AUTOMATIC RECORDING OF THE KINETICS OF ACID RESISTANCE OF AN ERYTHROCYTE POPULATION

V. P. Nefedov, V. N. Petushkov,
Yu. L. Gurevich, V. A. Samoilov,
and I. L. Yasnikov

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A method of automatic recording of a differential form of acid erythrogram has been developed, to show the dispersion of acid resistance among a population of erythrocytes. A number of differential erythrograms concerning the high accuracy and sensitivity of the method are shown.

The erythrogram method to demonstrate the dispersion of resistance of erythrocytes to various chemical hemolytics among a population was suggested earlier [6]. The effectiveness of this method was confirmed by many investigators on an extensive experimental and clinical material [1-4]. At the same time, a search has been made for an automatic method of obtaining erythrograms, which would increase the sensitivity of the method, and make it less laborious and more objective.

An integral form of automatic erythrogram (a curve showing changes in optical density with time) was obtained as long ago as in 1957 [5]. However, although the erythrogram in an integral form contains all information regarding the kinetics of the process, it still requires considerable additional processing, and for this reason, erythrograms are used in practice in a differential form, obtained by plotting visually the change in optical density over an assigned time interval. Instruments for the automatic recording of the erythrogram in the differential form are not available, so far as the writers are aware.

The object of the present investigation was to develop a method of automatic recording of the differential erythrogram.

The scheme developed by the writers for automatic recording of the erythrogram in the differential form was based on the following premise: the rate of change of optical density can be described by a change in the value of optical density D over a sufficiently short time interval $\Delta t = t_2 - t_1$:

$$V = \frac{dD}{dt} = \lim_{\Delta t \to 0} \frac{\Delta D}{\Delta t}. \quad (1)$$

If a whole series of measurements is made, if $\Delta t = \text{const.}$, the rate of change of optical density (rate of hemolysis) can be determined from the value of its change ΔD:

$$V = K\Delta D \quad (2)$$

where K is a constant determined by the value Δt.

To record the rate V automatically it is convenient to have the value of ΔD at each moment of time. Let us assume that the integral curve of the erythrogram is displaced along the time axis by a definite time interval Δt_1, as shown in Fig. 1a, when the difference between the ordinates at each moment of time will determine the mean rate of change in optical density (the mean rate of hemolysis) during the time interval Δt_1.

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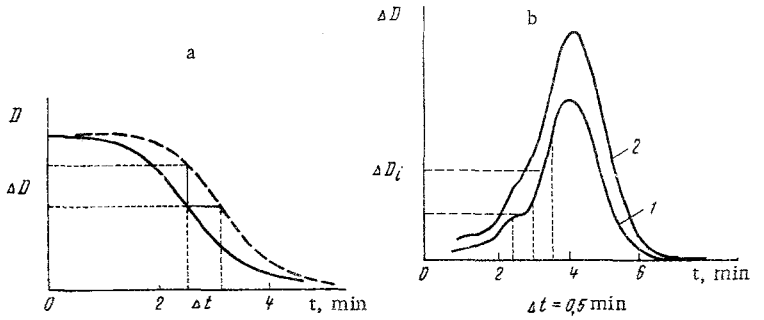

Fig. 1. Determination of the differential form of the erythrogram from the integral form (a) and recording of differential erythrograms (b). 1) $\Delta t = 20$ sec; 2) $\Delta t = 40$ sec. Explanation in text.

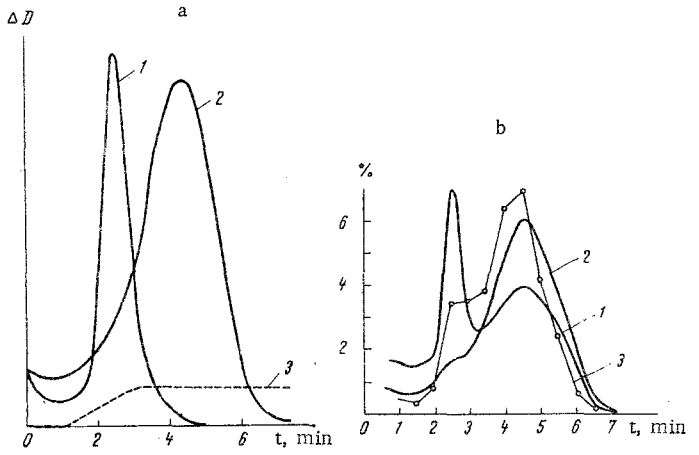

Fig. 2. Records of differential erythrogram for blood of a guinea pig and rabbit (a) and erythrograms of mixed blood (b). In a: 1) guinea pig; 2) rabbit; 3) curve of error of measurement of recording system. In b: 1) automatic recording of erythrogram for mixture of rabbit and guinea pig blood in ratio 1 : 1; 2) ditto in ratio 1 : 10; 3) manual recording of erythrogram for mixture of rabbit and guinea pig blood in ratio 1 : 10.

To obtain such a shift of the integral erythrograms in the scheme of automatic recording of the rate of hemolysis, the blood for testing is hemolyzed in two cuvettes having a time shift of Δt. The cuvettes were placed in the optical channels of an FÉK-M photoelectric colorimeter. As a result, the instrument measured the difference between optical densities in the two channels, giving the required value ΔD.

The apparatus used for measuring and recording the kinetics of hemolysis consisted of an FÉK-M photoelectric colorimeter and a type PDS-021 two-coordinate potentiometer.

The signal from the colorimeter was plotted on the y coordinate, and time was plotted along the x coordinate. The remainder of the analysis was carried out in the usual way, i. e., a 0.004 N solution of HCl was used as the hemolytic, and photometry was carried out in two thermostatically controlled 20-mm cuvettes, placed in the two channels of the instrument.

Hemolysis was carried out at a temperature of $24 \pm 0.2°C$.

As in the usual method, the concentration of erythrocytes in the left cuvette of the colorimeter was adjusted to 0.700 D on the left hand scale, and then by rotation of the left drum, the scale reading was returned to the "O" mark. The difference of optical densities arising under these conditions was then compensated by addition of the suspension of erythrocytes to the right cuvette (2 ml of suspension in each cuvette). The hemolytic in a volume of 2 ml is taken up into two pipets and then one portion of hemolytic is

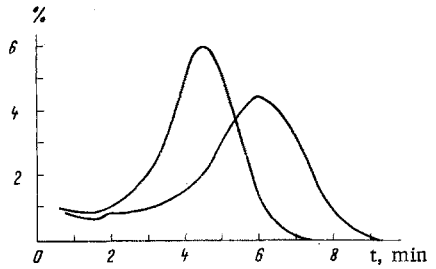

Fig. 3. Erythrograms recorded for two concentrations of hemolytic.

released into the left cuvette and simultaneously the time base of the recording instrument is switched on. After 20 sec the other portion of hemolytic is poured into the right hand cuvette. Thereafter the process is automatic.

It must be especially noted that when recording the automatic erythrogram it is essential to keep very careful watch on the accuracy of determination of the initial concentration of erythrocytes, and to make perfectly sure of the identical thermostabilization of the 2 cuvettes, which, as experience showed, is best done by arranging them in series and not in parallel in the flow of a water thermostat.

Another matter which requires careful attention is that the volumes of hemolytic added to the two cuvettes must be identical. This is ensured by the use of more accurate (2-ml) pipets with one mark.

As a rule, all these conditions must be satisfied also when ordinary, as well as automatic, erythrograms are recorded [4].

The dispersion of deviation of the time of maximum hemolysis of one specimen of blood in 18 measurements was $\sigma \pm 2$ sec (total time of hemolysis 7.5 min). The confidence interval for the maximum of the differential hemolysis curve was ± 0.91 sec, with a probability of 0.95.

The error or recording the differential hemolysis curve, introduced by the measuring system, was determined by the following method. Hemolysis of the blood was started in one cuvette (volume of reaction mixture 8 ml), and half of this suspension was then immediately transferred to the second cuvette. In this way, hemolysis curves in the right and left cuvettes must coincide, and the signal recorded by the measuring system (Fig. 2a) gives the error of the system (primarily the optical nonhomogeneity of the cuvettes).

Records of two erythrograms with $\Delta t = 20$ and $\Delta t = 40$ sec are shown in Fig. 1b. Subsequently the measurements were carried out with $\Delta t = 20$ sec.

To compare erythrograms obtained by the automatic and ordinary methods, it was necessary to express the automatic erythrogram in per cent. To do this, starting from the beginning of hemolysis, the mean value of ΔD_i was determined for each time interval of 30 sec on the graph of the velocity of hemolysis (Fig. 1b). Next, the sum of all values ΔD_i $\left(\sum_{i=1}^{n} \Delta D_i\right)$ was obtained. The percentage for each time interval was then determined from the formula:

$$\% \Delta D_i = \frac{\Delta D_i \cdot 100\%}{\sum_{i=1}^{n} \Delta D_i}.$$

This treatment is analogous to that used in the ordinary method, the only difference being that the values of ΔD are determined from the graph obtained automatically.

To express the curve of the rate of hemolysis in per cent more accurately, the measurements can be made more frequently than every 30 sec. This is done easily on the graph. When comparing erythrograms obtained automatically, there is no need to convert into percentages. All that is necessary is that all measurements are carried out at the same Δt value (for example, 20 sec, as was adopted in the present measurements).

By recording the erythrogram in the differential form automatically, from the graph thus obtained it is possible, without any further processing of the results, to record with great accuracy the displacement of the peak, the appearance of supplementary peaks, changes in the shape of the hemolysis curve and in the time of hemolysis, and so on in experiments with one object.

Erythrograms obtained automatically for guinea pig and rabbit blood are showed in Fig. 2a, and erythrograms with two peaks of hemolysis obtained automatically after mixing rabbit's and guinea pig's blood in the proportion of 1 : 1, and also erythrograms obtained automatically and in the usual way for rabbit's and guinea pig's blood mixed in the ratio of 1 : 10, are shown in Fig. 2b.

Displacement of the peak of an automatically obtained erythrogram with a change in the concentration of hemolytic is shown in Fig. 3.

This investigation shows that the erythrogram obtained by the automatic method is highly sensitive and accurate and that no special apparatus is required for its recording (instead of the PDS-021 instrument, any other type of self-writing potentiometer with a rectangular system of coordinates can be used).

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