# CIRCADIAN RHYTHM OF PHOSPHOLIPID CONTENT AND NONSPECIFIC PHOSPHOMONOESTERASE ACTIVITY IN THE RAT LIVER

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A circadian rhythm of the content of total and individual phospholipids and of alkaline and acid phosphatase activity was found in the albino rat liver by biochemical and histochemical methods. The phospholipid content and enzyme activity were higher during the daytime (9 a.m. to 3 p.m.). The results indicate harmony between the structural and metabolic organization of the liver during the period of maximal functioning activity of the organ.

KEY WORDS: liver; circadian rhythm; phospholipids; acid and alkaline phosphatases.

Cyclic processes have been discovered at all levels of organization of living matter, and the rhythm of such processes is accordingly one of its fundamental characteristics [9]. Fluctuation of phospholipids (PL), the substrate of the most important physiological processes of the cell — transformation of energy, structural metabolism of biomembranes, permeability, etc. [2, 6, 7] — and changes in nonspecific phosphomonoesterase activity in the rat liver which follow a circadian physiological rhythm have not yet been studied. The importance of PL and the marker properties of acid and alkaline phosphatases (AcP and AlP respectively) for subcellular structures [10] is responsible for their wide use as tests in the most varied investigations. In our opinion, the study of the circadian rhythm of these metabolic parameters in the rat (as a commonly used laboratory animal) liver under normal physiological conditions could be useful in order to obtain reliable information on the temporal aspect.

TABLE 1. PL Concentration (in μg/g inorganic phosphorus) and Phosphomonoesterase Activity (in μmoles inorganic phosphate/g/h) in Rat Liver at Different Times of 24-h Period (M ± m)

| Index | Time of day | | | | Mean value for 24-h period | Deviation from mean for 24 h | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 9 a.m. ($n$=32) | 3 p.m. ($n$=32) | 9 p.m. ($n$=32) | 3 a.m. ($n$=34) | | 9 a.m. | 3 p.m. | 9 p.m. | 3 a.m. |
| PA | 83,00 ±2,20 | 99,50 ±2,00 | 91,00 ±3,30 | 81,00 ±2,50 | 88,63 ±2,50 | —5,63 | +10,87 | +2,37 | —7,63 |
| CL | 75,00 ±2,90 | 79,00 ±2,30 | 85,00 ±4,90 | 82,00 ±5,50 | 80,25 ±3,90 | —5,25 | —1,52 | +4,74 | +1,75 |
| PEA | 153,00 ±6,20 | 173,00 ±1,40 | 140,00 ±1,50 | 152,00 ±4,00 | 154,50 ±3,27 | —1,50 | +18,50 | —14,50 | —2,50 |
| PC | 561,00 ±13,40 | 530,00 ±8,40 | 567,00 ±16,60 | 476,00 ±11,50 | 533,50 ±12,47 | +27,50 | —3,50 | +33,50 | —57,50 |
| PS | 104,00 ±9,50 | 134,00 ±13,70 | 122,00 +10,50 | 105,00 ±2,80 | 116,25 +9,12 | —12,25 | +17,75 | +5,75 | —11,25 |
| SPH | 74,00 ±4,65 | 162,00 ±8,10 | 123,00 ±10,90 | 94,00 ±8,60 | 113,25 ±8,06 | —39,25 | +48,75 | +9,75 | —19,25 |
| LL | 140,00 ±10,00 | 140,00 ±12,10 | 155,00 ±7,10 | 134,00 ±8,00 | 142,25 ±9,30 | —2,25 | —2,25 | +12,75 | —8,25 |
| Total PL content | 1190,00 ±31,00 | 1317,00 ±25,00 | 1283,00 ±25,00 | 1124,00 ±18,50 | 1228,63 ±24,90 | —38,87 | 188,87 | +54,37 | —104,63 |
| AlP | 10,60 ±1,25 | 10,30 ±1,33 | 7,40 ±1,03 | 8,30 ±1,11 | 9,15 ±1,18 | +1,45 | +1,15 | —1,75 | —0,85 |
| AcP | 186,50 ±8,60 | 194,70 ±14,08 | 177,28 +11,17 | 180,72 ±7,57 | 184,80 +10,35 | —1,70 | +9,90 | —7,52 | —4,08 |

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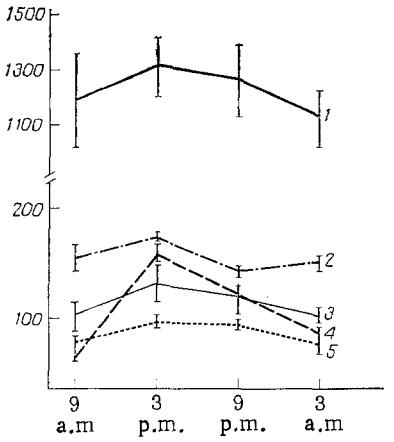


Fig. 1. Changes in PL content in rat liver during 24-h period. 1) Total PL, 2) PEA, 3) PS, 4) SPH, 5) PA. Ordinate, total PL content and their individual fractions (in μg/g lipoid phosphorus); abscissa, time of day.

In the investigation described below changes in PL content and nonspecific monoesterase (AcP and AlP) activity in the rat liver were investigated at different times of the 24-hour period.

## METHODS

Experiments were carried out on 130 noninbred male albino rats weighing 130-150 g. The animals were decapitated at 3 and 9 a.m. and 3 and 9 p.m. after preliminary starvation for 24 h. The material was treated by biochemical and histochemical methods. PL were separated by thin-layer chromatography on "Silufol" plates [2, 5] in a system of chloroform—methanol—water (65 : 25 : 4). Individual PL isolated from bovine brain were used as reference substances [3]. Lipid phosphorus was mineralized by a modified method [11]. The lipid content in the fractions was determined as inorganic phosphorus and expressed in μg/g [1]. AcP and AlP activity was determined in whole liver homogenates by Bodansky's method [12]. Homogenates were prepared in a homogenizer with Teflon pestle revolving at 3000 rpm and at 0°C. Phosphomonoesterase activity was expressed in micromoles inorganic phosphate hydrolyzed per gram tissue per hour at 37°C. AcP were detected histochemically by Burstone's simultaneous azo-coupling reaction in frozen sections after fixation in formol—calcium solution (18 h at 4°C); AlP was determined by Gomori's method (fixation in 90° alcohol at 4°, 20 h) [14]. Phospholipids were detected histochemically by Elftman's method [13]. The results of the biochemical tests were subjected to statistical analysis by the Student—Fisher method and to correlation analysis [4]. The histochemical data were evaluated by the method described previously [8].

## RESULTS

Altogether seven PL fractions (Table 1) were revealed in the rat liver by thin-layer chromatography, and in their phosphorus content they were arranged in the following order: phosphatidylcholine (PC), phosphatidylethanolamine (PEA), lysolecithin (LL), phosphatidylserine (PS), sphingomyelin (SPH), cardiolipin (CL), and phosphatidic acids (PA). The level of total PL and individual fractions showed considerable fluctuations during the 24-h period relative to the mean (Table 1, Fig. 1). The total PL content reached a maximum at 3 p.m. ($P < 0.05$) and a minimum at 3 a.m. ($P < 0.05$), and this was confirmed histochemically (Fig. 2A, B). The contribution of individual PL to the increase and decrease in the total PL level varied (Table 1). The main role in this fluctuation was played by PL with high metabolic activity [6], namely PA, PEA, and PS (Table 1), and also by a stable PL [6], namely SPH (Table 1). Changes in the levels of the remaining PL fractions during the 24-h period were characterized by fluctuations of low amplitude (Table 1).

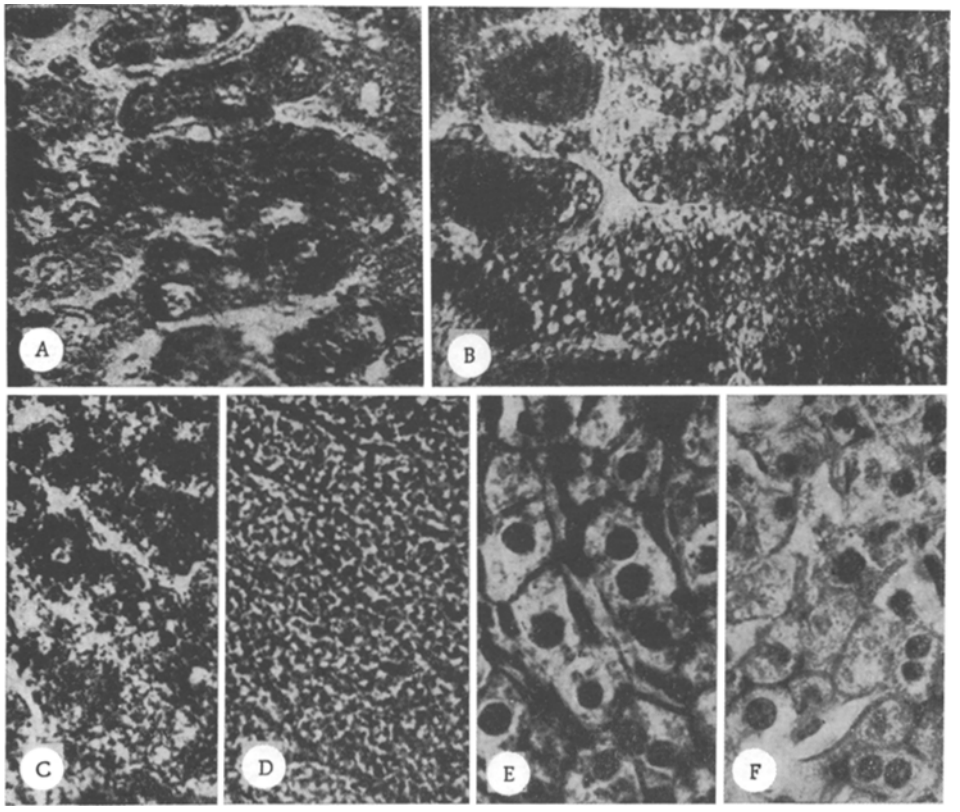


Fig. 2. Histochemical distribution of PL, AcP, and AlP during 24-h period in rat liver. A, B) Distribution of PL in rat liver at 3 p.m. (A) and 3 a.m. (B); fixation with 2.5% potassium bichromate solution (pH 3.5) at 56°C; staining with acid hematein and Weigert's solution; 420 ×; C, D) distribution of ACP activity in rat liver at 9 p.m. (C) and 3 a.m. (D); fixation with formol—calcium, stained by simultaneous azo-coupling reaction (substrate: naphthol-AS-MX phosphate, diazonium salt Fast red RC); 420 ×; E, F) distribution of AlP in rat liver at 3 (E) and 9 (F) p.m.; fixation with 90° alcohol at 4°C; stained by Gomori's method, 420 ×.

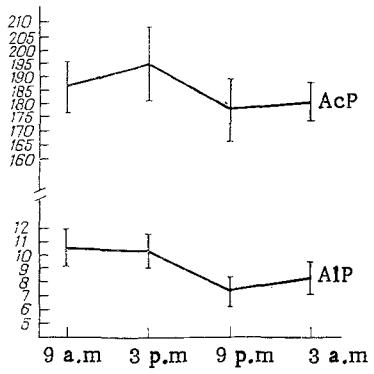


Fig. 3. Changes in phosphomonoesterase activity in rat liver during 24-h period. Ordinate, phosphomonoesterase activity (in micromoles inorganic phosphate hydrolyzed by 1 g tissue at 37°C in 1 h); abscissa, time of day.

According to the biochemical data, total nonspecific phosphomonoesterase activity fluctuated during the 24-h period so that it reached a maximum in the morning and afternoon (9 a.m. to 3 p.m.) and a minimum at 9 p.m. (Table 1, Fig. 3). Fluctuations of AcP and AlP activity during the 24-h period took place mainly synchronously. The maximum of AcP activity, both in the hepatocytes (especially centrilobular) and in the Kupffer cells, reached a maximum detectable histochemically at 9 p.m. (Fig. 2C, D). The enzyme reaction product appeared as discrete granules, perinuclear in distribution or in the region of the portal pole of the hepatocytes. Minimal AcP activity occurred at 3 a.m. and was characterized by the presence of solitary small granules close to the biliary capillaries. The difference between the fluctuations in AcP activity as revealed histochemically in the course of the 24-h period from the corresponding biochemical results can probably be attributed to inhibition of AcP in the cell matrix by formalin and the resistance of the corresponding lysosomal enzyme to it [10]. Histochemical investigation of AlP activity showed the presence of reaction products in the walls of the biliary tubules and in the endothelium of the capillaries and small veins at a maximal level at 3 p.m. and minimal at 9 p.m., when they were found in the endothelium of solitary sinusoids and at the vascular pole of the hepatocytes (Fig. 2E, F). The character of the changes in AlP activity in the hepatocytes, revealed histochemically, in the course of the 24-h period agreed with the biochemical data described above.

Correlation analysis revealed the presence of negative correlation ($r = -0.69$, $P < 0.01$) between the AcP and AlP content at 9 a.m. AcP activity was found to be inversely proportional to the LL concentration at 3 a.m. ($r = -0.72$, $P < 0.05$), and the PC concentration at 3 p.m. ($r = -0.73$, $P < 0.05$), whereas at 9 p.m. the AcP content was directly proportional to PS ($r = 0.69$, $P < 0.05$). Significant negative correlation also was found at 9 a.m. between the AlP content, on the one hand, and PA ($r = -0.81$, $P < 0.01$), CL ($r = -0.79$, $P < 0.01$), and PS ($r = -0.78$, $P < 0.01$) on the other hand.

Comparison of the character of the circadian rhythms of concentration of total and individual PL and phosphomonoesterase activity (Figs. 1 and 3) showed that the highest PL level coincides in time with maximal enzyme activity (3 p.m.).

The synchronous change in PL and phosphomonoesterase activity during the period of daylight suggests harmony between the structural and metabolic orgnization of the hepatocytes in the period of maximal functional activity of the liver.

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