectiveness and/or toxicity of CNS drugs might be related to endogenous levels of brain biogenic amines and other neurotransmitters during different times of the day since it has been shown[8-11] that some CNS drugs can alter the circadian patterns of brain biogenic amines. It will, therefore, be interesting to investigate in a future study the effect of diazepam on the circadian patterns of brain biogenic amines.



Chronotoxicity of diazepam in mice. The maximum toxicity is at 1 h after the onset of the light phase (09.00 h). The minimum toxicity is around 21.00 h which is 1 h after the onset of the dark phase.

1 Present address: Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan (Nigeria).
2 To whom correspondence should be addressed.
3 E.S. Vessell, Fed. Proc. *27*, 349 (1968).
4 A.H. Friedman and C.A. Walker, Fed. Proc. *28*, 44 (1969).
5 C.A. Walker, Exp. Pharmac. *1*, 247 (1971).
6 C.A. Walker and J.O. Owasoyo, JRCS, Med. Sci. *2*, 2354 (1974).
7 C.A. Walker and J.O. Owasoyo, Int. J. Chronobiol. *2*, 125 (1974).
8 A.H. Friedman and C.A. Walker, J. Physiol. *202*, 133 (1969).
9 J.O. Owasoyo and C.A. Walker, Pharmacologist *15*, 197 (1973).
10 J.O. Owasoyo and C.A. Walker, Pharmacologist *16*, 271 (1974).
11 J.O. Owasoyo, C.A. Walker and U.G. Whitworth, Jr, Life Sci. *25*, 119 (1979).
12 J.R. Litchfield and F. Wilcoxon, J. Pharmac. exp. Ther. *99*, 99 (1949).

Total atresia of the ovaries of *Tilapia leucosticta* (Cichlidae) after intoxication with the insecticide Lebaycid®

Dorothee Kling[1]

Institut Biologie III der Universität, Lehrstuhl für Zoophysiologie, Abteilung Physiologische Ökologie, Auf der Morgenstelle 28, D-7400 Tübingen (Federal Republic of Germany), 10 March 1980

Summary. Intoxication with sublethal Lebaycid® concentrations led to total atresia of the ovaries in 90% of the treated specimens of *Tilapia leucosticta.* The gonads were filled with atretic follicles and mature eggs were never found. In regeneration trials, intoxicated fish proved to be unable to spawn for at least 9 weeks.

The organophosphorus insecticide Lebaycid® (Fenthion) is being employed in the combat against Anopheles-, Culex- and Simulium-larvae in many tropical freshwaters[2-6]. The preparation is introduced repeatedly into the water and thereby it also effects non-target organisms especially fish, which are an important source of protein for human nutrition in many countries[7,8]. The highly toxic effects of Lebaycid on the spawn and different developing stages of Cichlid fishes have already been shown[9]. The aim of this investigation was to examine to what extent the periodical output of sublethal concentrations of Lebaycid affects the ovaries and reproductive activity of *Tilapia leucosticta.*

Materials and methods. Within a period of 14 days 20 individually marked females of *Tilapia leucosticta* (overall length 8.6± 1.2 cm, total weight 9.6± 3.8 g) were treated 5 times with sublethal 'pesticide-shots', each lasting 24 h. The shots were always interrupted by a freshwater-phase of 2 days duration. In each experiment 4 females were put in an aerated glass aquarium with a capacity of 10 l (adaptation period 1 day). In the 1st series, 8 females were exposed to a Lebaycid concentration of 7 ppm during each pesticide-shot. To make sure that the ovaries were in a comparable state at the beginning of the treatment, in a 2nd series, the gonads were visually controlled. The peritoneal cavity of anaesthetized females was opened by an incision in the ventral line under the Zeiss Dissecting Microscope. Through the transparent ovarian sack the different oocyte stages could be recognized and photographed. Afterwards the edges of the wound were approximated with catgut. 9–14 days after the operation, 8 females with maturing ovaries were exposed to the pesticide programme. In this 2nd series the Lebaycid concentration was reduced to 4 ppm. In a 3rd series, the possible stimulating influence of males on the ovarian cycle was taken into account. 4 females (visual control of the ovary) together with one male were treated with Lebaycid. 20 females (12 with ovaries visually examined) served as controls. After the experiments, the fish were killed and their total weight and overall length determined. The ovaries were dissected, measured and weighed. The usual histological methods (fixation in Bouin, Azan-staining) were employed. In the 3rd series, half of the