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CIRCADIAN RHYTHM OF LIVER PHOSPHOLIPIDS IN NORMAL HAMSTERS AND HAMSTERS WITH OPISTHORCHIASIS

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Opisthorchiasis modifies circadian changes in activity of the enzymes responsible for plastic functions of the liver [5] and cell renewal in the digestive system [4, 9] in the acute phase of the disease. Changes may accordingly be expected in the circadian rhythm of phospholipids (PL), which play a major role in the activity of membrane-bound enzymes [6].

The object of this investigation was a biohistochemical analysis of PL in the liver of golden hamsters at different times of the 24-h period in the acute and chronic phases of opisthorchiases, and also after dehelminthization.

EXPERIMENTAL METHOD

Experiments were carried out on 204 sexually mature male golden hamsters, divided into five groups. Groups 1 and 2 consisted of intact animals aged 3 and 8 months respectively; groups 3 and 4 were animals infected 30 and 150 days respectively after infestation, treated with chloxyle (0.4 g/kg, 2-day course) on the 45th day after infection; they were investigated

TABLE 1. Phospholipid Concentration (in μg/g inorganic phoshporus) in Liver of Normal Golden Hamsters (aged 3 months, series I) and Hamsters in the Acute Phase of Opisthorchiasis (30th day of infection, series II) during the 24-h Period (M ± m)

PL	Series of experiments	Clock time				M
		9 a.m. ($n=20$)	3 p.m. ($n=20$)	9 p.m. ($n=20$)	3 a.m. ($n=22$)	
Total PL	I	1030±35,0	1210±45,0	1190±15	1130±30	
	II	820±25,0	640±25,0	880±70	840±40	
PA	I	99,0±4,1	89,8±4,4	101,6±5,6	84,0±4,3	93,2±4,0
	II	52,4±4,5	41,9±3,2	45,9±2,7	70,6±5,0	54,0±2,8
CL	I	78,7±3,6	104,0±5,8	107,8±5,3	88,3±5,0	94,4±5,6
	II	50,8±4,2	40,9±3,1	44,7±2,6	68,8±5,8	52,5±2,9
PEA	I	141,9±7,7	166,4±7,9	135,3±10,9	156,7±6,8	148,6±6,2
	II	164,6±8,8	132,3±11,4	199,1±20,4	120,2±10,2	151,7±9,5
PCh	I	338,5±13,5	430,4±15,7	413,1±16,0	372,3±13,2	387,8±24,3
	II	183,0±8,9	144,1±16,2	203,5±27,5	258,5±34,1	201,4±8,6
PS	I	136,9±5,1	133,4±12,9	151,1±8,9	157,5±11,3	145,5±3,1
	II	157,3±13,5	122,5±14,8	167,3±20,7	101,2±15,8	134,6±6,1
SM	I	103,5±2,2	151,6±10,6	143,8±13,4	139,4±10,6	134,8±5,7
	II	102,6±7,1	123,6±6,4	96,3±9,3	81,5±4,1	90,4±3,0
LL	I	116,6±3,2	129,4±9,0	140,0±10,8	140,5±9,3	132,1±2,3
	II	120,3±13,0	92,0±13,0	142,3±19,9	133,5±14,5	122,8±7,9

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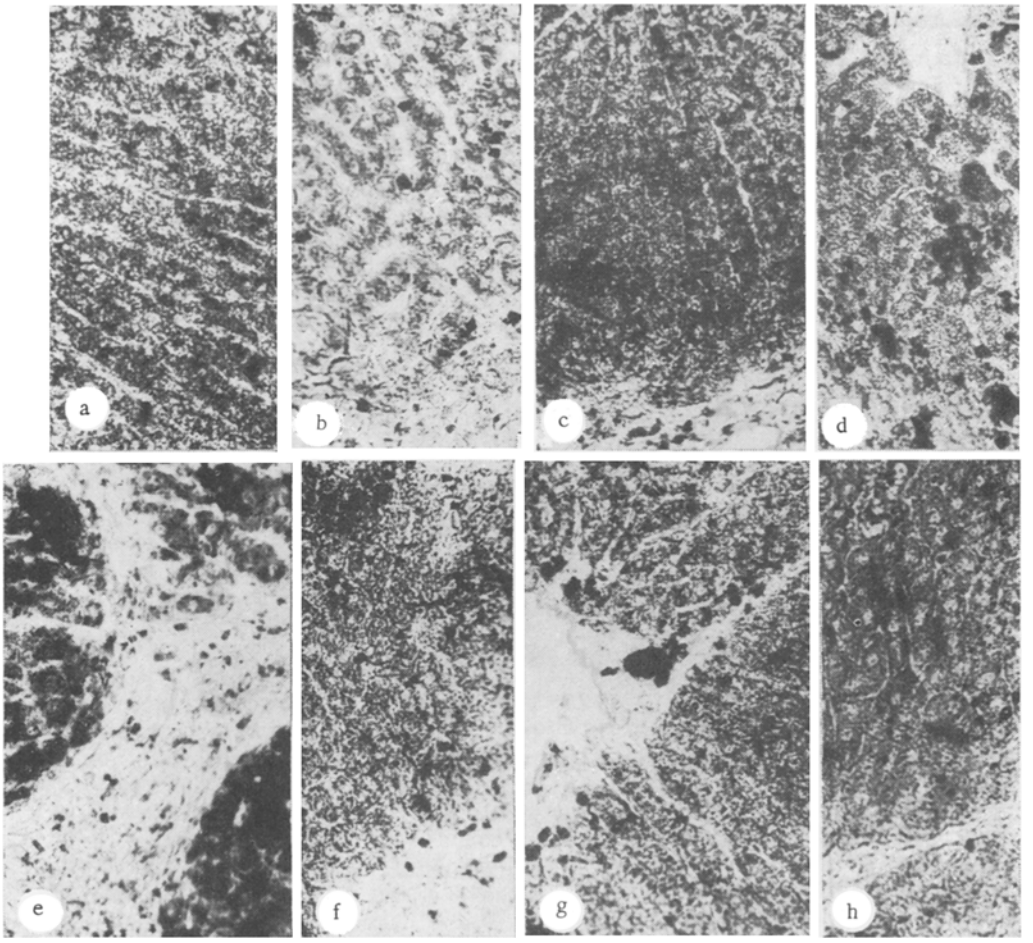

Fig. 1. Distribution of PL during 24-h period in liver of intact golden hamsters and hamsters infected with opisthorchiasis and treated with chloxyle. a, b) Histochemical distribution of PL in liver of intact golden hamsters aged 3 months at 3 p.m. and 9 a.m. respectively; c, d) the same in the liver of golden hamsters on the 30th day after infection with opisthorchiasis, at 9 and 3 p.m. respectively; a, b, c, and d) fixation with 2.5% potassium bichromate (pH 3.5) at 56°C. Staining with acid hematein and Weigert's solution. e, f) Histochemical distribution of PL in liver of golden hamsters on 150th day after infection with opisthorchiasis at 9 a.m. and 9 p.m. respectively; g, h) histochemical distribution of PL in liver of golden hamsters infected with opisthorchiasis and treated with chloxyle, on 92nd day after treatment at 9 p.m. and 9 a.m. respectively; e, f, g, h) fixation with calcium-formol solution by Baker's method at 4°C, staining with an alcoholic solution of Sudan black B.

92 days after the end of treatment. Group 5 consisted of animals completely free from helminths. The technique of infection with opisthorchiasis was described previously [2]. Hamsters of all groups were decapitated at 3 and 9 a.m. and 3 and 9 p.m. during the 24 h after preliminary starvation, also for 24 h. Total PL were determined histochemically [10] and by staining with Sudan black B [8]; they were fractionated by thin-layer chromatography on Silufol plates [3]. The results were subjected to statistical analysis and the significance of differences was determined by Student's t test; the mean daily value (M), the coefficient of circadian periodicity (CCP), the index of circadian adaptiveness (ICA), and the coefficient of synchronization of functions (CSF) also were calculated [1].

EXPERIMENTAL RESULTS

On the 30th day of infection (the acute phase of opisthorchiasis) a decrease in M for total PL (800 ± 27 compared with 1140 ± 45, $P < 0.001$), phosphatidic acid (PA), cardiolipin

TABLE 2. Phospholipid Concentration (in μg/g inorganic phosphorus) in Liver of Normal Golden Hamsters (aged 8 months, series I) and of Hamsters with Opisthorchiasis (150th day of infection, series II), and after Dehelminthization* (series III), during the 24-h Period (M ± m)

PL	Series of experiments	Clock time				M
		9 a.m. (n=30)	3 p.m (n=30)	9 p.m. (n=30)	3 a.m. (n=32)	
Total PL	I	990±25	1170±20	1160±20	990±30	
	II	1050±40	730±50	680±20	770±50	
	III	620±40	660±80	750±30	690±30	
PA	I	93,9±3,6	77,2±4,5	89,7±4,1	88,3±3,7	87,3±1,9
	II	102,3±9,4	47,0±4,4	63,6±9,7	89,1±14,6	75,7±5,0
	III	61,5±7,2	60,1±8,8	64,7±2,9	60,2±2,0	61,6±2,9
CL	I	71,7±3,4	86,8±2,3	83,1±1,4	78,8±5,0	81,5±1,7
	II	94,3±8,8	38,8±7,6	53,3±4,2	82,4±7,0	67,2±4,9
	III	60,7±7,1	58,4±5,2	59,5±2,6	55,6±1,8	58,6±2,3
PEA	I	134,2±8,6	170,3±6,6	157,3±5,5	151,0±7,4	153,2±3,4
	II	193,4±15,7	93,8±11,2	95,0±8,9	115,3±21,2	124,4±9,7
	III	91,2±10,7	119,5±27,6	117,3±2,6	117,7±6,0	112,2±7,5
PCh	I	282,6±29,1	498,3±15,2	398,9±11,0	325,7±16,5	376,4±16,0
	II	206,7±12,7	147,3±17,1	139,7±9,1	209,4±21,0	175,8±9,8
	III	149,4±17,6	150,9±21,6	129,8±7,0	130,8±3,3	140,2±7,1
PS	I	141,6±4,9	79,8±3,8	150,1±9,2	101,8±9,5	118,3±3,5
	II	140,4±16,9	131,2±29,8	127,0±6,0	137,0±11,4	133,9±8,6
	III	108,2±13,5	91,0±12,4	128,6±7,1	111,3±3,1	109,8±5,3
SM	I	117,0±17,1	158,6±11,6	150,2±9,1	106,3±7,0	133,0±5,0
	II	158,0±11,0	126,2±14,8	96,6±8,2	111,0±13,7	123,0±5,7
	III	77,9±5,8	84,6±16,2	133,0±7,8	116,2±3,5	102,7±5,9
LL	I	129,9±10,0	107,0±6,5	132,7±4,07	136,3±5,6	126,5±3,6
	II	165,9±11,4	127,2±11,4	104,6±8,3	104,9±10,4	125,7±6,4
	III	85,7±7,4	88,1±16,1	155,9±15,3	112,5±2,0	110,6±7,2

*92 Days after treatment with chloxyle (0.4 g/kg).

(CL), phosphatidylcholine (PCH), and sphingomyelin (SM), and desynchronization of the biorhythm of PA, CL, phosphatidylethanolamine (PEA), and SM were observed (Table 1, $P < 0.001$). Circadian changes in the phosphatidylserine (PS) content, not present in uninfected hamsters, also appeared. At 9 p.m. the maximal PL content was distributed histochemically chiefly in the periportal hepatocytes, which accumulate fat in stromal cells and Kupffer cells (KC) (Fig. 1c), whereas in hamsters aged 3 months (as in those aged 8 months) at the 3 p.m. maximum PL were uniformly distributed in the cytoplasm of the hepatocytes in all zones of the acinus (Fig. 1a). Under normal conditions PL were most clearly observed histochemically at 9 a.m. (minimum) in KC (Fig. 1b), whereas in the animals of group 3 heterogeneity of the hepatocytes was clearly manifested at 3 p.m. (minimum) (Fig. 1d). The circadian rhythm of PL in animals of group 2 was intense in character: an increase in ICA and CCP of all fractions except SM, an increase in CSF at each point of time: 9 a.m. (PA—CL = —0.93, CL—PS = —0.99, PS—lysolecithin (LL) = —0.89, $P < 0.01$); 3 p.m. (PA—PEA = 0.95, $P < 0.01$; PEA—PS = —0.73, SM—LL = —0.77, $P < 0.05$); 9 p.m. (3 p.m.) (PA—CL = 0.92, $P < 0.01$) and, in particular, at the M level: PA—CL = 0.92, PCH—PS = —0.68 ($P < 0.01$); PS—SM = 0.56, PEA—PS = 0.53, CL—SM = —0.35, PEA—SM = 0.37, PEA—LL = 0.3, PCH—LL = 0.37 ($P < 0.05$). The decrease in ICA and CCP of SM (65.39% and 0.95 compared with 80.49% and 1.14 respectively) was a sign of overstrain of the organ at this period. In the hamsters of group 1 the value of CSF was significant only at 9 a.m. (PA—PS = 0.95, $P < 0.01$; PS—LL = 0.69, $P < 0.05$; and at 9 p.m. (PA—PCH = —0.69, $P < 0.05$), and at the M level (PA—PS = —0.61, CH—PCH = 0.59, PA—SM = —0.59, $P < 0.01$; PCH—LL = 0.36, PS—LL = 0.32, $P < 0.05$). Comparison of these results with those of previous investigations [5] leads to the conlusion that the biorhythm of PL is not in harmony with that of the nonspecific and lysosomal phosphatases of the golden hamster liver in the acute phase of opisthorchiasis, especially during the period of maximal functional activity of the liver.

With the development of cirrhosis in the liver (group 4) the content of total PL fell significantly (830 ± 30 compared with 1070 ± 12 in the animals of group 2, $P < 0.01$), M fell for PA, CL ($P < 0.05$), LL, and PCH (Table 2, $P < 0.01$), whereas M increased by 1.4 times for PEA ($P < 0.05$, Table 2). The circadian rhythm of CL, PEA, PCH, SM, and total PL in the animals of group 4 was desynchronized in both phase and amplitude with these parameters for the animals of group 2 (Table 2). The histochemical distribution of PL changed synchronously (Fig. 1e, f). At the maximum, most PL was observed in hepatocytes of pseudolobules, stromal cells, and fat-accumulating cells (Fig. 1e); at the minimum most PL was observed in KC and

in the region of the outer cell borders (Fig. 1f). Liver function remained intensive: ICA was increased for PA (117.6% compared with 21.6%), CL (143.0% compared with 21.1%), PEA (106.2% compared with 26.9%), SM (63.5% compared with 35.5%), LL (56.6% compared with 27.4%), and CCP for PCH also increased (0.98 compared with 0.93). The decrease in ICA for PCH and PS (49.9 and 10.6% compared with 76.3 and 88.1% respectively) and the decrease in CCP for PA, PEA, PS, SM, and LL (1.02, 0.73, 0.97, 0.73, and 0.71 compared with 1.04, 1.01, 1.14, 0.93, and 1.13 respectively), and weakening of CSF at the minimum at 3 p.m. (PA–PEA = 0.65, PA–SM = 0.67, $P < 0.05$; PA–LL = 0.87, $P < 0.01$ compared with PA–SM = 0.69, $P < 0.05$; PA–LL = –0.91, $P < 0.01$; PEA–PA = 0.73, $P < 0.05$) indicate latent insufficiency of the liver in the chronic phase of the helminthiasis. The disturbance of homeostasis was compensated either by a readjustment and strengthening of correlations between individual PL fractions at 9 a.m.: (PA–CL = 0.89, $P < 0.01$, instead of CL–PCH = 0.66, $P < 0.05$), and also at 3 a.m.: PA–SM = –0.83, $P < 0.02$; PCH–LL = 0.64, $P < 0.05$, compared with PA–LL = 0.75 and PEA–PS = 0.66, $P < 0.05$, or by the appearance of new correlations at 3 p.m. (CL–PEA = 0.84, $P < 0.01$; CL–PCH = 0.74, $P < 0.02$) or an increase in CSF at 9 p.m. (PA–PEA = –0.79, PA–SM = –0.71, $P < 0.02$; PEA–PA = 0.68, PCH–PS = –0.68, PCH–LL = 0.65, $P < 0.05$; CL–PS = 0.80, $P < 0.02$, compared with PA–PS = –0.75, CL–SM = 0.82, CL–LL = –0.88, $P < 0.02$.

Dehelminthization smoothed the circadian fluctuations in PA, CL, and PCH (Table 2), reflecting exhaustion of the adaptive resources of the liver. This is also confirmed by a decrease in the value of ICA for PA, CL, PCH, and PS (7.6, 9.2, 16.2, and 41.3% compared with 21.1, 76.3, and 88.1% respectively*), a decrease in CCP for PA, CL, and PCH (1.03, 0.86, 0.87 instead of 1.04, 1.03, 0.93), and in the M level of total PL compared with its values in hamsters of group 2 (696 ± 24 compared with 1070 ± 12, $P < 0.01$), although a tendency was found for the rhythm of total PL to return to normal. Histochemically, the maximum and minimum of the lipid content did not coincide with the normal state (Fig. 1g, h), although the distribution of the lipids was comparable. The high value of ICA for SM and LL (57.2 and 79.7% compared with 35.5 and 27.4%) and of CCP for PEA, PS, SM, and LL (1.13, 1.2, 1.53, and 1.54 compared with 1.01, 1.14, 0.93, and 1.13) in the animals of group 5 was evidently compensatory and adaptive in character. Fluctuations in the PL content after dehelminthization differed from those in intact animals of comparable age (group 2) and also with respect to CSF values. For instance, at 9 a.m.: PA–CL = 0.9 ($P < 0.1$), SM–LL = 0.96, PEA–SM = –0.66 ($P < 0.05$), at 3 p.m.: PA–PCH = 0.7, CL–PCH = 0.67, PS–SM = 0.68 ($P < 0.05$), at 9 p.m. PS–SM = 0.65 ($P < 0.01$), and at 3 a.m.: PA–PCH = 0.7, CL–PCH = 0.69, CL–SM = 0.68 ($P < 0.05$). The results of analysis of the circadian rhythm of PL in the hamsters of group 5 suggested that resistance of the host–parasite system is connected not only with antigenic [7] but also with membrane mimicry of the hepatocytes.

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