USE OF THE METHOD OF POLAROGRAPHIC COULOMETRY FOR THE STUDY OF OXYGEN RELEASE BY THE BLOOD

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The polarographic method has started to be used to study the respiratory function of the blood. Thus, Colman and Longmuir [4], by adding ground myocardial tissue to the investigated blood, induced a uniform removal of oxygen from the blood, and, by measuring polarographically the oxygen tension (pO_2), were able to record the oxyhemoglobin dissociation curve.

A polarographic study of the capacity of the blood to release oxygen can be carried out even without adding a "breathing" preparation to it. The device we proposed for this purpose [2] is a closed polarographic microelectrolyzer with a quite small working chamber (15-30 mm^3), having a relatively large polarizable mercury surface (on the order of 20 mm^2) equipped with a magnetic mixer.

The electrolyzer (Fig. 1) is a glass vessel on legs. A cylindrical depression 1 is in its elevated bottom. The bottom of the vessel is ground down to surface 2. The depression contains pure mercury with sufficient room left for the blood and magnetic mixer 7. For this purpose, when the depression is filled with mercury, a steel ball of a known volume and an excess of mercury are placed in it; the excess of mercury is removed by pressing with a flat ground glass, then the ball is removed. Electrical contact with the mercury is accomplished through sealed-in platinum wire 5 and mercury column 6 in a side capillary. The depression contains the mercury, magnetic mixer, and investigated blood, which is restricted at the top by a moistened cellophane strip 3 laid under plate 4 made of microporous ceramic. The electrolyte 8 (physiological salt solution or 0.14 M solution of HCl) poured into the vessel and the salt connector 9 permit conducting the anode potential to the electrolytic cell. A calomel electrode with a mercury-calomel dividing surface at least 50 times greater than the surface of the electrode in the electrolyzer is used as the anode.

The electrical circuit of the electrolyzer consists, consequently, of the following links: mercury column 6, platinum wire 5, mercury 1, investigated blood, electrolyte in the pores of plate 4 and in the circular section of the moistened cellophane strip 3 (which offer a small resistance for the electrical current and a larger resistance for the dissolved oxygen) electrolyte 8, and salt connector 9 of the calomel electrode.

The entire vessel is placed in brass water bath 10 in which the temperature necessary for electrolysis is automatically maintained. Magnet 12, which drives the magnetic mixer, rotates under the bottom of the bath at a constant rate.

Electrolysis is usually carried out at a constant potential of 1.3-1.6 V (relative to the calomel electrode), at 37°, and rotation speed of the magnet not below 750 rpm.

Before starting the work, a drop of investigated blood is applied with an excess on the surface of the mercury in the depression and covered with a plate, under which the cellophane strip moistened with the physiological solution (without an excess of moisture on it) is placed. The plate is pressed with tweezers; the solution is carefully poured into the vessel, the salt connector inserted, and after equalizing the temperature, the magnetic mixer is switched on first and then the circuit of the polarograph. If there is no automatic recorder, the current of the galvanometer is recorded visually every 15 or 30 sec with the use of a stopwatch. This recording is the initial one for all subsequent calculations.

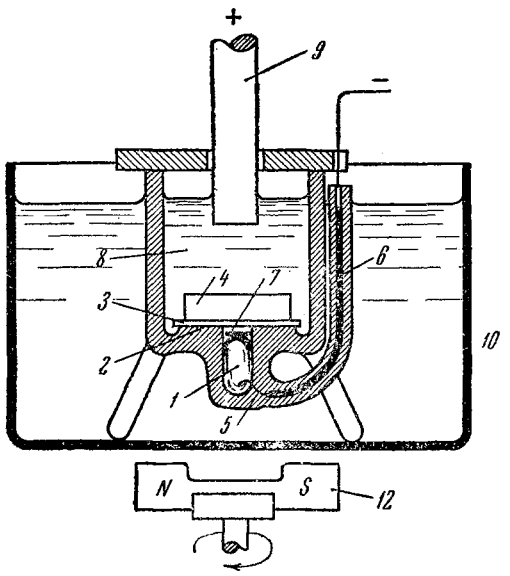

Fig. 1. Electrolyzer for studying the release of oxygen by blood. Explanation in text.

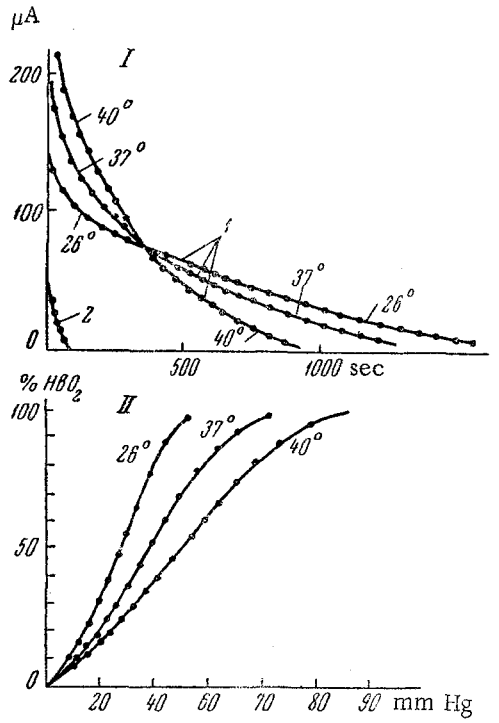

Fig. 2. I) Change of the depolarization current strength in time depending upon the temperature during electrolysis of donor blood saturated by a mixture of air with 5% CO_2 in a closed electrolyzer. The volume of the chamber is 32 mm^3, diameter 5.5 mm, rate of rotation of the magnetic mixer 100 rpm, Hb 10 g%; emf 1.375 V; 1) blood; 2) physiological solution saturated with air; II) curves of the nonuniform dissociation of oxyhemoglobin obtained on the basis of the curves of graph I.

If blood saturated only with air is investigated, there is no need to take special measures to maintain pO_2 constant in the sample when loading the electrolyzer. However, when more accurate measurements of pCO_2 are required, the instrument can be charged with blood after saturating with gases in a table box blown with a gaseous mixture of a given composition. If electrolysis is carried out at a voltage of 1.3-1.6 V relative to the calomel electrode, the oxygen is reduced at the electrode according to the overall reaction.

$$O_2 + 4e - 4OH^-$$

The ions of the hydroxyl are neutralized by the buffer system of the blood or by buffer additions.

The polarized mercury surface of the cathode on which pO_2 is maintained equal to zero owing to polarization, "absorbs" by diffusion of the oxygen from the blood plasma. The loss of plasma oxygen is made up by its uptake from erythrocytes as a result of dissociation of oxyhemoglobin.

Before coulometric electrolysis of the blood, it is neccessary to make more precise the magnitude of the cathode potential at which the entire electrolysis will be carried out. For this purpose, the investigated blood (column height 0.5 cm) is poured into the instrument; the magnetic mixer is placed in the mercury; the cellophane strip and plate 4 are not used. The position of the area of the diffusion current reducing O_2 of the blood to H_2O is determined in an open vessel with a large volume of blood during recording of the polarogram from 0 to 2 V. Having determined the necessary cathode potential, the gas-saturated blood is electrolyzed at a constant potential in a closed chamber, using the cellophane strip and plate 4. From the theoretical investigations of the kinetic current [3,5] and from direct experiments [4], it follows that the strength of the depolarization current passing through the galvanometer is proportional to pO_2 of the blood.

The readings of the change of current in time permit plotting the first graph of the relative changes of the rate of oxygen released by the blood (Fig. 2, I). These data can be used for plotting the graph of the curve of oxyhemoglobin dissociation under conditions of continuous oxygen released by the blood (Fig. 2, II). We will give an example of plotting the curve of oxyhemoglobin dissociation of blood by our method. Conditions: blood with sodium oxalate, Hb 11%, volume of cell 16.7 mm^3, diameter 5.5 mm; polarization potential 1.375 V relative to a calomel electrode; the blood placed in the cell of the instrument is saturated by air with pCO_2 40 mm Hg; temperature 37°, rate of rotation of magnetic mixer 800 rpm.

Upon switching on the circuit, the light indicator of the M-95 micrometer shows 375 μA (see table), then the current strength begins to drop, the rate of which decreases with time. During the first min, the current strength is recorded every 10 sec, then every 30 sec (see table). After 780 sec of electrolysis, recording is stopped, the magnetic mixer is switched off,

table

Time from start of electrolysis (in sec)	Current strength (I) In μA	Current strength with correction for residual current In μA	$\log \frac{I_0}{I_t}$	Micro-coulombs $30 \cdot \sum_{+=0}^{+=\infty} I$	HbO_2 (%)	Time from start of electrolysis (in sec)	Current strength (I) In μA	Current strength with correction for residual current In μA	$\log \frac{I_0}{I_t}$	Micro-coulombs $30 \cdot \sum_{+=0}^{+=\infty} I$	HbO_2 (%)
Switched on	375	373		1387,8	100,0	390	42	40		245,5	18,4
10	213	211 (I_0)		1325,6	95,5	420	39	37		205,5	14,8
20	170	168		1255,2	90,5	450	37	35		168,5	12,1
30	155	153		1199,2	86,5	480	30	28		133,5	9,6
40	143	141		1148,2	82,8	510	23	21		105,5	7,6
50	133	131		1101,2	79,5	540	19	17		84,5	6,2
60	128	126		1057,5	76,2	570	15,5	13,5		67,5	4,9
90	113	111		973,5	70,3	600	13	11		54,0	3,9
120	100	98		862,5	62,3	630	10,5	8,5 (I_1)	1,394	43,0	3,1
150	90	88		764,5	55,2	660	8,7	6,7			
180	82	80		676,5	48,8	690	7,5	5,5			
210	76	74		596,5	43,2	720	6,5	4,5			
240	69	67		522,5	37,7	750	5,7	3,7		↑	
270	63	61		455,5	32,2	780	5,2	3,2 (I_2)	1,818		
300	57	55		394,5	28,5						
330	52	50		339,5	24,5	$t \to \infty$	2,0	0	∞	0	0
360	46	44		289,5	20,9						

and electrolysis is carried out for 5-10 min without mixing. The established current strength (2 μA) is taken as the residual current, i.e., as zero. Then the entire second column of the table is rewritten with the subtraction of 2 from each number and the readings of the third column are obtained. We calculate the value $\log (I_0/I_t)$ for two numbers, for example, of the last 150 sec, taking as I_0 the current strength, for example, every 10 sec after the start of electrolysis. These values are needed to calculate the quantity of remaining microcoulombs with respect to two values of the current strength: 8.5 μA (630 sec) and 3.2 μA (780 sec).

Calculation of the "residue" is performed by the formula

$$Q = I_1 \cdot \tau_K \cdot \frac{Q}{30} = \frac{0.43 \cdot I_1 \cdot t \text{ sec.}}{30 \left(\log \frac{I_0}{I_2} - \log \frac{I_0}{I_1}\right)} = \frac{0.43 \cdot 8.5 \,(5 \cdot 30)}{30 \cdot (1.818 - 1.394)} = 43.0.$$

Then we perform graphic integration by simple sequential summation from the bottom up of the numbers of the third column of the table up to the figure 111 inclusively. Since the intervals varied from 10 to 30 sec when recording the current strength, we add to the sum not 126 but $\frac{2}{3} \times 126$, i.e., 84. Then we add $\frac{1}{3}$ of 131, 141, 153, 168, and 211 since their recording was carried out with 10-sec intervals. From the value of the original reading of the galvanometer, we add $\frac{1}{6}$ of its value (373/6 = 62.2). These rules of summation follow from calculating the value of the integral by the trapezoid method (see M. Ya. Vygodskii, Handbook on Higher Mathematics, Moscow, 1961, p. 48) as the average of the values obtained by adding the row of numbers of the third column of the table, whereby it is done in one case from the bottom up and in another case from the top down.

To determine the total quantity of microcoulombs equivalent to the oxygen contained in the oxyhemoglobin, it is necessary to multiply 1387.8 (in the fifth column of the table) by 30 sec, i.e., by the main interval used in recording the current strength. Having multiplied any number of the fifth column of the table by 30, we obtain the quantity of microcoulombs corresponding to it, which is equivalent to the quantity of oxygen in the oxyhemoglobin at a given instant of time.

To plot the dissociation curve, it suffices to take the figure 1387.8 as 100% oxyhemoglobin. On the axis of the abscissa, we plot the readings of the current strength given in the third column of the table which are proportional to pO_2 (Fig. 3) and HbO_2% on the axis of the ordinate.

To calibrate the scale of pO_2, we can perform electrolysis with normal donor blood and establish to what value of the diffusion current corresponds, for example 50% of the total quantity of coulombs. In normal dissociation curves 50% HbO_2% corresponds to pO_2 37 mm Hg [1].

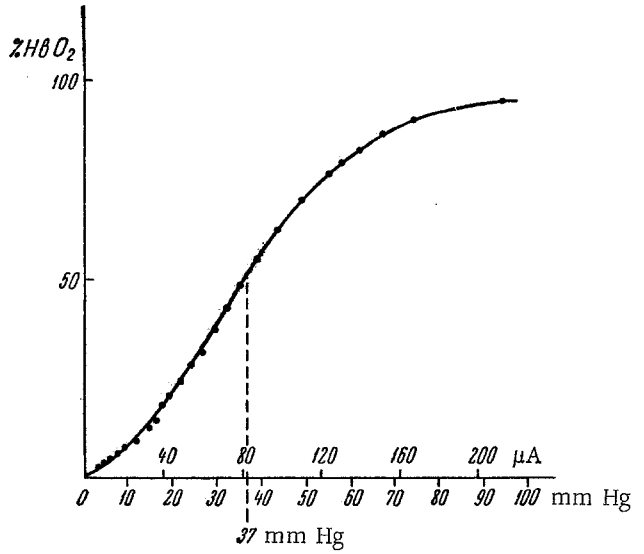

Fig. 3. Plotting of the oxyhemoglobin dissociation curve from the data of the table. Explanation in text.

This method permits judging the affinity of hemoglobin for oxygen based on the current strength at the start of electrolysis, the half-life of HbO_2, and oxyhemoglobin dissociation curve.

In addition to defibrinated blood, we can investigate hemolyzed blood or a solution of oxyhemoglobin.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
