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USE OF SCATTERING OF LIGHT TO RECORD ERYTHROCYTE DESTRUCTION BY HEAT

A. E. Gromov

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The study of resistance of erythrocytes to harmful factors is widely used in biological and medical research because it provides important information on the physicochemical properties of erythrocytes. The best known methods of determining resistance are osmotic, acid, mechanical, and certain others [1, 2-4]. Each of these methods provides different information and each has its advantages and disadvantages. For example, in osmotic hemolysis the erythrocyte ghosts remain in suspension, indicating incomplete destruction of the cells. From our point of view, the soundest method of studying erythrocyte resistance is that involving the use of heat, which also causes hemolysis and much more complete destruction of the cell membranes [2, 3, 5].

In the investigation described below, to determine the resistance of erythrocytes they were destroyed by heat and a method based on scattering of light was used to measure the course of the process.

EXPERIMENTAL METHOD

The method is essentially as follows. A suspension of erythrocytes in a solution of NaCl (or any other substance) is exposed to a constant high temperature (58-60°C) and changes in scattering of light by the suspension are recorded over a period of time. In this way the resistance of erythrocytes can be studied in different salt concentrations (i.e., at different osmotic pressures), including in isotonic solutions, in the presence of any other substances, over a wide range of pH values, and so on. Erythrocyte destruction during osmotic and acid hemolysis can also be recorded automatically by this method.

In Fig. 1 a diagram of the apparatus for recording erythrocyte destruction by the scattering of light method is shown. The tube containing the specimen, 0.5 ml in volume, is placed in a constant temperature cuvette 1. The cuvette is made of duralumin and blackened electrolytically. Temperature is kept constant by the U-3 ultrathermostat. The suspension is stirred by the mixer 7. A beam from the source of light (OI-19 illuminator) passes through the condenser lens 2, red filter 3, and light guide 4 and falls on the test object. Scattering of light is recorded at an angle of 20° to the incident beam by a cadmium sulfide (type SF-3-1) photoresistor 6 through the light guide 5, protecting the photoresistor against heat. The light guides are made from transparent plastic, covered with black nitroenamel; the ends of the light guides are polished with GOI paste.

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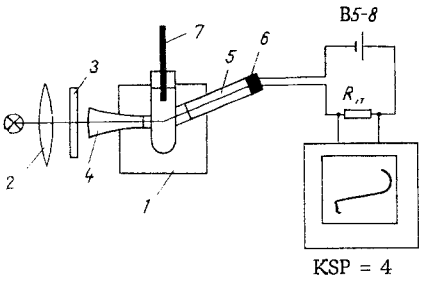

Fig. 1. Diagram of apparatus for recording scattering of light during erythrocyte destruction by heat (explanation in text).

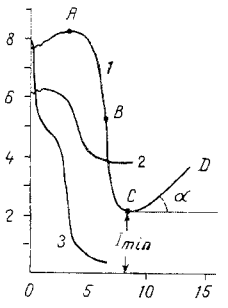

Fig. 2

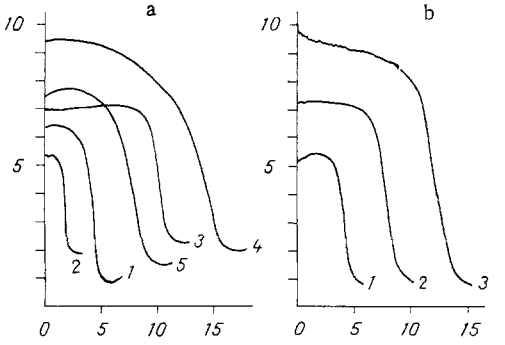

Fig. 3

Fig. 2. Changes in scattering of light during destruction of rabbit erythrocytes by heat (58°C) in physiological saline (1), during osmotic destruction in 0.4% NaCl solution at 20°C (2), and during acid hemolysis at 20°C (3). Here and in Fig. 3: abscissa, time (in min); ordinate, intensity of scattering of light (in relative units).

Fig. 3. Changes in scattering of light during destruction of rabbit erythrocytes by heat in different media. a: 1) serum, 2) Polidez, 3) Gemodez, 4) Gelatinol, 5) physiological saline; b: 1,2,3) 0.6%, 0.9%, and 1.8% KCl solutions, respectively.

The voltage on the photoresistor is supplied from a stabilized voltage source of the B5-8 type. The sensitivity of the photometer is controlled by changing the supply voltage between 1 and 20 V. The voltage from the load resistance (R_1 = 200 Ω) is led to a type KSP-4 self-recording potentiometer (0-10 mV). Since the change in resistance of the photoresistor is proportional to the intensity of incident light for low values of photic flux, the readings of the automatic writer are a linear function of the intensity of light scattered by the specimen, i.e., of the erythrocyte concentration in the illuminated volume of the sample. Measurement of erythrocyte resistance by the suggested method is conducted as follows: The blood sample or washed erythrocytes are diluted with the solution in which the measurement is made, introduced into a constant temperature cuvette at 58°C, and changes in the scattering of light with time are recorded.

The quantitative parameters which characterize the properties of erythrocytes during destruction by heat are: time (in min) elapsing from the beginning of the process to destruction of half of the erythrocytes (point B), which characterizes resistance of the erythrocytes; the minimal intensity of scattering of light (I_{min}) (the point C), determined by the mean size of the erythrocyte destruction products; the angle of slope α of region CD of the curve to the abscissa, which is connected with the rate of denaturation of the hemoglobin molecules after erythrocyte destruction and passage of the hemoglobin into solution (Fig. 2).

EXPERIMENTAL RESULTS

To illustrate the possibilities of the method, curves showing changes in scattering of light during destruction of rabbit erythrocytes by heat in isologous serum, various plasma expanders, and physiological saline are given in Fig. 3a. It will be clear that the resis-

tance of erythrocytes in serum may be lower than resistance of the same erythrocytes in physiological saline, and that different plasma expanders may either increase or decrease resistance of the cells.

Curves for rabbit erythrocytes suspended in KCl solutions of different concentrations are given in Fig. 3b. Besides different resistance in the various media studied, the erythrocytes also have different density, which can be estimated from the initial intensity of scatter. Information of this kind cannot be obtained by any of the existing methods of measuring erythrocyte resistance. The study of the effect of various substances on erythrocyte resistance in experiments *in vivo* and *in vitro* showed that the method which the writer has developed can be used to record changes in erythrocyte resistance that cannot be detected by the study of osmotic resistance or acid hemolysis of erythrocytes.

The suggested method can thus be used to study erythrocyte resistance in solutions of different substances (for example, to study the action of drugs), and the measurements may be made over a wide range of pH values, to study the action of substances not chemically connected with erythrocytes and not penetrating into them (molecules of plasma expanders, protein macromolecules, and so on), and to measure resistance of erythrocytes in homologous or heterologous serum over a wide range of hematocrit values, i.e., under conditions most nearly approximating to those *in vivo*, as well as to estimate the density of erythrocytes.

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