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EFFECT OF CULTIVATION TEMPERATURE ON BLAST TRANSFORMATION OF LYMPHOCYTES FROM PEOPLE OF DIFFERENT AGES

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The effect of the cultivation temperature on blast transformation of lymphocytes from persons aged 20-35 and 90-102 years induced by phytohemagglutinin was studied. Cultivation at 39°C was found to increase, but at 41°C to reduce sharply the index of blast transformation compared with the control (37°C). The effect of cultivation temperature on the blast-transformation process has certain features which depend on the donors' age.

KEY WORDS: *blast transformation; lymphocytes; age; temperature.*

The intensity of blast transformation of lymphocytes under the influence of phytohemagglutinin (PHA) is known to depend to a definite degree on the conditions of cultivation: the PHA concentration, the density of distribution of the cells, the composition of the nutrient medium and of the gaseous phase [6-12, 14]. According to some reports, the mitotic activity of PHA-stimulated lymphocytes depends on the incubation temperature [2, 3].

The object of this investigation was to study the effect of the cultivation temperature on the intensity of blast transformation of the lymphocytes after incubation for different periods. Data in the literature [1, 5, 13, 15] and the writer's own observations [4] are evidence of a change in the response of lymphocytes to PHA during aging, and it was therefore decided to carry out the present investigation from an age aspect.

EXPERIMENTAL METHOD

Altogether 457 cell cultures from 20 people aged 20-35 years and from 12 people aged 90-102 years were investigated.

Leukocytes were isolated from heparinized blood by sedimentation with gelatin (1 ml of 10% gelatin to 10 ml blood) and were cultivated in medium No. 199 containing 25% group IV serum. The final concentration was $1 \cdot 10^6$ cells in 1 ml culture. Difco PHA-P in a dose of 0.002 ml to 1 ml of culture was used as the stimulator. Incubation was carried out at 37 ± 0.3, 39 ± 0.3, and 41 ± 0.3°C for 24, 39, 48, 54, 66, 72, and 78 h. At the end of cultivation the cell suspension was centrifuged and films prepared from the residue and stained by the May–Gruenwald method. At least seven cultures were used at each time. Blast cells and intermediate forms were regarded as transformed lymphocytes. The criteria of transformation were the size of the nucleus and cytoplasm and the structure of the nuclear chromatin. The number of transformed lymphocytes in 1000 cells was counted.

The numerical results were subjected to statistical (dispersion) analysis. The significance of differences was assessed by Fisher's criterion and by the χ^2 criterion in Fisher's modification.

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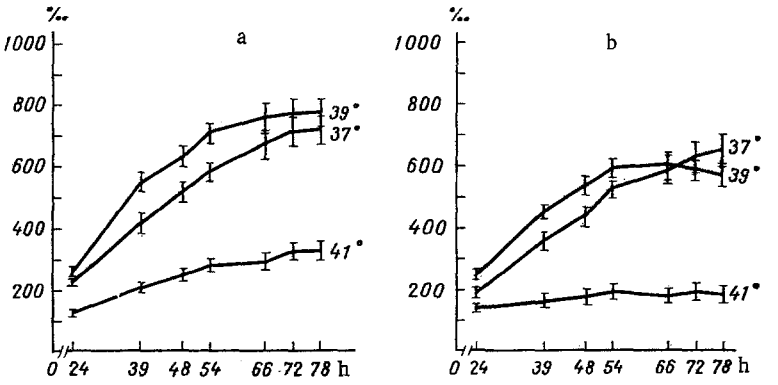

Fig. 1. Blast transformation of PHA-stimulated lymphocytes from persons aged 20-35 (a) and 90-102 (b) years depending on temperature and time of cultivation. Abscissa, cultivation period (in h); ordinate, index of blast transformation (in %).

TABLE 1. Distribution of People of Different Ages by Reaction of Lymphocytes to Elevation of Cultivation Temperature to 39°C Compared with Control (37°C) after Incubation for 78 h

Age, years	Increase in blast transformation index	Decrease in blast transformation index	P
20—35	11	1	<0,01
90—102	3	6	

EXPERIMENTAL RESULTS

The results (Fig. 1) show an increase in the blast transformation index at 39°C compared with the control (37°C). This rule was manifested most clearly after incubation for 39-54 h. Cultivation of lymphocytes at 41°C led to a sharp decrease (by 50-75%) in the blast transformation index, observable as early as 24 h after the beginning of incubation but particularly marked at the end of the third day.

Dispersion analysis showed that the intensity of blast transformation depends highly significantly on the cultivation temperature ($P < 0.001$) for each of the seven incubation times in both age groups. The degree of the effect of temperature of the blast transformation index increased with the duration of cultivation; the index varied from 40-50% in the initial stages of cultivation to 65-76% in the final stages.

Because of the diversity of the changes in blast transformation at 39 and 41°C, these changes cannot be unequivocally linked with the effect of temperature either on the intensity of the process itself or on the differences in mortality of the intact and transformed cells; this problem requires further study.

Age differences in the temperature dependence of PHA blast transformation in the initial stages of cultivation were hardly manifested at all, but later they became substantial. First, in the older age group the stimulating effect of a temperature of 39°C on blast transformation of the lymphocytes was replaced toward the end of the third day by inhibition. This rule, observed in respect of the mean indices, was reliably confirmed by comparing the response of lymphocytes of people of different ages in each experiment (Table 1). Second, the inhibitory effect of a temperature of 41°C on blast transformation of lymphocytes from subjects of the older age group was much more marked. For instance, in the older age group the index of blast transformation was virtually unchanged between 24 and 78 h of cultivation, whereas in the younger age group it increased by a statistically significant degree ($P < 0.005$).

The cultivation temperature thus has a significant effect on the intensity of blast transformation in tissue culture; the effect of temperature on blast transformation has certain special features which depend on the donors' age.

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PROLIFERATIVE ABILITY OF NUCLEATED RAT BONE MARROW CELLS AFTER FREEZING AND THAWING

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The proliferative power of rat bone marrow myelokaryocytes after freezing and thawing under the protection of hydroxyethyl derivative of tetrahydric alcohol (HEDTA) was studied in experiments on mouse—rat radiation chimeras. Rat bone marrow cells were shown to preserve their proliferative power.

KEY WORDS: *bone marrow; proliferative activity; freezing—thawing; radiation chimeras.*

The criterion of effectiveness of low-temperature conservation of bone marrow cells is the assessment of their biological activity, whereby the functional integrity of the cells can be determined. The functional activity of the conserved cells is characterized by their proliferative ability in the recipient.

The object of this investigation was to study the proliferative ability of nucleated rat bone marrow cells after freezing and thawing (to —196°C) under the protection of the hydroxyethyl derivative of tetrahydric alcohol (HEDTA).

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