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CIRCADIAN VARIATION OF MITOCHONDRIAL SUCCINIC DEHYDROGENASE AND MICROSOMAL CYTOCHROME P-450 DEPENDENT MONOOXYGENASE ACTIVITY IN THE LIVER OF SEXUALLY IMMATURE AND MATURE RATS

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The experiments were carried out on 60-day and 6-month old male Wistar rats within 48 h in one season of the year (autumn). Material was collected every four hours, beginning from 10:00 a.m. The present results indicate that the fluctuations of cytochrome P-450 content in liver microsomes in both age groups occurred in a 12 h rhythm with peaks at 10:00 and 22:00. Similarly, the activity fluctuations of NADPH-cytochrome c reductase showed the 12 h rhythm, with maximal values at 10:00 and 22:00, too. The cytochrome b_5 content in a younger group of rats oscillated apparently in the 12 h rhythm with the maximal values at 06:00 and 18:00. The activity course of cytochrome b_5 in 6-month-old rats revealed a 24 h rhythm and two maxima of the activity were found: the first one at 14:00 and the second one at 02:00. NADH-cytochrome b_5 reductase in both age groups showed a 24 h rhythm, also the activity fluctuations of succinic dehydrogenase showed a tendency to 24-h rhythmicity, and the differences between minimal and maximal values were statistically insignificant.

The results of our experiment have shown some correlations between the activity of microsomal system of mixed-function oxidases and mitochondrial respiratory enzyme.

Key words: *Circadian rhythm, liver mitochondrial succinic dehydrogenase (SDH), microsomal system mixed-function oxidases.*

INTRODUCTION

The rhythmic variations in several frequency ranges found in the activity of many enzymes are of considerable physiologic and pathophysiologic interest. Many of the variations of enzyme recur in regular intervals and represent rhythms which are predictable for the time of the day as well as for the seasons (1—3). According to Hardeland [4], a lot of enzymes show a 24-hour rhythm

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of activity, but little is known about its mechanisms. Generally, no acceptable explanation has been presented for the mechanism causing the rhythm of enzyme as yet (5). Among various organs of the body diurnal rhythms of hepatic enzyme activities have been most extensively studied (6—9). Previous works indicate that the variability of some liver enzyme activity in the circadian cycle depends on e.g. age, sexual maturity, and diet (10,11). We have found no publications in the literature concerning the daily course of the enzyme activity which would be connected with energetic processes in sexually immature and mature rats.

The structural connections between the mitochondria and endoplasmic reticulum (particularly RER) were described many years ago. They were found both in embryonal livers (12), in mature rat livers (13) as well as in regenerating livers [14]. It was shown, that these connections are not an accidental event but an extensive system, including most — if not all — mitochondria (15). The peculiar function of these two cell compartments was not definitively been eluciated. For example there is some evidence showing the role of RER in the synthesis and transport of mitochondrial proteins into the mitochondria (16). Some authors suggest that the role of the mitochondria-RER complex is a synthesis of proteins which are involved in the processes of oxidative detoxication (17). Picket et al (18) believe that the mitochondria-RER complexes are the primary site of apocytochrome P-450 synthesis.

This study is an attempt to show the presence of the circadian rhythm in the activity of microsomal system of mixed-function oxidases and of mitochondrial respiratory enzymes.

MATERIAL AND METHODS

The experiments were carried out in autumn (November) on 120 Wistar males 60-day and 6-month old. All animals were bred under stable conditions, illumination (LD 12:12, 08:00—20:00 day, 20:00—8:00 night), at a steady temperature (about 20°C) and stable humidity (60%). The experiments were carried out in the 48-hour cycle beginning at 10:00 a.m. every four hours. During the whole breeding season the animals were kept in plastic single cages with free access to food given at 10:00 and to water. All the animals were deprived of food for 12 hours before the experiments.

The rats were killed by decapitation and then 3 mm long liver sections were taken from the left lobe for histochemical investigations. The material was frozen with carbon dioxide and cut into serial sections 7 μm thick in cryocut at −18°C and then a reaction to succinate dehydrogenase (EC 1.3.95.1), was performed according to Pearse [19]. The enzymatic activity was examined with the method of surface optic density measurements always in the 3rd zone of hepatic acini, according to the rule that optic density of the examined surface is directly proportional to the reaction material within the examined zone. All the measurements were taken on the 0.07 mm^2 surface of the preparation using a picture computer analyser: Quantimet 720, type 30 (Cambridge Instruments) and HP 9825 A computer.

The microsomal fraction was prepared using Dallner's method (20), and then the cytochrome P-450 and cytochrome b_5 content were determined according to Estabrook's and Werringloer's method (21). The activities of NADPH-cytochrome c and NADH-cytochrome b_5 reductases were estimated using Hodges and Leonard's (22) method.

The protein content was determined according to Lowry et al. (23) method by the use of bovine serum albumine as the standard.

To obtain maximal and minimal computer values the calculations were made with F-Fischer and Student's t-tests with statistical significance taken as $p < 0.05$. Function matching:

$$Y = M + A^{*}\cos(\omega^{*}t + \varphi)$$

suggested by Cornelissen (24) was applied in all the series showing significant temporal changes.

RESULTS

The fluctuations of cytochrome P-450 content in liver microsomes in both age groups occurred within 12-h rhythm with peaks at 10:00 and 22:00 (*Fig. 1 and 2*). While in a younger group the peak activity during the light phase was the lower one, in the 6-month-old group the activity of this hemoproteid was higher at 10:00 than at 22:00. Similarly to the changes in the cytochrome P-450 content the activity of NADPH-cytochrome c reductase acting jointly with this hemoproteid showed fluctuations. In this case the 12-h rhythm was observed, with maximal values at 10:00 and 22:00. In both age groups higher values occurred in the dark phase, but the differences were statistically insignificant (*Fig. 1, 2; Tab. I*). The differences between minimal and maximal values for particular elements of the I-st chain of electron transport were statistically significant ($p < 0.05$).

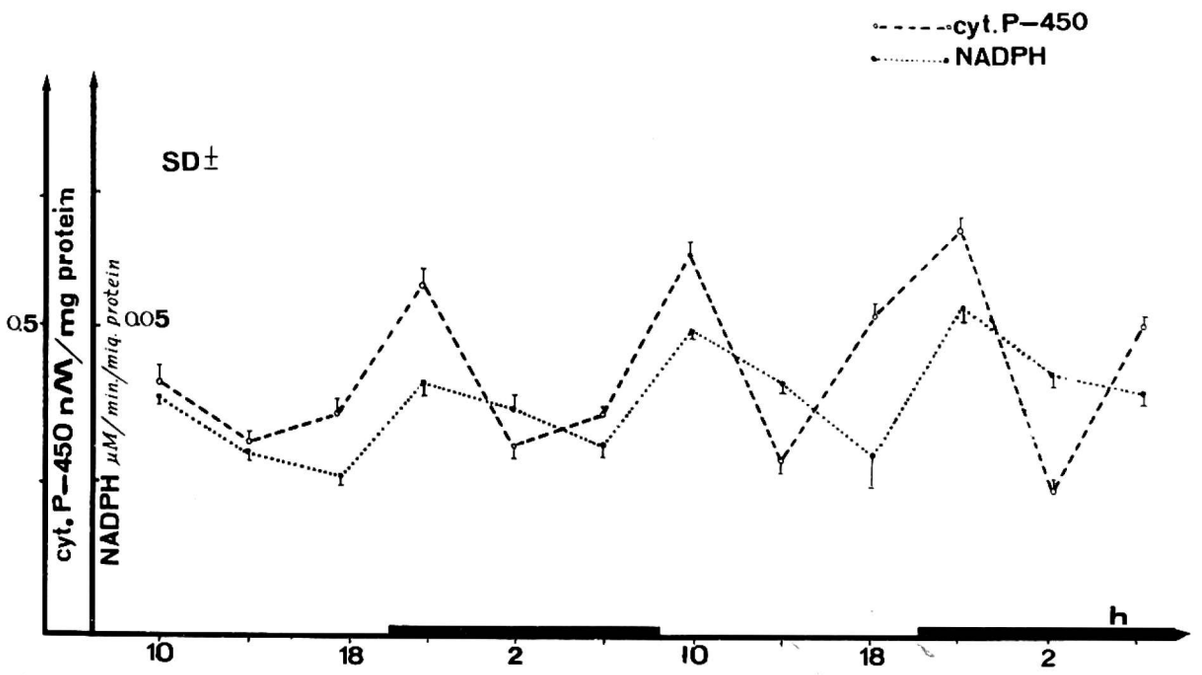

Fig. 1. Circadian fluctuations of microsomal cytochrome P-450 content and NADPH-cytochrome c reductase activity in rat liver of 60 days old rats.

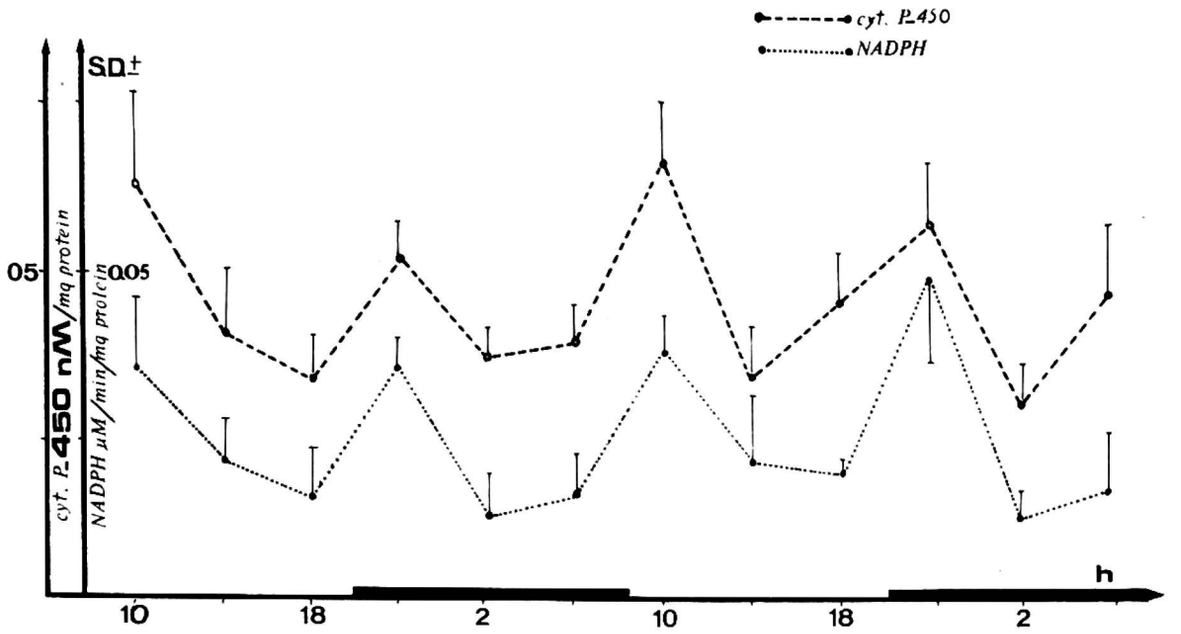

Fig. 2. The rhythm of microsomal cytochrome P-450 content and NADPH-cytochrome c reductase activity in liver of 6 months old rats

The cytochrome b_5 concentration in 60-day-old rats apparently oscillated in the 12-h rhythm. Te maximal values were observed at 06:00 and 18:00. In the group of 6-months-old rats two maxima of the cytochrome b_5 activity were found: the first one at 14:00 and the second one at 02:00. During the two-day cycle the first activity peak was the higher one. The minimal value of this enzyme activity was observed at 02:00 in both experimental days. The course of fluctuations revealed the characteristics of the 24-h rhythm (*Figs 3 and 4, Tab. I*).

Table 1. Parameters of approximative functions: $y = M + A^{*}\cos(\omega^{*}t + \varphi)$

Age	Enzyme	Mesor	Amplitude	Period [hr]	Acrophase [hr/min]
60 days	cyt. P-450	0.4094	0.1497	12	8hr 57 min
	NADPH	0.0372	0.0070	12	10hr 55 min
	cyt b_5	0.3272	0.1107	12	5ht 14 min
	NADH	0.3939	0.0970	12	0hr 46 min
	SDH	0.0400	0.0100	24	6hr 35 min
6 months	cyt. P-450	0.536	0.173	12	9hr 33 min
	NADPH	0.024	0.010	12	10hr 00 min
	cyt b_5	0.372	0.020	24	11hr 43 min
	NADH	0.272	0.040	24	1hr 49 min
	SDH	0.030	0.010	24	17hr 52 min

Abbreviations:

cyt. P-450 = cytochrome P-450
NADPH = NADPH-cytochrome c reductase
b_5 = cytochrome b_5
NADH = NADH-cytochrome b_5 reductase
SDH = succinic dehydrogenase

In case of NADH-cytochrome b_5 reductase in the group of younger rats a distinct 24-h rhythm was observed in autumn, with the maximal values occurring at 14:00 and 02:00. The peak activity in the dark phase was clearly higher. In the group of older animals the activity of that reductase showed the 24-h rhythm, as it was analysed separately in two subsequent days. Similarly to cytochrome b_5 maximal values were observed at 14:00 (*Figs 3 and 4, Tab. I*).

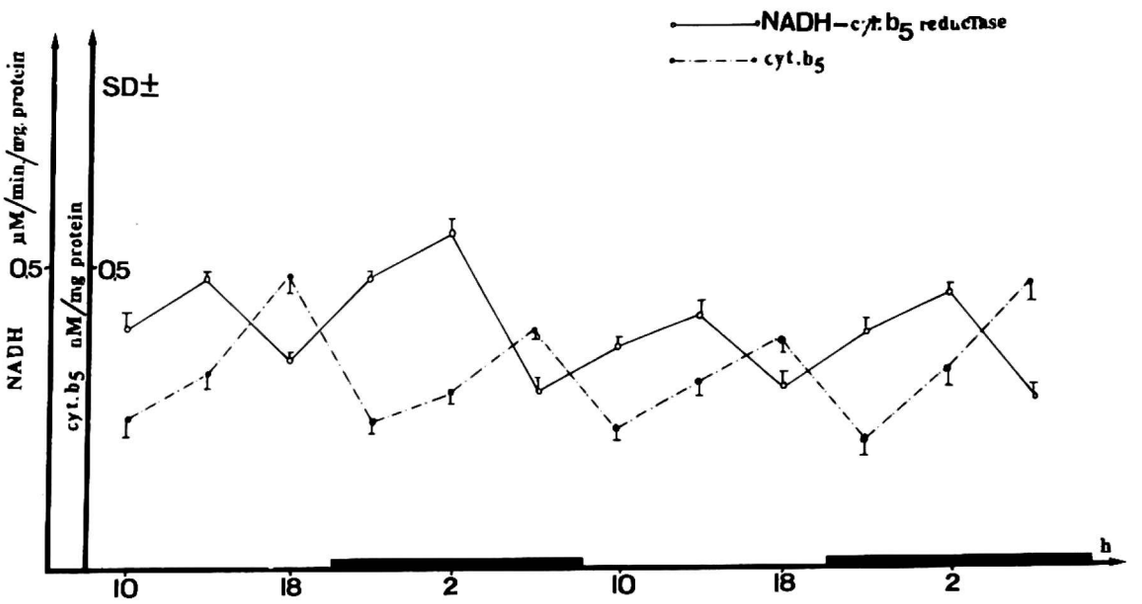

Fig. 3. Circadian variation in the microsomal cytochrome b_5 content and NADH-cytochrome b_5 reductase activity in liver of 60 days old rats.

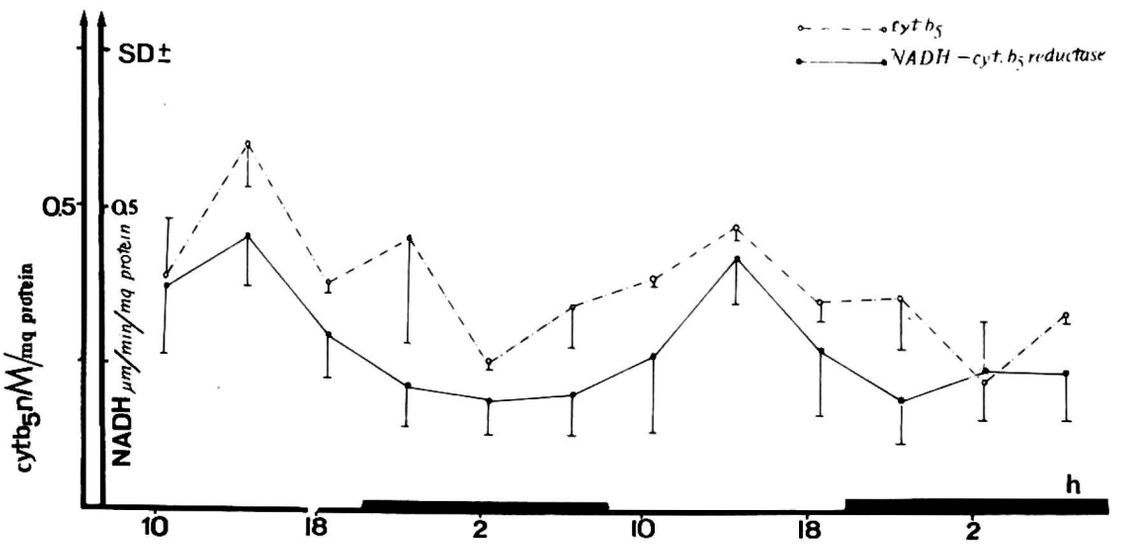

Fig. 4. The circadian rhythm of microsomal cytochrome b_5 content and NADH-cytochrome b_5 reductase in liver of 6 months old rats.

In both age groups the course of succinic dehydrogenase activity change showed a weak tendency to 24-h rhythmicity. The highest SDH activity in 60 day-old rats occured at 02:00. The differences between maximal and minimal

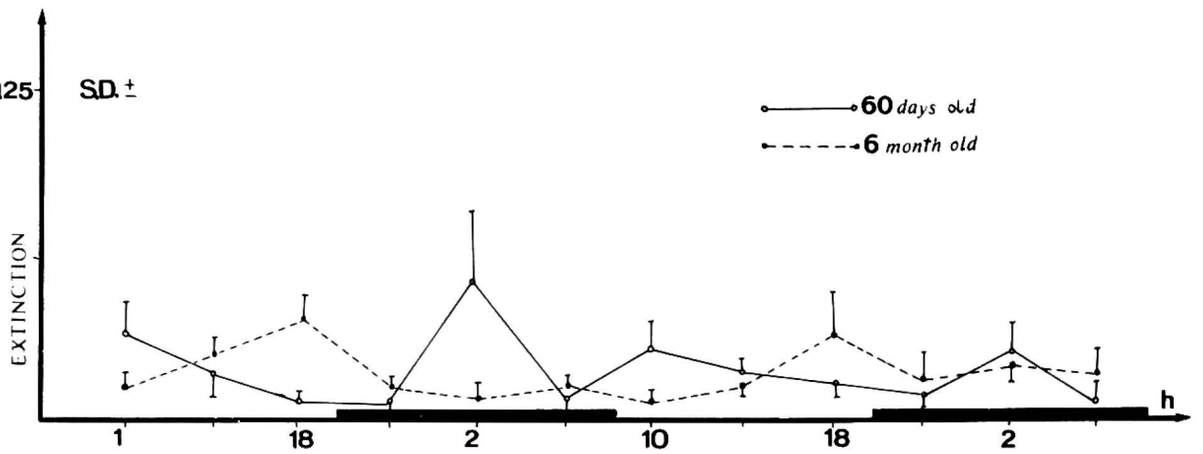

Fig. 5. Circadian activity of succinic dehydrogenase (SDH) in livers of 60 days old and 6 months old rats.

activity values were insignificant. In 6-month-old animals the activity maximum was observed at 18:00. The differences in activity values were also insignificant (*Fig. 5, Tab. I*).

DISCUSSION

A detailed discussion of the present results is rather difficult because of lack of bibliographical data concerning the estimation of circadian activity fluctuations in the particular elements of functionally mixed oxydases system.

The 12-h circadian rhythmicity of NADPH-dependent electron transport chain enzymes activity in our study is in part confirmed by Beil et al (25) and Kagan et al (26) who have found that NADPH-cytochrome c reductase alters its activity during the day parallel to the fluctuations in the content of a cooperating cytochrome. In our case this event refers to the 60 day-old rats only. There is much indirect evidence concerning the contribution of xenobiotics to the liver metabolism. This problem is broadly discussed in the paper by Guskova and Liberman (27).

Birt and Maines (28) observed a circadian rhythm in the liver weight as well as in the content of microsomal proteins and cytochrome P-450.

In our study the activity fluctuations both in cytochrome P-450 and NADPH-cytochrome c reductase in 60-day- and 6-month-old rats show a 12-h rhythm with closely correlated activity maxima at 10:00 and 22:00. In case of the second element of microsomal chain of electron transport i.e. NADH-cytochrome b_5 reductase, the course of its activity was not so closely correlated. It was found that it differed with respect to age. In 60-day-old animals with a maximum of cytochrome b_5 content the activity of NADH-cytochrome b_5 reductase was minimal. This results from two distinct rhythms: for cytochrome (12 hr) and for reductase (24 hr), observed in our

experiment. In 6-month-old animals both rhythms of the activity are of a 24-h character, with maxima at 14:00 and 02:00 for cytochrome b_5 and at 14:00 for NADH-cytochrome b_5 reductase. The present results suggest that the studied elements of microsomal electron transport chain share some common features of time relations and show some distinctions in their activity level.

Electron microscopic studies (29) revealed that the amount of SER varied with the time of the day, and regional differences in the distribution of SER and RER within the hepatic lobule were also observed. The authors imply, in general, that within the same lobule a centroportal gradient in the distribution of SER developed and it was higher at 10:00 p.m. in the pericentral areas and to a certain extent in the midzones, but the RER — in contrast — showed less variability. Studies of Kast et al. (30) have shown, that the SER in hepatocytes was significantly increased at 22:00 but in mitochondria it was increased at 14:00 in zone 1 and 3 but at 22:00 only in zone 3. We agree with Philippens (31) that mitochondria operating as respiratory units may show heterogenous fluctuations of their constituting elements, and that in the morning hours the mitochondrial activity is lower than at night.

The results obtained in our experiment indicate that the SDH activity fluctuation in both age groups show a weak tendency towards a 24 hour rhythm. Some correlation between the activity of SDH and microsomal enzyme activity was found.

Harisch et al (32) showed a daily rhythm for microsomal NADPH-cytochrome c reductase and succinic dehydrogenase, with maximum values being reported during the second half of the day, minimum during the first half. Similar results were obtained by Glück et al. (33) in the mitochondria of the adrenal glands and Philippens (31) in liver mitochondria using both histochemical and biochemical methods.

In our experiments the higher activity of SDH was observed in the second half of the light phase (at 18:00) only in 6-month-old rats, whereas in younger animals it occurred in the middle of the dark phase, i.e. at 02:00.

At the age of 60 days, the animals are sexually mature and adapted to a new course of life, i.e. to independent life. In addition, androgens, estrogens and adrenocortical hormones began to play a significant role in the animal metabolism (11). A similar dependence was observed by Hoffman and Hardeland (34). Maxima of the SDH activity occur in the middle of the dark phase.

Harisch et al (32) suggest that the activity of the flavin enzymes at each time of the day is most probably determined by the actual concentration of serum corticosteroids. The well-known daily rhythm of serum corticosterone is congruent with the rhythm of the flavin enzymes (7, 33).

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