DETERMINATION OF FRAGILITY OF ERYTHROCYTES FROM THE CATALASE ACTIVITY OF BLOOD

S. I. Krainev

From the Department of Biochemistry (Chairman: Prof. N. P. Pyatnitsky), Kuban Medical Institute, Krasnodar

(Received March 27, 1955. Presented by S. E. Severin, Member Acad. Med. Sci. USSR)

Existing methods for determining the osmotic stability of erythrocytes are based on comparison of the coloration of hypotonic sodium chloride solutions containing intact erythrocytes and their breakdown products.

It appears from our observations that the catalase activity of unhemolyzed blood is 7-10 times lower than of hemolyzed blood. The most abrupt changes in catalase activity due to hemolysis are observed within a relatively narrow concentration range of hypotonic solutions (Fig. 1).

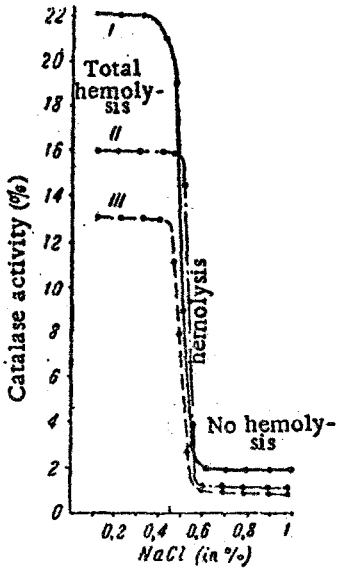

Fig. 1. Changes in catalase activity of blood suspensions in solutions of sodium chloride. Data from three experiments (I, II, III), with different catalase activities.

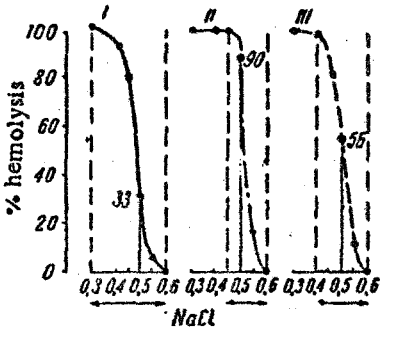

Fig. 2. Intensity of erythrocytolysis and the "zone of hemolysis" of blood in hypotonic sodium chloride solutions. The zones of hemolysis are indicated by arrows. Data from three experiments (I, II, III).

The existence of a correlation between osmotic stability of erythrocytes and catalase activity of hemolysates suggests a possibility of applying it to the objective evaluation of degree of hemolysis.

Individual differences between the bloods of different persons appear not only in their catalase activity before and after hemolysis, but also in the width of the "zone of hemolysis", i.e. the range of concentrations of sodium chloride within which hemolysis is achieved. If we assume that the catalase activity of blood suspensions corresponds with the proportion of cells hemolyzed, it is more convenient to express the latter value as percentages.

According to our observations, particular interest pertains, within the "zone of hemolysis", to 0.5% sodium chloride solutions (Fig. 2).

Procedure for Determination of Erythrocyte Fragility

Finger blood (20 μl) is taken in a Sahli pipette, and transferred to a vessel containing 10 ml of 1% sodium chloride.

A series of hypotonic solutions is prepared in 9 other 100 ml beakers, by mixing 1% NaCl solutions with water, in the proportions indicated in the table.

No. of beaker	1	2	3	4	5	6	7	8	9
Ml of 1% NaCl solution	7	6	5	4.5	4	3.5	3	2	1
Ml of water	2	3	4	4.5	5	5.5	6	7	8
% NaCl content after addition of blood	0.8	0.7	0.6	0.55	0.5	0.45	0.4	0.3	0.2

It is more convenient to prepare the series of solutions previously, and to measure out 9 ml into each beaker.

Three drops of 2% ethanol are then added to each beaker, followed after mixing by 1 ml of the blood suspension. The systems are then well mixed, and are left to hemolyze for 15-20 minutes.

Three drops of 2% ethanol and 9 ml of 1% NaCl are then added to the residue of the original suspension; this system serves as the control.

A porcelain crucible is placed on the bottom of each beaker, containing 2 ml of 0.05 N hydrogen peroxide in 0.006 M phosphate buffer at pH 7.0.

Five ml of 10% sulfuric acid is added to the control system.

After 15-20 minutes catalase activity is determined in all 9 beakers.

The beakers are taken up at exactly zero time, and swirled round, so as to upset the crucibles and, without interrupting mixing, 5 ml of 10% sulfuric acid is added from a test tube, during 30 seconds, after which mixing is continued for a further 5-10 seconds.

The contents of the control and the experimental systems are titrated with 0.1 N permanganate. The difference between the readings for the control and the experimental systems, in ml, multiplied by 10, gives the catalase activity of the system, expressed as % of the hydrogen peroxide decomposed in 30 seconds.

The highest catalase activities will be found in the systems with the lowest sodium chloride concentrations, i.e. where total hemolysis has taken place (A), and the lowest with high concentrations of NaCl, where there is no hemolysis (B). The difference between these values, (A–B), represents the change in catalase activity due to hemolysis (C). Designating catalase activity of the systems in the "zone of hemolysis" as D, we calculate the degree of hemolysis, expressed as a percentage, from the equation:

$$\text{Percentage hemolysis} = \frac{D-B}{C} \cdot 100.$$

Example of calculation. The catalase activity was 2 in systems containing 0.8, 0.7, and 0.6% NaCl (zero hemolysis), and was 22 in systems containing 0.3 and 0.2% NaCl (100% hemolysis). Within the "zone of hemolysis", catalase activity was 3 in 0.55% NaCl, and 19 in 0.45% NaCl. Percentage hemolysis was $\frac{3-2}{22-2} \cdot 100 = 5$ for the former system, and $\frac{19-2}{22-2} \cdot 100 = 85$, for the latter system.