COORDINATION OF PHASES OF SOLAR ACTIVITY AND OF MONTHLY RHYTHMS OF BLOOD SERUM CHOLESTEROL LEVELS IN RABBITS

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Changes in the serum cholesterol concentration constitute a complex oscillatory process [14]. Besides circadian fluctuations [9], distinct seasonal fluctuations of this parameter also are observed [10, 11]. Natural fluctuations of the blood cholesterol concentration in experimental animals make interpretation of the data difficult when the influence of various factors on cholesterol metabolism is investigated, for there is as yet no substantiated approach to the prediction of oscillations of the blood cholesterol level during the course of a chronic experiment. On the basis of data of helio-biology [12] we postulated that such prediction is possible if correlation can be established between the phases of the monthly cholesterol biorhythms and monthly rhythms of change of a concrete parameter of solar activity. However, because of the difficulty of conducting long-term biological investigations and because of the absence of any single mathematical approach, parameters of the basic cholesterol biorhythms of varied frequency have not yet been discovered, nor has their possible correlation between rhythms of solar activity been established. The solution to this problem is essential for the creation of a reliable dynamic background against which it will be possible to judge changes in the blood cholesterol level of rabbits in model investigations to study the pathogenesis of atherosclerosis.

This paper describes a first attempt at mathematical determination of individual differences and group patterns of temporal organization of serum cholesterol biorhythms with periods of 1 month and many months in healthy rabbits and rhythms of heliogeophysical parameters with similar frequency.

EXPERIMENTAL METHOD

The investigation was carried out in two stages with the phase of a "minimum" of the 11-year cycle of solar activity as the background for comparison. Experiments were carried out on 13 male Chinchilla rabbits weighing 3.8-4.2 kg. The animals were kept under standard animal house conditions (temperature 18-20°C, daily pellet diet with water ad libitum + 100 g of fresh cabbage). Blood was taken in a volume of 0.5-0.8 ml from the marginal vein of the ear at 9 a.m. before feeding, and ensuring that the time intervals between blood sampling were equal. In the first stage of the investigation (from September 6, 1973 to January 10, 1974) eight rabbits were studied. To detect low-frequency biorhythms (seasonal and monthly) blood from these animals was taken every 3 days for 127 days. In the course of an experiment lasting several months, blood was taken daily from these same animals for 15 days (from September 6 to September 21) to detect any weekly cholesterol biorhythms. Total, esterified, and free cholesterol levels were determined in the blood serum by the method in [5]. Parallel with the biological experiment, the dynamics of the external environmental factors were studied: Wolf (sunspot) numbers, A_p index (mean diurnal amplitude of variation of the earth's magnetic field), the solar radioemission at a wavelength corresponding to 2800 MHz, atmospheric pressure, relative humidity, and air temperature in Moscow.

In the second stage (December 14, 1984 to April 9, 1985) five male rabbits of the same weight were investigated every 3 days in accordance with the same experimental scheme. Parallel with the taking of blood samples, heliogeophysical and climatic factors were studied. Individual series of cholesterol levels and also the analogous temporal series of heliogeo-

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TABLE 1. Hierarchy of Biorhythms of Blood Serum Cholesterol of Intact Rabbits and Rhythms of Heliogeophysical and Meteorological Factors (September 6, 1973 to January 10, 1974), $\bar{M} \pm 2\,m$

Rhythm	Parameter of rhythm	Total cholesterol, mmoles/liter	Free cholesterol, mmoles/liter	Bound cholesterol, mmoles/liter	Wolf number, conventional units	Radioemission at 2800 MHz, 10^{-22} W/m² Hz	AP index, nT	Atmospheric pressure, gPa	Temperature, °C	Relative humidity, %	Wind velocity, m/sec
	C	1,26±0,08	0,52±0,02	0,73±0,07	32,8±2,3	87,6±1,24	14,16±0,97	991±10	2,52±0,51	82,5±0,9	2,39±0,12
1/3 year	T	120,1±1,8	119,7±1,6	118,6±2,2	121,8±1,2	120,0±1,9	119,9±3,5	119,5±1,8	120,0±2,2	119,0±1,3	119,0
	A	0,43±0,14	0,10±0,03	0,35±0,11	10,5±0,9	4,78±0,15	3,18±0,07	8,5±0,2	1,19±0,03	2,8±0,1	0,45
	φ	1±38	8±64	3±35	353±4	338±2	114±4	80±5	356±3	258±4	244
1/4 year	T	93,2±2,7	94,6±8,1	93,8±3,1	90,6±1,1	90,1±1,1	87,0	91,4±2,3	90,1±1,00	90,4±2,6	95,0
	A	0,51±0,17	0,10±0,03	0,44±0,14	14,0±2,2	6,16±0,05	2,46	7,7±0,2	2,63±0,40	4,3±0,3	0,44
	φ	46±18	56±28	49±18	13±7	27±7	150	112,3±3,0	61±8	259±4	281
10 weeks	T	72,8±0,9	73,4±0,9	72,3±0,2	72,5±0,1	72,2±1,4		73,0±2,1	70,0±1,3	69,0±1,8	n/d
	A	0,58±0,18	0,10±0,03	0,48±0,12	8,4±0,4	5,05±0,05	n/d	6,5±0,2	1,17±0,09	7,2±0,3	
	φ	122±18	120±15	136±13	28±8	44±2		121±1	114±5	305±6	
2 months	T	59,0±0,4	59,3±2,2	58,4±2,2	59,6±0,2	58,7±1,0			58,9±2,4	58,0±0,9	n/d
	A	0,39±0,10	0,10±0,03	0,31±0,09	4,9±0,5	5,40±0,25	n/d	n/d	0,85±0,05	5,5±0,2	
	φ	207±14	172±16	212±16	75±8	29±1			170±18	330±5	
1,5 months	T	48,2±1,3	47,5±2,4	47,6±3,5	48,0±0,7	48,5±0,7	n/d	47,8±1,1	47,8±0,7	n/d	48,5
	A	0,37±0,09	0,09±0,02	0,27±0,09	4,0±0,1	3,35±0,09		5,1±0,4	2,93±0,14		0,31
	φ	269±26	266±33	314±59	338±11	47±4		132±5	109±13		266
5 weeks	T	36,4±2,6	38,0±2,4	35,8±3,7	36,1	n/d	n/d	36,3±0,5	35,2±0,6	40,0±1,0	n/d
	A	0,28±0,08	0,09±0,02	0,22±0,09	3,7			6,8±0,3	1,48±0,13	4,3±0,1	
	φ	14±32	359±57	328±21	288			216±7	337±2	65±8	
1 month	A	27,9±0,8	26,9±0,3	28,5±0,3	28,5±0,3	28,3±0,4	29,1±0,4	29,7±0,4	28,6±0,3	n/d	n/d
	T	0,28±0,03	0,07±0,01	0,24±0,05	22,6±0,3	2,24±0,10	3,20±0,24	5,0±0,3	1,33±0,06		
	φ	86±14	43±48	66±20	315±4	355±15	257±22	293±7	354±7	n/d	
2 weeks	T	14,2±0,3	14,6±0,4	13,8±1,1	13,7±0,1	13,8±0,1	13,1±0,1	13,3±0,1	14,1±0,2	14,6±0,1	14,3
	A	0,22±0,08	0,07±0,02	0,15±0,02	10,2±0,6	2,63±0,11	5,33±0,32	3,7±0,3	1,12±0,05	3,6±0,5	0,45
	φ	158±18	141±16	158±24	338±9	321±7	71±8	198±20	333±15	241±12	212
1,5 weeks	T	10,3±0,3	10,1±0,7	9,8±0,6	10,8±0,1	10,8±0,2		10,5±0,2	10,4±0,1	10,3±0,2	n/d
	A	0,19±0,06	0,06±0,01	0,14±0,03	5,6±0,2	2,27±0,41	n/d	2,9±0,1	1,19±0,05	4,1±0,2	
	φ	321±18	174±51	340±61	293±8	326±23		11±23	231±15	276±10	
1 week	T	7,0±0,2	7,2±0,5	7,1±0,2	7,3±0,1	n/d	6,9±0,4	6,6±0,1	7,1±0,1	7,4±0,1	7,5
	A	0,25±0,04	0,06±0,01	0,20±0,06	4,5±0,2		2,87±0,06	3,7±0,2	0,85±0,13	2,4±0,2	0,30
	φ	179±27	346±40	125±2	228±9		220±18	354±17	289±58	274±28	160

Legend. 0) Mean statistical value of parameter in absolute units; T) duration of period in days; A) amplitude of oscillations in absolute units; φ) angle of shift of cosinusoid on September 6, 1973, in deg.; n/d) rhythm not detected.

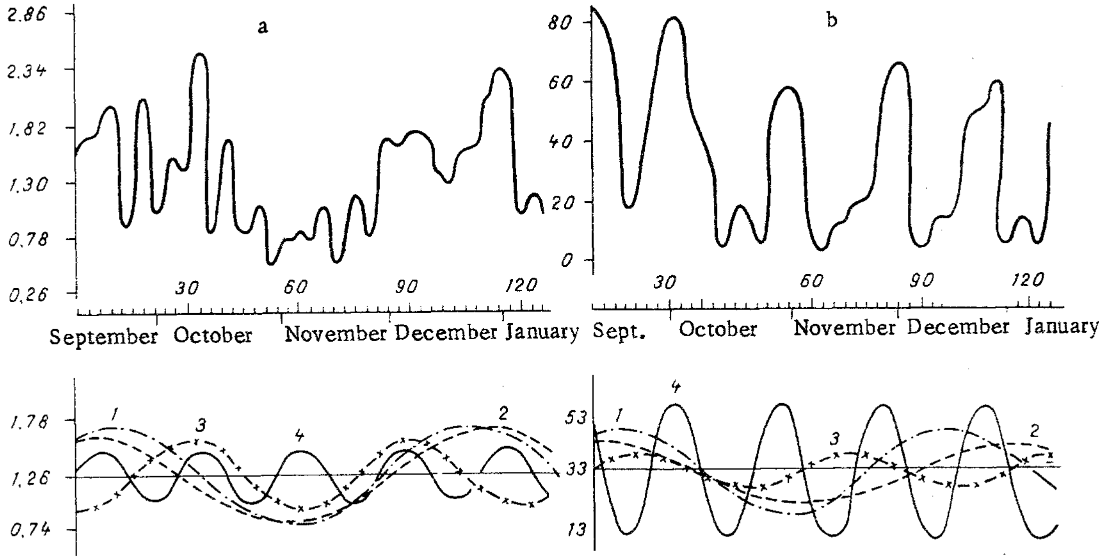

Fig. 1. Natural time course (above) and hierarchy of isoalted rhythms (below) of total serum cholesterol concentration of a rabbit (a) and Wolf numbers (b): 1) 4-month rhythm; 2) 3-month rhythm (seasonal); 3) 2-month rhythm; 4) monthly rhythm. Abscissa, calendar time of recording parameters (in days and months); ordinate, absolute values of parameters; on left — total cholesterol (in mmoles/liter), on right — Wolf numbers.

physical factors (corresponding to the year, month, day, and hour of taking blood from the rabbits) were analyzed by an original mathematical method [8], using a special program for the EC computer. Only rhythms differing from "noise" with a significance of over 95% were taken into account.

EXPERIMENTAL RESULTS

The time course of any oscillatory process may be the result of summation of a corresponding set of sinusoidal curves; the individual sinusoidal curves, moreover, may give more information for the elucidation of the process than the resultant summation curve, characterizing the integral dynamics of the parameter studied [4]. Accordingly, in the mathematical approach used, the aim was to discover the largest possible set of latent biological rhythms, most frequently not multiples of each other, with hitherto unknown periods. This approach differs in principle from those usually adopted in order to discover biorhythms [7].

On the basis of Fourier's theorem, and adding to it a complex procedure of "searching" for individual lengths of periods, phases, and amplitudes of rhythms with assessment of the probability (p) that they belong to the "noise" category, sets of individual cosinusoidal oscillations ($p < 0.05$), each of which is described by the familiar equation

$$X_t = G + A\cdot\cos\left(\frac{2\pi\cdot t}{T} - \varphi\right),$$

[in which X_t denotes the value of the parameter on particular days of the investigation; G the mean statistical value of the parameter; A the amplitude of the oscillations (in absolute units); T the period of the oscillations (in days); t the number of days from the beginning of the investigation; φ the phase (the angle of shift or position of the first maximum on the cosinusoid)], were isolated from the natural time course of the serum cholesterol concentration of each rabbit and from the natural time course of each external environmental factor.

After summation of the set of cosinusoids, individual for each animal and each heliogeophysical factor, theoretical curves were obtained and these were subsequently compared by the stepwise regression method with the original curves. In all cases a high degree of correlation was obtained between the theoretical and experimental curves, indicating that the set of latent rhythms constituting the dynamics of the process under study was sufficiently complete.

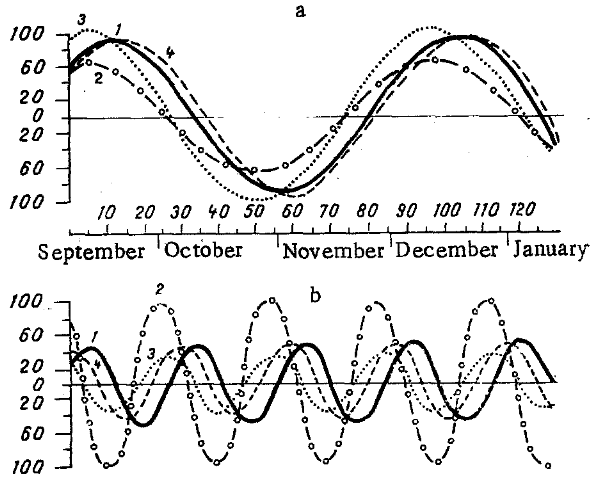

Fig. 2. Phase coordination of seasonal (a) and monthly (b) total serum cholesterol biorhythms of healthy rabbits and rhythms of some external environmental parameters (September 6, 1973 to January 10, 1974). 1) Total cholesterol; 2) Wolf numbers; 3) solar radioemission at a wavelength corresponding to 2800 MHz; 4) air temperature in Moscow. Abscissa, calender time of experiment (in days and months); ordinate, conventional amplitude of oscillations (in % of maximal amplitude of rhythms discovered for each parameter).

To obtain a clear picture of the character of the individual time course of the total serum cholesterol of the rabbits and parameters of solar activity, experimental curves and diagrams of phase relations and amplitudes of significant rhythms, isolated from the natural time course of the parameters, are given in Fig. 1.

It will be clear from Fig. 1 that the time course of total cholesterol and that of Wolf numbers over a period of several months consists of the sum of several cosinusoids. However, the harmonics given in Fig. 1 do not constitute the whole of the "ensemble" of cholesterol biorhythms revealed. To determine the general principles governing fluctuations in the concentration of total, free, and bound cholesterol in the rabbits blood, summation of the individual parameters of the homonymous rhythms in all the animals investigated was carried out (Table 1).

As Table 1 shows, in the time course of cholesterol over a period of many months 10 dimensionalities of biorhythms were discovered (the same number of rhythms also was found in the simultaneously recorded time course of Wolf numbers). The statistical homogeneity of the duration of the periods and the matching of the phases of the monofrequency biorhythms and heliorhythms will be noted with the exception of the 2-month rhythm and that with a period of under three weeks.

Comparison of the parameters of the biorhythms of the different forms of cholesterol shows that all low-frequency biorhythms of total, free, and bound cholesterol were in phase, but the phase of the weekly biorhythms of free and bound cholesterol did not coincide. This may indicate that it is within these dimensionalities of biorhythms that transition processes and self-adjustments of phases of oscillations of free and bound cholesterol take place in response to regulatory influences of external factors, including pathogenetic influence affecting one or other form of cholesterol metabolism.

Of all the biorhythms discovered (Table 1), rhythms with a period of several months were found to be the most important (they had the highest amplitudes and were discovered in all animals). Monthly biorhythms of free cholesterol were found in only half of the animals studied. Despite the great amplitude of the monthly rhythm of Wolf numbers and of the solar radioemission at a wavelength corresponding to 2800 MHz, the amplitude of the monthly cholesterol biorhythms was comparatively low. Rhythms with a period of several months, which together give maxima of the increase in total cholesterol concentration at the end of September or beginning of October, and at the end of November or beginning of December, and a minimum at the end of October or beginning of November, are thus the most characteristic for free cholesterol and its esters.

In the experiment conducted in 1984-1985 to test the stability of some general principles governing cholesterol biorhythms recorded in 1973-1974, it was found that the duration of the periods of the whole hierarchy of rhythms and also the phase of the low-frequency cholestrol biorhythms were unchanged after 11 years. However, the amplitude of the monthly biorhythms of total cholesterol in 1984 was doubled compared with the amplitude in 1973 against the background of a threefold decrease in amplitude of monthly oscillations of Wolf numbers.

The statistical identity of the duration of the periods and phases of the low-frequency cholesterol biorhythms and rhythms of change of heliogeophysical factors (Table 1) may provide further indirect confirmation of the theoretical concept that biorhythms are linked with rhythmic changes in solar activity [12], the information function of natural electromagnetic fields [6] and, finally, the electromagnetic nature of biological clocks and of cellular differentiation, so that the living organism can respond with resonance to a change in the frequency and intensity of external electromagnetic fields of the biosphere [1, 2].

It is from this standpoint that the possibility of a particularly important role of cholesterol in the mechanism of transmission and assimilation of radiant information by living cells, connected with the ability of cholesterol (cholesteric) esters to exhibit the properties of liquid crystals. In a cholesteric liquid crystal the directions of the long axes of the molecules are known to form a three-dimensional spiral with a step (period) corresponding to the range of wavelengths from optical to infrared radiation [3], and for that reason the structures of living cells containing liquid cholesterol crystals may react precisely to weak electromagnetic (including temperature) influences.

The cholesterol biorhythms described in this paper relate to intact animals, but it follows from other publications [13, 14] that monthly biorhythms of cholesterol during hypercholesterolemia differ from "normal" only in the sharply rising amplitude of the oscillations around the mean level. An increase in amplitude of the oscillations of total serum cholesterol of patients with atherosclerosis compared with that of healthy subjects also is mentioned in [10] and, what is particularly important, the stability of the phase of the annual fluctuation of the blood cholesterol level of patients and healthy subjects irrespective of the geographic location where they live on the planet and the seasonal variations in diet, is pointed out. This state of affairs emphasizes once again the regulatory role of a planet-wide factor, acting on an organism at any point on earth (for example, seasonal and monthly changes in solar activity).

The effect of solar activity on cholesterol biorhythms is illustrated in Fig. 2 by a model of phase coordination observed between seasonal and monthly cholesterol biorhythms and rhythms of external environmental parameters. It will be clear from Fig. 2 that the presence of correlation between biorhythms for different forms of serum cholesterol with one another and with the heliorhythms can be assessed not only on the basis of coincidence of frequencies of the hierarchy of biorhythms discovered, but also and to an even greater degree, on the basis of stability of phase coordination of heliorhythms and biorhythms. Because of correlation between the phases of the biorhythms for all forms of cholesterol studied, it is possible to judge the shape of the time course of serum levels of free and esterified cholesterol in normal rabbits on the basis of the dynamics of the total cholesterol concentration. Stability of the phase coordination of seasonal cholesterol biorhythms and seasonal rhythms of solar activity indicate the possibility of long-term prediction of the tendency of the change in the serum cholesterol concentration in normal rabbits can be undertaken in relation to the phase of oscillations of Wolf numbers.

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EFFECT OF ADRENALIN ON INHIBITION OF MITOSIS BY A CHALONE IN EHRLICH'S ASCITES CARCINOMA

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In 1964 Bullough and Laurence [7] postulated that cell proliferation is under the control of a chalone-adrenalin complex. This suggestion was confirmed by data obtained during the study of the action of a chalone-containing preparation from vertebrate skin on cell division in the epidermis [8, 9]. It was also shown that hepatic chalone is activated by adrenalin in vitro [13]. At the same time, granulocytic, erythrocytic [12], lymphocytic [10], and even epidermal [11] chalones are effective even in the absence of adrenalin.

The study of the kinetic parameters of cell proliferation under the combined influence of adrenalin and chalone showed that interaction between these substances is expressed not only as weakening or strengthening of chalone effects, but also as a change in the duration of action of chalones [3, 4].

In the investigation described below the effect of adrenalin on inhibition of mitosis by a chalone-containing preparation (CCP) isolated from Ehrlich's ascites carcinoma (EAC) was studied with respect to the time interval between administration of these substances.

EXPERIMENTAL METHOD

Experiments were carried out on 350 noninbred male albino mice weighing 18-20 g. The animals were kept on a daily schedule of 12 h daylight (6 a.m. to 6 p.m.) and 12 h of darkness, at a temperature of 18°C, and received food and libitum. All the animals were inoculated intraperitoneally with a diploid strain of EAC (the tumor was obtained from the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR). The tumor was used on the 5th day of its development (in the exponential phase of growth). The CCP was obtained from a 13-day tumor by alcoholic fractionation, which was first used for purification of chalones by Bullough et al. [6], in the modification of Savchenko et al. [5]. The lyophilized CCP was dissolved in physiological saline and injected in a dose of 15 mg per animal in a volume of 0.5 ml. The 0.1% adrenalin solution was diluted with physiological

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