EFFECT OF TEMPERATURE ON MITOTIC ACTIVITY OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES IN CULTURE

P. A. Borodkin

Cultivation of human lymphocytes at a temperature of 39-40°C leads to an increase in mitotic activity over that observed during cultivation at 37°C.

The optimal temperature for cultivation of human cells is considered to be 37°C. This paper gives data on the cultivation of human circulating blood lymphocytes at 37-43°C.

EXPERIMENTAL METHOD

A culture of lymphocytes was prepared from blood taken from the vein of a healthy donor in a volume of 10-20 ml by the usual method. The number of flasks with the culture was divided into two equal batches: one batch was incubated at 37°C (control), the other at 38-43°C. Colchicine was added 3 h before hypotonic treatment. The control and experimental cultures were fixed simultaneously after 36, 41, 45, and 48 h. Films were stained with 10% azure-eosin solution by Romanovsky's method.

Medium No. 199, issued by the Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR, to which phytohemagglutinin (PHA) of series M (Park Laboratory, General Biochemicals, USA) was added, was used for cultivation.

Five repetitions of each experiment were set up. Blood was obtained from different donors.

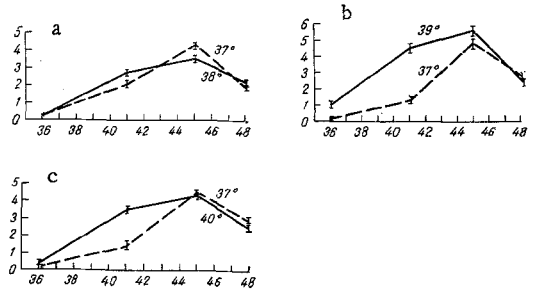

Fig. 1. Mitotic activity of human lymphocytes as a function of cultivation temperature (a, b, c). Ordinate, mitotic index (in percent); abscissa, time of fixation of culture (in h) after beginning of incubation.

EXPERIMENTAL RESULTS

Human lymphocytes are highly thermostable. Ability of the lymphocytes to divide by mitosis was completely lost only if cultivated at 43°C. At 42°C mitotic activity was considerably inhibited: only single mitoses were found among several 100 cells. At temperatures of 38 and 41°C mitotic activity was not significantly different from the control (Fig. 1a), although the mitotic wave began a little earlier at 38°C.

Interesting results were obtained when the culture was incubated at 39 and 40°C. In these cases the mitotic index was 3-4 times higher (fixation of the culture at 41 h of cultivation) than in the control. If the culture was fixed after cultivation for 45 h the mitotic indices were almost identical in the experimental and control variants, although

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in the control mitotic activity was considerably increased while in the experimental series it was almost unchanged. After cultivation for 48 h a decrease in the mitotic index was found in both variants (Fig. 1b, c).

The points of fixation of the cultures cannot be regarded as constant. They depend on the quality of the PHA and of the nutrient medium, so that they must be determined empirically in each case.

When PHA (Difco) was used the times of fixation of the culture were different (44, 48, 52, and 56 h), but the relationship between mitotic activity and the cultivation temperature was similar to that described above. The mitotic index in all experimental cultures fixed after 52 h, except in that cultivated at 41°C, was significantly higher than in the control.