Rhythms of enzymatic activity in maternal and umbilical cord blood

R. Wilf-Miron[a], L. Peleg[b, c], B. Goldman[2] and I. E. Ashkenazi[c, *]

[1] *Division of Pediatrics and* [2] *The Genetic Institute, Chaim Sheba Medical Center, Tel-Hashomer, and* [3] *Unit of Chronobiology, Department of human Genetics, Tel-Aviv University, Tel-Aviv (Israel)*
Received 30 August 1991; accepted 18 October 1991

Abstract. The 24-h activity patterns of various enzymes were determined in human serum, red blood cells and white blood cells of maternal and umbilical cord blood. Blood was drawn from the brachial vein of mothers and from the umbilical cord within ten minutes after delivery. Corresponding blood specimens were obtained from 83 spontaneous labors, occurring at different hours over a period of 60 days. For each variable (variable = activity of a specific enzyme in one of the blood components) the results were grouped according to delivery hour, forming a 24-h pattern which was analyzed to elucidate time dependency. Five out of six corresponding maternal and fetal variables were similar with regard to pattern and peak time. The activity rhythms of glyceraldehyde-3-phosphate dehydrogenase and glucose phosphate isomerase in red blood cells of mothers and fetuses possessed a significant bimodal pattern. The activity rhythms of the latter enzyme in white blood cells and sera exhibited a significant 24-h period. Hexosaminidase activity exhibited a distinct 24-h rhythm in maternal white blood cells, but no significant rhythm could be detected in the fetal white blood cells. The activity of hexosaminidase showed, identical 24-h patterns in maternal and cord serum when analyzed by best fit cosine, and no significant time-dependency when analyzed by ANOVA.
Key words. Circadian rhythms; cord blood; maternal blood; enzyme activity pattern; entrainment.

Many physiological and behavioral variables exhibit circadian rhythmicity in humans and animals[1–6]. Some of these rhythms have been shown to be endogenous, in that they persist in the absence of time cues[4]. Circadian rhythms have also been detected in animal[7,8] and human[9–13] fetuses; for example, in concentration of plasma hormones[7], heart rate[10], deoxy-d-glucose uptake by the suprachiasmatic nuclei[16] and levels of plasma cortisol of human maternal and cord blood[14]. However, as fetuses are not exposed directly to external zeitgebers, it is assumed that the mother's biological clock paces and coordinates the fetus' rhythm[7,8,14–19].

The present study was designed to examine whether enzyme activities exhibit 24-h rhythms in human cord blood, and to compare these rhythms with those in maternal blood at the time of delivery. The enzyme activity patterns were measured in red blood cells, white blood cells and sera. All the selected enzymes (glyceraldehyde-3-phosphate dehydrogenase, glucose phosphate isomerase and hexosaminidase) have been shown to exhibit circadian rhythms in mouse tissues[20,21]. A circadian rhythm of hexosaminidase activity has also been shown in human plasma[3]. The endogenous nature of the rhythmicity has been documented for two of these enzyme activity rhythms[20].

Materials and methods

Blood was drawn within ten minutes after delivery from the brachial vein of the mother and from the umbilical cord after it had been clamped and cut. All deliveries were spontaneous at term (38–42 weeks gestation). The pregnant women rested in the delivery room, exposed to normal daylight and darkness, until the delivery. They were given the normal hospital meals at 08.00, 12.30 and 17.30 hours. The mothers gave their consent in each case.

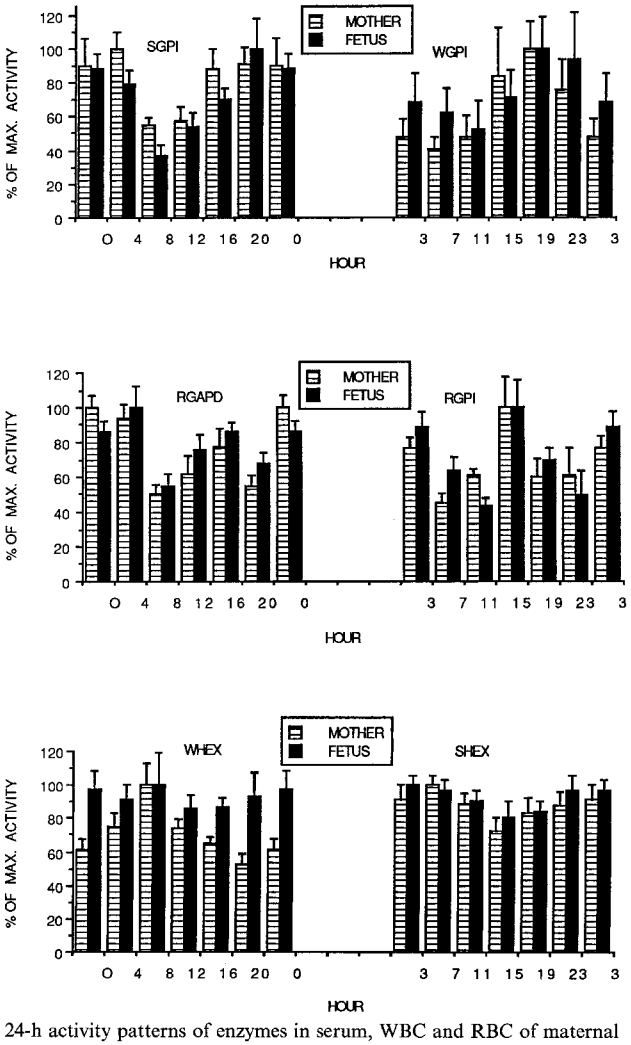
Each specimen was divided between a heparinized test tube (to obtain blood cells) and a non-heparinized one (for serum). White blood cells (WBC) were isolated by differential centrifugation with polyvinyl pyrolidol[22], and the clean pellets were suspended in 1 ml of distilled water. The suspensions were then sonicated for 30 s (Branson B-12 sonifier, 8 microns peak to peak) and centrifuged (10000 g for 10 min). The supernatants were used for enzyme activity determinations. Red blood cells (RBC) were washed twice with SKG solution (Physiological saline with 0.04% KCl and 0.1% glucose) and hemolyzed with 10 vols of water. RBC and serum were obtained from 83 paired maternal and fetal samples, and WBC from 63 pairs. Glucose phosphate isomerase (GPI) activity was assayed in all three blood components[23]. Glyceraldehyde-3-phosphate dehydrogenase (GAPD) activity was measured only in RBC hemolysates[24] and beta-N-acetyl-glucosaminidase (hexosaminidase, HEX) activity was assayed in serum and WBC[25,26]. GPI and GADP activities are monitored by a Gillford spectrophotometer and that of HEX by a fluorescence spectrometer (Perkin Elmer LS-5B).

Activity units of RBC enzymes were defined as change in OD/min/g% hemoglobin, those of WBC enzymes as change in OD/min/mg protein, for GPI activity, and nmols of substrate cleaved per hour per mg protein for HEX activity[27]. Serum GPI activity was defined as change in OD/min/ml and that of HEX activity as nmols of substrate cleaved per ml per hour. The values obtained for each variable were pooled according to time of delivery for six time-points equally spaced over twenty-four hours, to produce a 24-h activity pattern. The activity units of each class were averaged, the highest value of each pattern was defined as 100% and the rest of the values were computed accordingly. The same procedure

was applied separately to maternal and cord samples. The patterns obtained were analyzed by one-way ANOVA to elucidate the presence of any significant time-dependent enzymatic activity. In addition, best fit cosine analysis[28,29] was used to determine whether a pattern exhibited a significant 24-h periodicity, and if it did to compute the peak time (acrophase) and amplitude. In the case of bimodal patterns the best fit cosine analysis as modified by Vokac and Vokac was applied[30]. Two-way ANOVA was used: one factor with 2 levels (mother vs fetus), the second factor with 6 levels (time points), to elucidate similarities or differences between maternal and fetal activity patterns of each enzyme.

Results

The 24-h patterns of the examined variables are presented in the figure. ANOVA analysis of each pattern revealed significant time-dependent patterns in all except those of cord blood HEX and maternal serum HEX. RBC enzymes showed a significant bimodal pattern



24-h activity patterns of enzymes in serum, WBC and RBC of maternal and cord blood. GPI; Glucose phosphate isomerase. GAPD; Glyceraldehyde-3-phosphate dehydrogenase; HEX; hexosaminidase.

Table 1. ANOVA analysis of activity patterns

Variable	Source	Pattern	p value=
RGPI	MO	bimodal	0.0163
RGPI	CO	bimodal	0.0079
RGAPD	MO	bimodal	0.0005
RGAPD	CO	bimodal	0.0035
SGPI	MO	unimodal	0.0035
SGPI	CO	unimodal	0.0001
WGPI	MO	unimodal	0.0003
WGPI	CO	unimodal	0.0500
WHEX	MO	unimodal	0.0001
WHEX	CO	--------	0.9802*
SHEX	MO	--------	0.2239*
SHEX	CO	--------	0.3466*

*not significant; MO-mother, CO-cord; S-serum, W-WBC, R-RBC.

Table 2. Rhythm parameters of enzyme activity in mothers and fetuses analyzed by best fit cosine

Enzyme	Period	Amplitude	peak-time
SGPI-MO*	circadian	27.0 (6.6)	0.4 (0.5)
SGPI-CO*	circadian	39.8 (8.1)	0.2 (0.4)
RGPI-MO	semi**	29.6 (2.9)	14.3 (0.3)
RGPI-CO	semi	38.9 (4.3)	15.5 (0.3)
WGPI-MO	circadian	47.0 (3.4)	18.4 (0.2)
WGPI-CO	circadian	30.0 (3.2)	20.3 (0.4)
RGAPD-MO	semi	26.4 (8.0)	2.3 (0.3)
RGAPD-CO	semi	23.6 (5.8)	2.4 (0.2)
SHEX-MO	circadian	11.1 (2.9)	5.5 (1.0)
SHEX-CO	circadian	9.1 (2.0)	6.1 (0.5)
WHEX-MO	circadian	27.3 (5.0)	8.2 (0.4)
WHEX-CO	--------	--------	--------

*MO-maternal, CO-cord; S-serum, W-WBC, R-RBC; **semi-semicircadian. All periods were significant ($p < 0.05$); The term circadian was applied to unimodal patterns and semi to bimodal; -------- no significant rhythm. The numbers in parentheses are standard errors; Amplitude is expressed as percentage of the mesor (the pattern's mean activity); Peak time is given in hours and minutes.

while the others had unimodal patterns. Best fit cosine analysis, detailed in table 2, gave the same results except that by this analysis HEX activity in sera also exhibited a significant 24-h pattern (though with a very low amplitude).

The amplitude and peak time of PGI activity rhythms were different among the three blood components (table 2; SGPI, RGPI, WGPI). These results are in agreement with previous studies which demonstrated individual tissue control of enzyme activity rhythm[2,20,21,31]. Two-way ANOVA demonstrated that except for WBC-HEX, all other corresponding examined variables, in mother's and cord blood, exhibited identical rhythm patterns with regard to peak times (table 3). WBC-HEX of umbilical cord had no defined rhythm pattern, while the activity pattern of the same enzyme in the mother's WBC possessed a significant 24-h rhythm.

Four of the variables (WBC-GPI, serum-GPI, RBC-GAPD and WBC-HEX) exhibited similar 24-h activity-means (mesors) in both cord and maternal blood, while

Table 3. Comparison of maternal and fetal enzyme activity patterns (two-way ANOVA)

Enzyme	p value
RGPI	0.793
RGAPD	0.950
SGPI	0.579
WGPI	0.318
SHEX	0.570
WHEX	0.008*

*Patterns differ significantly; S-serum; R-RBC; W-WBC.

the activity-mesors of RBC-GPI and serum-HEX in cord blood were 1.3 times higher then that of the respective mean activities in maternal blood.

Discussion

When individuals of the same species are exposed to similar environments, they usually exhibit comparable rhythms for given variables. Thus, if sequential samplings from a single individual are not possible, a rhythm for a variable can be constructed by combining results obtained from different individuals, each examined at a different time[32]. This approach was adopted in the present study in order to measure enzymatic activity patterns in three components of maternal and cord blood. These three surveyed blood components are different biological entities. Serum is an extracellular milieu; WBC contain nuclei and protein synthesis apparatus, whereas RBC do not. Since the activity of an enzyme may exhibit different rhythm parameters in different tissues[2,20,21,31], we use the term 'variable' to define the activity pattern of an enzyme in a specific blood component and consider each as a separate variable. For most variables which have been examined, and which exhibited defined rhythms in both mothers and their fetuses, the patterns of the respective rhythms were similar, both in humans and in animals[10,14,17,24]. In some, like the rhythm of adrenal steroids in humans and rhesus monkeys[7,34], a phase shift between mothers and fetuses was recorded.

In the present study the peak times of four of the examined variables were similar in both maternal and cord blood (ANOVA and best fit cosine analysis), all having significant unimodal (SGPI WGPI) or bimodal (in RBC) rhythms. It should be noted that the significant 24-h rhythm of serum- HEX activity elucidated in our study by best fit cosine analysis (but not by ANOVA) has also been documented in human plasma[3].

The lack of any defined rhythm in WBC-HEX activity of umbilical cord suggests either that the oscillatory pattern of this variable develops postnatally[34], or that the oscillator had not yet been entrained and still exhibited a free-running rhythm. Only repeated sampling of a single fetus (and not group sampling) could distinguish between these two possibilities.

Our observations cannot yet conclusively determine whether the rhythms represent an output of the circadian system (persist under constant conditions) or result from being entrained by endogenous circadian metabolic signals. It should be noted, however, that in humans only a few variables have been examined with regard to the presence of endogenous rhythmicity, and in none of these studies were enzymatic activities analyzed. On the other hand, animal studies do demonstrate that the activity of many enzymes posseses an an endogenous circadian rhythm[35,36]. For example, we have documented the presence of circadian rhythmicity of GAPD and GPI in murine tissues[20]. Assuming that similar mechanisms govern the circadian rhythms in human and other mammals[4,6] we can deduce that the activity rhythms of human enzymes reflect, either directly or indirectly (modulated by metabolites or cofactors), the presence of an endogenous circadian pacemaker. Consequently, it is reasonable to suggest that the rhythms of the variables examined in our study should also be included in the domain of circadian rhythms. Furthermore, the fact that the fetus' rhythms are very similar to the respective rhythms of the mother seems to support the idea that maternal entrainment of fetus functions occurs in humans as well as in other mammals[15–17].

Acknowledgment. We are grateful to the nurses of the maternity ward of Sheba medical center for their kind assistance in this project.

* To whom all correspondence should be addressed.

1 Haus, E., Nicolau, G. Y., Lakatua, D., and Sacket-Lindeen, L., A. Rev. Chronopharmacol. *4* (1988) 333.
2 Ashkenazi, I. E., Ramot, B., Brok-Simoni, F., and Holtzman, F., J. Interdiscipl. Cycle Res. *4* (1973) 193.
3 Goi, G., Fabi, A., Lombardo, A., Burlina, A. B., Tettamanti, G., Montalbetti, N., Cavallery, M., and Halberg, F., Clin. chim. Acta *176* (1988) 1.
4 Reinberg, A., and Smolensky, M. H., in: Biological Rhythms and Medicine, p. 1. Springer-Verlag, Berlin 1983.
5 Takahashi, J. S., and Zatz, M., Science *217* (1982) 1104.
6 Minors, D. S., and Waterhouse, J. M., Experientia *42* (1986) 1.
7 Walsh, S. W., Ducsay, C. A., and Novy, M. J., Am. Obstet. Cynecol. *150* (1984) 745.
8 Honma, S., Honma, K. I., Shirakawa, T., and Hiroshige, T., Endocrinology *114* (1984) 1791.
9 Visser, G. H. A., Goodman, J. D. S., Levine, D. H., and Dawes, G. S., Am. J. Obstet. Gynecol. *142* (1982) 535.
10 Carmichael, L., Campbell, K., and Patrick, J., Am. J. Obstet. Gynecol. *148* (1984) 675.
11 Nasello-Paterson, C., Natale, R., and Connors, G., Am. J. Obstet. Gynecol. *158* (1988) 312.
12 Patrick, J., Campbell, K., Carmichael, L., and Richardson, B., Am. J. Obstet. Gynecol. *142* (1982) 363.
13 Chamberlain, P. E., Manning, F. A., Morrison, I., and Lange, I. R., Obstet. Gynecol. *54* (1984) 657.
14 Goretzlehner, V. U., and Wodrig, W., Zentralbl. Gynakol. *109* (1987) 802.
15 Weaver, D. R., and Reppert, S. M., Am. J. Physiol. *253* (1987) 401.
16 Reppert, S. M., and Schwartz, W. J., Science *220* (1983) 969.
17 Hiroshige, T., Japanese J. Physiol. *36* (1986) 237.
18 Elliot, J. A., and Goldman, B. D., J. exp. Zool. *252* (1989) 237.
19 Davis, F. C., and Gorski, R. A., J. comp. Physiol. *162* (1988) 601.
20 Peleg, L., Ashkenazi, I. E., Nesbitt, M. N., and Dotan, A., Chronobiol. Int. *3* (1986) 39.
21 Vaughan, M. K., Chambers, J. P., Tsin, A. T., Vaughan, G. M., and Reiter, R. J., Brain Res. *417* (1987) 321.
22 Padeh, B., and Navon, R., Israel J. med. Sci. *7* (1971) 259.
23 Carter, N. D., and Parr, C. W., Nature *216* (1967) 51.
24 Velick, S. F., in: Methods in Enzymology, vol. 1, p. 401. Eds S. Colowick and N. Kaplan. Academic Press, New York 1955.

25 Nakagawa, S., Kumin, S., and Nitowsky, H. M., Clin. chim. Acta *75* (1951) 181.
26 Leabach, D. H., and Walker, P. G., Biochem. J. *78* (1961) 151.
27 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
28 Vokac, M. A., Chronobiol. Int. *1* (1984) 87.
29 Halberg, F., Am. Rev. Physiol. *31* (1969) 675.
30 Vokac, Z., and Vokac, M., Chronobiologia *12* (1985) 283.
31 Brok-Simoni, F., Ashkenazi, E. I., Ramot, B., and Holzman, F., Br. J. Haemat. *32* (1976) 601.
32 Minors, D. S., and Waterhouse, G. M., Rev. Environ. Hlth *7* (1987) 65.
33 Patrick, J., Challis, J., Campbell, K., Carmichael, L., Natale, R., and Richardson, B., Am. J. Obstet. Gynecol. *136* (1980) 325.
34 Attanasio, A., Rager, K., and Gupta, D., J. Pineal Res. *3* (1986) 251.
35 Margolis, R., Am. J. Anat. *149* (1978) 459.
36 Feuers, R. J., Delongchamp, R. R., Scheving, L. E., Casciano, D. A., Tsai, T. H., and Pauly, J. E., Chronobiol. Int. *3* (1986) 221.

0014-4754/92/050520-04$1.50 + 0.20/0

Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines

J. C. Dickens[a], R. F. Billings[b] and T. L. Payne[c]

[a] *U.S. Department of Agriculture, Agricultural Research Service, Boll Weevil Research Unit, Mississippi State (Mississippi 39762, USA),* [b] *Texas Forest Service, Pest Control Section, Lufkin (Texas 75901, USA), and* [c] *Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg (Virginia 24061, USA)*
Received 8 October 1991; accepted 12 December 1991

Summary. Green leaf volatiles were shown to interrupt responses to aggregation pheromones of three species of bark beetles (Coleoptera: Scolytidae) which infest pines in the southern United States [the southern pine beetle, *Dendroctonus frontalis* Zimm.; the four-spined engraver, *Ips avulsus* (Eichhoff); and the five-spined engraver, *Ips grandicollis* (Eichhoff)]. The order of effectiveness of the compounds tested for each species was hexanal > hexanal + hexan-1-ol > hexan-1-ol. Neither hexanal, hexan-1-ol nor hexanal + hexan-1-ol was as effective in interrupting pheromone responses of *D. frontalis* as verbenone, a known inhibitor of this species. Other than interspecific chemical signals, this is the first report of an interruptant for *Ips* species, and the only report of a pheromone interruptant active for both *Ips* and *Dendroctonus* species.
Key words. Inhibitor; antiaggregation pheromone; bark beetle; green leaf volatile; *Dendroctonus frontalis*; *Ips avulsus*; *Ips grandicollis.*

We have discovered that green leaf volatiles interrupt pheromone responses of three species of cohabiting bark beetles (Coleoptera: Scolytidae) [the southern pine beetle, *Dendroctonus frontalis* Zimm.; the four-spined engraver, *Ips avulsus* (Eichhoff); and the five-spined engraver, *Ips grandicollis* (Eichhoff)] which infest pines in the southern United States. This is the first report of an interruptant for *Ips* species other than response interruption by chemicals emitted by cohabiting *Ips* species[1,2], and the only report of a pheromone interruptant active for both *Ips* and *Dendroctonus* species. We hypothesize that the green leaf volatiles have their effect by providing an inappropriate host stimulus, i.e. a broad-leafed plant, and that, therefore, these compounds may serve to interrupt response of other forest insects to their attractants. The orientation of bark beetles to conspecifics and host trees is largely governed by insect-produced aggregation pheromones and host tree-produced volatiles which may enhance responses to the pheromones[3,4]. Among sympatric bark beetle species, interspecific interactions may occur in which pheromones produced by one species may either enhance responses of other species to their aggregation pheromones[5], or inhibit responses of other species to their aggregation pheromones[1,2]. Antiaggregation pheromones, such as verbenone for *Dendroctonus frontalis* (Zimm.)[6], also may be produced and function to prevent overcrowding by switching of attack from previously attractive trees.

Green leaf volatiles are six carbon alcohols, aldehydes, and their derivatives, e.g. acetates, which are produced by plants as a product of oxidation of surface lipids[7]. These compounds have been assigned several roles in insect behavior, including: 1) host plant finding by phytophagous insects[8-10]; 2) enhancement of insect pheromones[11-13]; and 3) host finding by parasitoids of lepidopterous larvae[14,15].

A series of field tests were conducted involving one or more of the sympatric bark beetle species to determine the effects of the green leaf volatiles, hexan-1-ol and hexanal, on their responses to aggregation pheromone blends. Our first field test investigated the effects of the green leaf volatiles on responses of *D. frontalis* to its aggregation pheromone, frontalin, and a host synergist, southern pine turpentine[16,17]. Verbenone, a known antiaggregation pheromone of *D. frontalis*[6], also was included. Both hexan-1-ol, hexanal, and the combination of the two odors, interrupted the pheromone response of *D. frontalis* (table, A.; $p < 0.05$). The interruptant effect