CONTENT OF T AND B LYMPHOCYTES IN HEALTHY HUMAN PERIPHERAL BLOOD

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It was shown by the rosette-formation method that the number of T and B lymphocytes in human blood varies with the time of day and season of the year. The number of T cells reaches a maximum in the morning and the number of B cells in the evening. The relative percentage of B cells is higher in the fall than in winter.

KEY WORDS: Human lymphocytes; diurnal and seasonal rhythms.

The intensity of function of many physiological systems including the immune system, shows diurnal and seasonal fluctuations [2-7].

The object of this investigation was to study whether a diurnal and seasonal rhythm exists in the relative percentage of T and B lymphocytes in human peripheral blood.

EXPERIMENTAL METHOD

Tests were carried out on 24 male volunteers aged between 18 and 21 years. Venous blood was taken 6 times during the 24-h period (at 3, 7, and 11 a.m. and 3, 7, and 11 p.m.). Altogether three series of experiments were carried out at different seasons (summer, fall, and winter).

TABLE 1. Diurnal and Seasonal Rhythms of Relative Percentage of T Lymphocytes in Healthy Human Peripheral Blood (M ± m)

Time of day or night	T-lymphocytes			Significance of seasonal differences	
	summer (Jun.)	fall (Sep.)	winter (Nov.)	groups compared	P
11 a.m.	57,6±1,35 (15)	71,1±1,29 (14)	60,2±1,45 (10)	summer–fall	<0,001
				fall–winter	<0,001
3 p.m.	54,6±1,58 (15)	69,8±1,36 (15)	58,0±1,38 (10)	summer–fall	<0,001
				fall–winter	<0,001
7 p.m.	46,3±3,03 (15)	60,3±1,26 (14)	48,6±3,36 (6)	summer–fall	<0,001
				fall-winter	<0,01
11 p.m.	46,2±1,96 (15)	66,4±1,42 (15)	48,3±1,52 (9)	summer–fall	<0,001
				fall–winter	<0,001
3 a.m.	54,5±1,39 (14)	65,2±3,42 (14)	56,7±0,88 (9)	summer–fall	<0,01
				fall–winter	<0,05
7 a.m.	54,1±1,30 (14)	71,4±1,55 (15)	59,4±0,96 (9)	summer–fall	<0,001
				fall–winter	<0,001

	Summer	Fall	Winter
$P_{11a.m.-7p.m.}$ h	<0,01	<0,001	<0,01
$P_{11p.m.-7a.m.}$ h	<0,01	<0,05	<0,001

Legend. Here and in Table 2 number of observations shown in parentheses.

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TABLE 2. Diurnal and Seasonal Rhythms of Relative Percentage of B Lymphocytes in Healthy Human Peripheral Blood (M ± m)

Time of day or night	B lymphocytes		P
	fall (Sep.)	winter (Nov.)	
11 a.m.	17,2±0,14 (15)	15,0±0,65 (10)	<0,01
3 p.m.	17,9±0,89 (15)	13,3±0,80 (10)	<0,001
7 p.m.	27,8±1,36 (14)	19,6±1,70 (6)	<0,01
11 p.m.	26,8±1,34 (15)	19,6±0,99 (9)	<0,001
3 a.m.	12,0±1,07 (14)	11,7±0,82 (9)	>0,05
7 a.m.	17,3±0,58 (15)	14,9±0,51 (9)	<0,01
		Fall	Winter
$P_{3p.m.-11p.m.}$ h		<0,001	<0,001
$P_{7p.m.-9a.m.}$ h		>0,05	>0,05
$P_{3a.m.-7a.m.}$ h		<0,001	<0,01

T lymphocytes were identified by their ability to form spontaneous rosettes with sheep's red cells [12, 15]. The method described by Lay et al. [17] was used. Lymphocytes of the B class were identified by the presence of receptors on their surface for the C_3 component of complement [8, 12]. For this purpose, sheep's red cells treated with subagglutinating doses of rabbit antiserum against sheep's red cells (hemolytic serum from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow) and with guinea pig complement were used. The red cells treated in this way, when mixed with lymphocytes carrying receptors for the C_3 component of complement on their surface, form rosettes [18]. The rosette-forming lymphocytes were identified in suspension under the phase-contrast microscope. Altogether 200 lymphocytes were counted: A lymphocyte with three or more adherent sheep's red cells was taken to be a rosette-forming cell.

EXPERIMENTAL RESULTS

The number of T and B lymphocytes in the peripheral blood were shown to vary during the 24-h period. The number of T cells reached a maximum in the morning and a minimum in the evening (Table 1). Conversely, the percentage of B lymphocytes reached the maximum in the evening, fall sharply at 3 a.m., and then (by 7 a.m.) increased a little and remained at about the same level during the daytime (Table 2).

The relative percentage of T lymphocytes also varied with the season of the year. The character of the diurnal fluctuation in the percentage of T lymphocytes did not change significantly at the different seasons (Table 1). The number of B lymphocytes was greater in the fall than in winter (Table 2).

Diurnal and seasonal rhythms in the relative percentages of T and B lymphocytes in healthy human peripheral blood were thus demonstrated; these rhythms are probably connected with diurnal and seasonal fluctuations in the synthesis of adrenocortical hormones [1, 9, 14, 16], which are known to have a considerable influence on the immune system [13]. For example, if added in vitro, glucocorticosteroids destroy immature lymphocytes and modify the mitotic and migratory activity of immunocytes [10, 11]. The importance of the fluctuations in the numbers of the subpopulations of lymphocytes circulating in the blood stream for the function of the immune system requires further investigation. However, they must be taken into account whenever human peripheral blood lymphocytes are studied.

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MECHANISM OF REVERSIBILITY OF COLCEMID-INDUCED COLCHICINE MITOSIS

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Restoration of the normal course of mitosis after its blocking by colchicine takes place through additional protein synthesis – through the formation of new microtubules of the division spindle. A study of the reversibility of the stathmokinetic reaction to colcemid shows that blocking of protein synthesis by puromycin has little or no effect on the rate of restoration of the normal mitotic regime, whereas copper ions retard this process. It can be concluded from these facts that the restoration of the colcemid-induced C mitosis takes place chiefly through the repolymerization of tubulins. The predominance of the formation of new microtubules or repolymerization during the restoration of the microtubules is evidently determined both by the degree of their destruction and by the character of binding of the subunits of the tubular protein pool with the harmful agent.

KEY WORDS: Mitosis; microtubules; tubulins; stathmokinetic reaction; colcemid.

In a previous communication [2] we examined the mechanism of reversibility of the colchicine mitosis (C mitosis) induced by colchicine. Electron-microscopic and autoradiographic investigations showed that for the normal course of mitosis to be restored after treatment with colchicine additional protein synthesis is required [1, 2]. The peak of intensity of protein synthesis preceded complete restoration of the normal mitotic regime and corresponded in time to the period of formation of microtubules of the division spindle and reconstruction of the mitotic apparatus. It was not made clear whether the formation of new microtubules is the universal mechanism of reversibility of the C mitosis or whether this process can also take place through the repolymerization of microtubules from the tubulin pool [3, 8, 12, 13].

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