Analysis of the results thus shows that of all the components of blood, Fe^{++}, Cu^{++}, hemoglobin, and phospholipids lead to intensification of LPO of the vitreous body, both when injected into the eye and when added directly to the isolated vitreous body. Hemoglobin and Fe^{++} act most rapidly on LPO reactions. The results are evidence that the first maximum of intensity of LPO which was observed is connected with the action of hemoglobin and Fe^{++}. Comparison of the kinetics of LPO after injection of autologous blood with its kinetics after injection of an emulsion of blood phospholipids, both *in vivo* and in the model, leads to the conclusion that the appearance of the second maximum is probably associated with oxidation of the lipids of autologous blood.

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USE OF PARAMETERS OF LIPID PEROXIDATION ACTIVITY TO STUDY HUMAN ADAPTATION TO NEW CLIMATIC AND GEOGRAPHIC CONDITIONS

V. N. Ushkalova and G. D. Kodachnikova

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KEY WORDS: lipids; peroxidation; adaptation

During adaptation to stress situations, including to new climatic and geographic conditions, various changes of a systemic character take place in the body, and are manifested as changes in various physiological and biochemical parameters. At the whole-body and cellular levels, this phenomenon is considered to be based on changes in activity of lipid peroxidation (LPO) of biological membranes. A promising way of studying the process of adaptation is accordingly to study parameters characterizing LPO activity [2, 3, 5, 10].

This paper gives the results of a study of the concentrations of LPO products in lipids of blood erythrocytes obtained from workers in the southern districts of the USSR in the course of their adaptation to conditions of life in Western Siberia.

EXPERIMENTAL METHOD

Altogether 250 clinically healthy individuals aged from 20 to 50 years were investigated during the period of adaptation from the 1st day until 40-50 weeks. The control group consisted of 50 local inhabitants of the corresponding age groups.

The optical density of solutions of lipid extracts, with absorption at 232 nm, is known to characterize the concentration of conjugated dienes, including diene perixides, whereas absorption at 268 nm is considered to be due to absorption of conjugated trienes [9]. Venous blood was taken by the standard method [1]. The analysis was carried out by the writers' modification of Placer's method [7]. Central blood, in a volume of 0.9 ml, was stabilized with 0.01 ml of a 3.8% solution of sodium citrate and centrifuged for 10 min at 3000 rpm. The plasma was removed and the pellet was washed twice in a 1 mm tube with 0.9% solution of NaCl and was centrifuged twice for at 3000 rpm for 5 min. The supernatant was discarded and 0.3 ml of erythrocyte suspension (ES) was diluted with phosphate buffer (pH 7.4) in the ratio of 1 : 33, and 1 ml of its was transferred into a tube with ground glass stopper, mixed with 0.9 ml of a mixture of heptane and isopropyl alcohol

Department of Organic Chemistry, Tymen' Medical Institute.

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TABLE 1. Changes in Blood LPO Parameters Depending on Age During Adaptation for 1 Year (M ± m)

Group of animals	Optical density					
	1 ml ES		per milligram total lipids in 1 ml of ES		not allowing for dilution	
	D_{232}	D_{268}	D_{232}	D_{268}	D_{232}	D_{268}
Local inhabitants (control)	45,51±20,81	21,01±19,72	11,35±5,20	5,25±4,93	0,14±0,06	0,06±0,05
Immigrants: Of all ages	225,92±43,85	39,04±7,95	56,48±10,96	9,76±1,99	0,68±0,13	0,12±0,02
Aged 20-25 years	182,11±47,90	31,62±9,39	45,53±11,97	7,90±2,35	0,55±0,14	0,09±0,03
Aged 26-30 years	195,89±56,83	31,68±7,21	48,97±14,21	7,92±1,80	0,59±0,17	0,09±0,02
Aged 31-35 years	287,00±51,71	50,58±13,36	71,75±12,92	12,64±3,34	0,86±0,15	0,15±0,04
Aged 36-40 years	238,73±85,72	42,27±9,36	59,68±21,43	10,56±2,34	0,72±0,26	0,13±0,03

(1: 1 by volume), vigorously shaken for 1 min, centrifuged for 5 min at 3000 rpm, and kept for 1 min, centrifuged for 5 min at 3000 rpm, and kept for 1 min to allow separation into phases. Next, 3 ml of the top heptane layer was transferred into another tube and optical density was measured at 232 and 268 nm.

In the present investigation, unlike in others [4, 6], optical density at 232 and 268 nm was calculated per ml of ES by equations 1 and 2 (see below) and was calculated per milligram of total lipids in 1 ml of ES. The concentration of total lipids was determined by a gravimetric method in the heptane layer after extraction by the binary solvent. The yield of lipids was 4 ± 1 mg/ml ES in all groups of subjects.

$$\frac{D_{232}}{V} = D_{232} \cdot K_d, \tag{1}$$

$$\frac{D_{268}}{V} = D_{268} \cdot K_d, \tag{2}$$

where K_d is the coefficient of dilution, namely 333, and V is the volume of ES. The results were subjected to statistical analysis by the method in [8].

EXPERIMENTAL RESULTS

Adaptation is associated with a raised level of LPO products (Table 1). The concentration of conjugated dienes of the immigrants, irrespective of the time of adaptation, was five times higher than in the local inhabitants, whereas the concentration of conjugated trienes was twice as high.

For comparison, the optical density at 232 and 268 nm of the lipid extract, without allowing for dilution of the original ES, as was done by other workers [4, 6], is given in Table 1.

The study of changes in the topical density of the lipid extracts at 232 and 268 nm, depending on the time of adaptation, showed that it was phasic in character: periods of acute and chronic adaptation were present (Fig. 1). During the first 5-8 days of the subjects'

Fig. 1. Changes in optical density of lipid extracts depending on times of adaptation. 1) D_{232}; 2) D_{268}. Here and in Figs. 2 and 3, optical density calculated per millileter of ES.

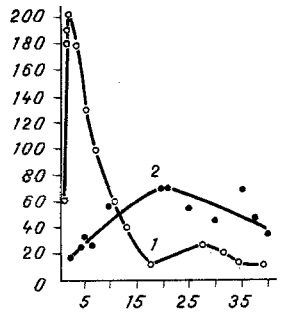

Fig. 2

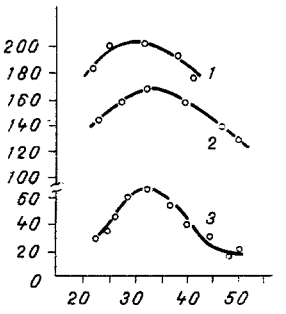

Fig. 3

Fig. 2. Concentrations of diene conjugates in erythrocytes depending on times of adaptation and seasons. 1) Spring; 2) fall.

Fig. 3. Concentrations of diene conjugates in erythrocytes depending on age for different times of adaptation. 1) 9-11 days; 2) 1 year; 3) control.

stay under new climatic and geographic conditions, their conjugated dienes level rose five-fold, whereas during the next 20 days it fell below its initial level and then slowly increased during the next 20 days of adaptation, with an extremum corresponding to 15 or 25 weeks depending on the season of adaptation. In the course of a stay of 2 years under new climatic and geographic conditions, periodization of the process of long-term adaptation takes place, but it does so at lower values of the parameters (Fig. 1).

The concentration of carbonyl compounds showed changes which were opposite in phase to the change in absorption at 232 nm. The acute period of adaptation (to 15 days) was characterized by a decrease of absorption, and subsequently absorption increased slowly.

Incidentally changes in the character of absorption of the lipid extracts at 268 nm were due to breakdown products of lipid hydroperoxides, and for that reason the decrease in absorption at 232 nm during adaptation may be accompanied by increased absorption at 268 nm. It was also found that in spring the period of acute adaptation was characterized by a sharp increase in the concentration of conjugated dienes; during the period of long-term adaptation the value of the parameter changed threefold in the course of 15 weeks, and during the next 20 weeks it reached its initial level. Differences in the character of the change in level of the conjugated dienes depending on the season could be connected with differences in the concentrations of bioantioxidants. Powerful stimulation of LPO in the period of acute adaptation in spring was probably due to vernal avitaminosis E. In the fall, against the background of a diet rich in vitamine E, there was a peaceful change in the level of conjugated dienes.

The study of changes in concentrations of diene conjugates depending on age, for each time of adaptation, showed that they were extremal in nature. The highest level of LPO products was characteristic of the age of 30-35 years. Probably in young and mature individuals the adaptation process takes place at a lower level of peroxidation (Fig. 3). The results of this investigation are evidence of the high sensitivity of LPO parameters when adaptation is assessed and they indicate that these tests may be used during the planning and organization of the emigration of workers into new districts.

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STRUCTURE OF INTERPHASE CHROMATIN OF PERIPHERAL BLOOD CELLS IN CHILDREN WITH ACUTE LYMPHATIC LEUKEMIA AND THEIR HEALTHY PARENTS

I. É. Yudina and K. N. Fedorova

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KEY WORDS: chromatin; blood cells; lymphatic leukemia

It has been shown by thermal denaturation of cellular deoxyribonucleoproteins (DNP) [6], in the writers' modification [2], followed by recording of changes in chromatin structure by cytofluorometry with acridine labeling [5], that binding of acridine orange (AO) with chromatin DNA of human peripheral blood lymphocytes between temperature of 20 and 100°C has clearly defined regular features and is represented by a curve with maxima in particular temperature intervals [2]. Analysis by computer showed that healthy persons with identical individual characteristics (number and location of the maxima) of their melting profiles of lymphocyte chromatin are distributed into groups. In 40% of cases (independent of sex) six maxima were obtained at particular temperatures (the modal class), whereas in 60% of cases various types of deviations (sex-dependent) were observed, and which, by their character, could be divided into a number of subgroups: a control group of five women and seven men, and not less than five identical cases in each subgroup.

Specific differences in cell chromatin melting profiles have been discovered in patients with various types of inborn and acquired [3, 4] chromosomal anomalies, and they correlate with similar characteristics of the curves in individual subgroups of healthy persons, so that the presence of genetic predisposition to particular diseases can be postulated. Accordingly structural features of cell chromatin were analyzed in children with acute lymphatic leukemia (ALL) and in their healthy parents.

EXPERIMENTAL METHOD

Nuclear chromatin of peripheral blood cells was studied in children with ALL aged from 0 to 12 years (21 children) at different stages of the disease, and in their healthy parents (19 families). Blood was taken in the Children's Hematology Department of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, where the diagnosis was made on the basis of clinical-hematologic and immunologic investigations. Changes in chromatin structure on heating (from 20 to 100°C) were recorded as the amount of luminescent label (AO) bound every 2-3°C. The tests were carried out on cells incubated for 1 h in Eagle's nutrient medium with the addition of 10% autologous serum. The intensity of luminescence of AO,

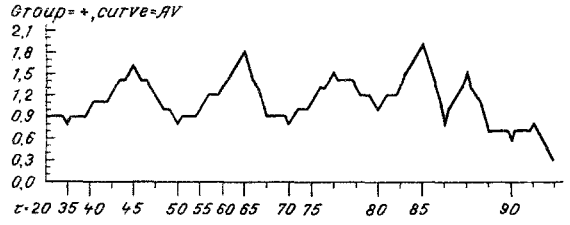

Fig. 1. Melting profile of interphase chromatin of healthy human lymphocytes (modal class), obtained by acridine labeling. Abscissa, temperature (in °C); ordinate, mean data of fluorescence at 530 nm (in relative units).

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