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SEASONAL CHANGES IN INTRALYSOSOMAL pH IN HEALTHY HUMAN PERIPHERAL BLOOD CELLS

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An important factor influencing the natural resistance of the body is seasonal changes in geophysical parameters. A seasonal rhythm of immune processes, influencing the time course of several diseases, has now been established [3]. An important contribution to the level of nonspecific resistance is due to the lysosomal bactericidal systems of the blood leukocytes and, in particular, of neutrophils and monocytes. A certain level of average pH of the granule population of these cells, being a necessary condition for the normal functioning of lysosomal enzyme systems, also may evidently vary in the course of the year. In this paper the terms "lysosome" and "granule" will be regarded as synonyms.

The aim of the investigation was to test the hypothesis relating to seasonal changes in pH of granules of leukocytes and platelets of healthy blood donors.

EXPERIMENTAL METHOD

Peripheral blood from 49 male and female blood donors aged from 18 to 38 years was studied. Blood was taken from a vein between 11 a.m. and noon in a volume of 10 ml into plastic tubes containing heparin (100 U) in 0.5 ml of physiological saline. Measurements were made 2-3 h after natural separation of the blood into fractions. Neutral red (NR), a vital stain accumulating in structures with acid pH and enabling lysosomes to be detected and their pH measured, was used as the pH-indicator [1].

The intralysosomal pH (pH_L) was measured after staining of the cells with NR by a microspectrophotometric method on the Univar instrument ("Reichert," Austria). Buffy coat (0.02 ml), isolated from the blood, was mixed on a slide with an equal volume of 0.025% NR, and incubated for 15 min, after which the preparation was covered with a coverslip, and the pH measured in each sample in 15-40 granules of neutrophils and in 5-10 granules of lymphocytes, monocytes, eosinophiis, an platelets. Incidentally, on contact of the blood cells with the surface of the slide, a so-called oxidative burst took place, so that the lysosomal pH measured by this method evidently reflected this metabolic state of the cells. On statistical analysis the mean value

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TABLE 1. Mean Annual Values of pH of Granules of Individual Cells of Human Peripheral Blood

Cells	Number of granules measured	Number of donors	$M \pm m$
Neutrophil (1)	1007	48	6,55±0,03
Eosinophil (2)	100	26	6,73±0,15
Monocyte (3)	84	24	6,83±0,10
Lymphocyte (4)	455	44	6,43±0,04
Platelet (5)	248	34	6,18±0,09

Legend. The following differences are significant: 1-3, 1-4, 1-5, 2-5, 3-4, 3-5, 4-5.

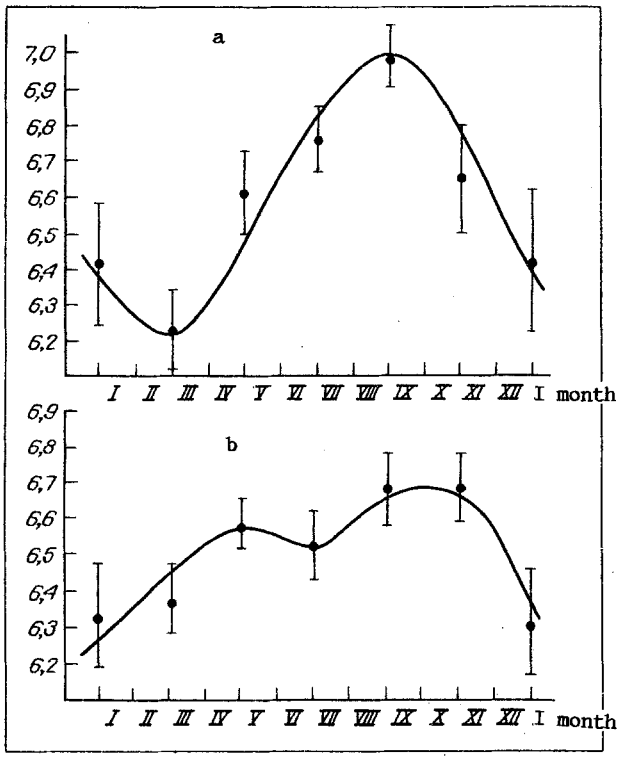

Fig. 1. Dependence of pH of leukocytic granules from donors on season of the year: neutrophils (a) and lymphocytes (b).

of pH of the granules of each type of cell in a given sample (pH_L) and the mean pH of the granules over a certain period of the year were calculated. Preliminary analysis showed that values of pH_L measured in neighboring months (February-March, April-May, and so on) showed virtually no difference, so that these values for neutrophiis and lymphocytes and the mean annual value (pH_L) for each type of cells could be pooled. The mean values were compared by Student's test, with a $p < 0.05$ level of significance.

EXPERIMENTAL RESULTS

The mean annual values of pH of the granules from peripheral blood lymphocytes and platelets from healthy donors, measured by the method described above, are given in Table 1 (no sex differences were found).

The highest value of pH_L was found for monocytes and eosinophiis, the lowest for platelets.

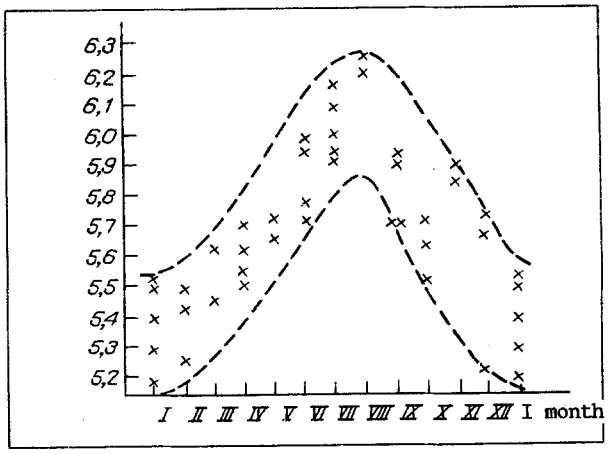

Fig. 2. pH of granules of hog embryonic kidney cells in culture

The values which we obtained for pH_L were significantly higher than pH_L of other types of cells, also measured with the aid of NR. This difference can be explained, on the one hand, by the specific features of lysosomes of blood cells, and on the other hand, by differences in the functions of the blood cells during the oxidative burst, as a result of which a change takes place in the intracellular pH [3], and may be accompanied by an increase in pH_L.

Investigation of the trend of pH_L of the donors' neutrophils showed that the mean values measured at different times of the year were different (Fig. 1a): the highest values were observed in August-September (6.98 ± 0.08), the lowest in February-March (6.23 ± 0.13, $p < 0.001$). In relation to lymphocytes, no marked rhythm could be observed, but in August-November pH_L was significantly higher (6.66 ± 0.06) than in December-March (6.34 ± 0.1, $p < 0.01$, Fig. 1b).

Thus in summer and the early fall higher values were obtained for the intralysosomal pH of the donors' peripheral blood neutrophils and lymphocytes. We showed previously that if conserved blood is stored, pH of the granules of the neutrophiis, measured by this same method (by neutral red on slides) fell, and this was accompanied by impairment of the phagocytic, chemotaxic, and bactericidal properties of the neutrophiis [4]. It evidently tot lows from this that higher values of pH of the granules correspond to a better functional state of the cells. Consequently, it can be tentatively suggested that the contribution of neutrophiis to the level of nonspecific resistance of healthy persons is greater in summer and the early fall than in winter and the beginning of spring.

Seasonal changes in lysosomal pH also are characteristic of cells in culture, for example, for hog embryonic kidney cells (HEKC) [2]. The highest lysosomal pH values in these cells also were recorded in the summer months (Fig. 2). The possibility cannot be ruled out that the seasonal dynamics of pH of granules in different types of animal cells is perhaps a reflection of an adaptive, phylogenetically determined response to a distinct geophysical rhythm.

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