MICROTHERMAL METHOD OF SEPARATING RED BLOOD CELLS INTO AGE GROUPS

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The microthermal method of separating red blood cells by age is based on the creation of a temperature gradient with the aid of a microthermistor. Red blood cells of different age fractions, differing in their biochemical indices, are successively localized in the zone of the microthermistor and sampled. The microthermal method of separating red blood cells can assist with their differential study.

KEY WORDS: *red blood cell; age; fractionation; temperature gradient; density.*

The red blood cells of man and animals exist in the blood stream for a long time and they are heterogeneous and functionally unequal [3]. Repeated investigations of erythrocyte metabolism have as a rule been undertaken on mature cells. Reticulocytes and young erythrocytes have been analyzed after enrichment of the blood with them under conditions of experimental anemia. These cells are mixed with mature red blood cells and their fractionation is not absolute [4]. A detailed study of the chemical composition and specific features of the metabolism of a population of red blood cells of different ages can be undertaken by the use of improved methods of fractionation.

To separate red blood cells into age groups, methods now available include centrifugation [9], electrophoresis [6], and a method of fractionation in biphasic systems consisting of polyethyleneglycol and dextran [1]. Evaluation of these fractionation methods has shown that there is still no effective, gentle, and labor-saving method whereby isolated populations of red blood cells of different age groups can be obtained.

The novelty of the suggested microthermal method, in principle, lies in the use of a temperature gradient created in the test cell suspension in order to separate the cells into groups on the basis of very small differences in density.

A temperature gradient is applied by means of a microprobe heater or microthermistor, hermetically sealed in a thin glass capillary tube. A microthermistor of the MT series, designed by V. G. Karmanov, was chosen as the heat flow generator because it enables energy to be introduced precisely into the system, on account of the sufficiently high electrical resistance of the transducer and, at the same time, its insulation from the biological system under investigation by the glass capillary tube wall.

A suspension of red blood cells washed to remove plasma, 0.2 ml in volume, is carefully introduced into a glass cuvette filled with buffer mixture (3 parts physiological saline to 1 part 0.067 M potassium phosphate buffer, pH 7.2-7.4). The cuvette is placed in a glass water bath until the microthermistor reaches a constant temperature. The microprobe, a type MT-54 or MT-64 microthermistor, is then immersed in the cuvette containing the suspension of red blood cells to a depth of 2-3 mm from the surface of the sedimented red cells. A bridge circuit is set up, with the microthermistor in the measuring arm and, in parallel with it, a milliammeter monitoring the current through the microthermistor. The microthermistor is kept at 30 mW (direct current), by which the local temperature is maintained at 4-5°C above the temperature of the surrounding medium.

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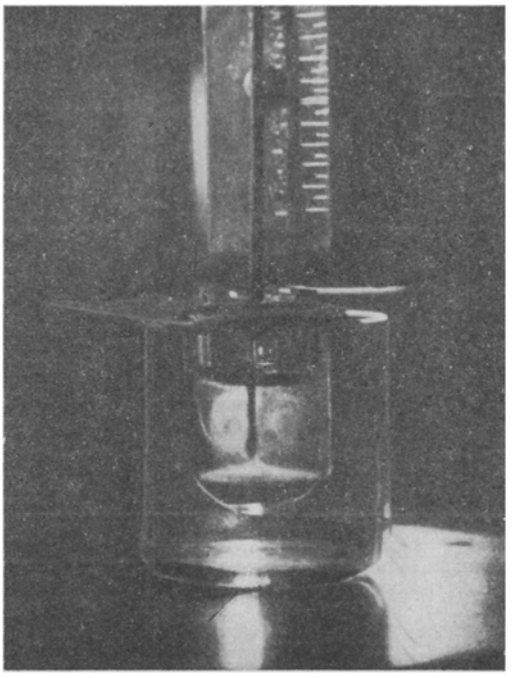

Fig. 1. Formation of fraction of light red cells in zone of microthermistor.

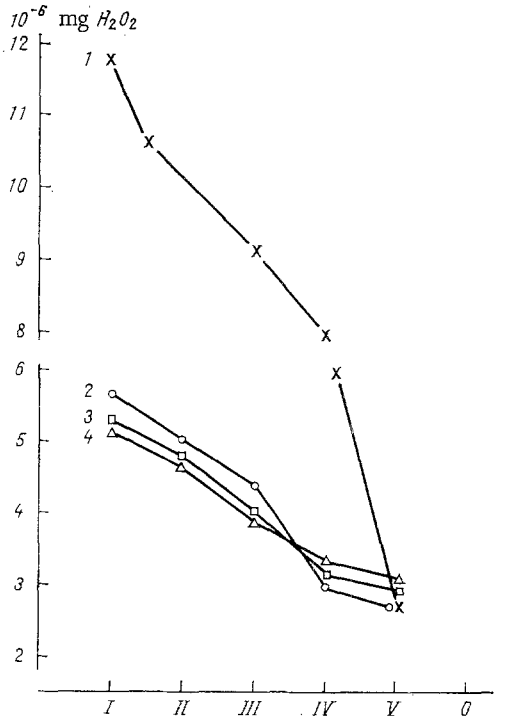

Fig. 2. Catalase activity of red cells (ordinate) of man and laboratory animals before and after fractionation. Here and in Fig. 3: I-V) numbers of fractions; 0) cell population before fractionation. Red cells from: 1) man; 2) rabbit; 3) guinea pig; 4) albino rat.

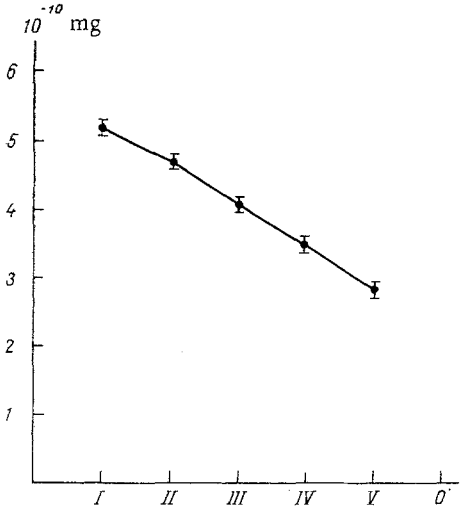

Fig. 3. Cholesterol content (ordinate) in erythrocytes of rabbit with hemolytic anemia in regenerative phase before and after fractionation.

Convection currents generated as a result of the temperature gradient at once attract the lighter cells into the region of lower density, namely the zone of the microthermistor, and after 60-90 sec a characteristic "cloud" consisting of cells of lower density is formed in the zone of the microthermistor (Fig. 1). This is recorded visually. The lightest cells, separated in this way and concentrated near the microthermistor, are aspirated with a Pasteur

pipet and transferred to a test tube (red cells of fraction I). After the current has been passed for a second time through the microthermistor for a few seconds the convection currents carry away the next group of red cells with lower density. The "cloud" formed by these cells is also aspirated (red cells of fraction II). Buffer mixture is added carefully to the cuvette containing the remaining red cells, the microthermistor is again switched on, and the fractionation procedure repeated.

By means of the microthermal method, red blood cells can be separated in a very short time (6-8 min) into five or more fractions corresponding to cells of different age groups.

To verify qualitative differences between the red cells separated by age by the microthermal method, two indices of red cell metabolism were chosen: catalase activity and cholesterol concentration.

Young red blood cells are known to have higher catalase activity, and blood with a high reticulocyte count also has higher catalase activity [8]. The cholesterol performs a diversified role in the red cell: In particular, it has a marked effect on oxidative processes and is linked with catalase function [7]. The cholesterol content in young red cells is twice or three times higher than in mature red cells. The cholesterol content is particularly high in reticulocytes [10].

Catalase activity [2] was expressed in milligrams of H_2O_2 decomposed during 30 min by the catalase contained in one cell, washed to remove plasma. The cholesterol content [5] was expressed in milligrams cholesterol per cell. Human, guinea pig, albino rat, and rabbit red cells were fractionated and studied (Fig. 2).

The catalase activity of unfractionated human and animal red cells was almost identical: human $4.09 \pm 0.176 \cdot 10^{-6}$ mg H_2O_2/cell; rabbit $4.04 \pm 0.023 \cdot 10^{-6}$ mg H_2O_2/cell; guinea pig $3.84 \pm 0.079 \cdot 10^{-6}$ mg H_2O_2/cell; and rat $4.09 \pm 0.046 \cdot 10^{-6}$ mg H_2O_2/cell.

After fractionation of the red cells by means of the microthermistor a marked difference was found in the catalase activity of the red cells belonging to the five age groups. The ratio between the catalase activity of the fraction I cells and that of the fraction V cells was 4.4 for human red cells, 2.3 for rabbit, 1.72 for rat, and 1.82 for guinea pig.

Comparison of the catalase activity of "young" and "old" red cells of man and laboratory animals leads to the conclusion that human red cells show the greatest biochemical heterogeneity. The reason is probably that the life span of human red cells in the blood stream is twice that of the red cells of the rabbit, guinea pig, and rat.

The data for the cholesterol content of the red blood cells of a rabbit in the regenerative stage of experimental phenylhydrazine anemia are given in Fig. 3.

The cholesterol content in the red cells before fractionation was $2.99 \pm 0.083 \cdot 10^{-10}$ mg/cell. Red cells of fraction I had the highest cholesterol content, and 94% of these cells were reticulocytes. The cholesterol content in them was $5.20 \pm 0.152 \cdot 10^{-10}$ mg/cell. The cholesterol content of the "old" red cells of fraction V was $2.71 \pm 0.034 \cdot 10^{-10}$ mg/cell.

Aging of red cells, which is accompanied by a decrease in their content of lipids, including cholesterol, changes the specific gravity of the cell, so that red cells of different age groups can be separated with the aid of the microthermal method.

Characteristic features of the microthermal method are a high speed of fractionation and absence of injury to the separated cells. The method facilitates the differential study of red blood cells.

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