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The Alteration of Red Blood Cells Charge in Patients with COPD of Varying Severity

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Abstract: The main goal of our study was to investigate erythrocytes charge balance in patients with COPD of different types and severity. Patients were divided into 5 groups according types of COPD (emphysematous and bronchial), severity (moderate or severe). The sixth group consisted of 32 healthy subjects. The erythrocytes charge balance was recorded based on ΔpH registration under heating. The first domain represented Δ pH in the range of temperatures from 37 to 58°C. The second domain represented the Δ pH after 20 minutes of incubation at 58°C. After erythrocytes suspension heating different types of thermograms were observed. In the range of 37 - 58°C prevalent type of thermograms of erythrocytes was found. Under incubation of erythrocytes at 58°C the different subtypes of thermograms were observed. We discussed the possible mechanisms of erythrocytes charge balance disorder.

Key words: Red Blood Cells Charge · Chronic Obstructive Pulmonary Disease

INTRODUCTION

Among the molecular and cellular events, determining the features of progression of chronic obstructive pulmonary disease (COPD), the changes of physicochemical properties of red blood cells (RBC) are consideredSantini M.T. *et al*. [1] demonstrated the change in the shape of RBC and guessed that alteration of shape of RBC and of blood rheological properties plays a key role for COPD progression.

The disorder of the RBC structural-functional state could lead to decreased deformability, increased tendency to aggregation, contributing to hemodynamic disorders, provokes further violations of the oxygen-transport function of blood, helping to hypoxia. It allowed us to consider the study of the biophysical characteristics of the state of erythrocytes in patients with COPD of varying severity as one of the promising directions of research.

Early we have reported about erythrocytes charge balance changing in patients with COPD of bronchial and mixed types [2]. Our results have demonstrated the prospects of further investigation of erythrocytes charge balance for understanding the mechanisms of RBC disorder at COPD.

The main goal of our study was to investigate erythrocytes charge balance in patients with COPD of different types and severity.

MATERIALS AND METHODS

Control subjects were healthy volunteers without any medication. All patients were on hospitalization and inspection. The syndrome of bronchial obstruction has been revealed at 100 percent of patients among receipted ones in hospitals. Respiratory insufficiency (RI) was established in reason of the syndrome of short wind in a rest condition and at the insignificant physical loading representing walking on 100 meters by slow rate (speed of movement is not higher than 5 steps in one minute). RI of I degrees was diagnosed at 25% of patients and RI of II degrees - at 75% of patients. Verification of the diagnosis was carried out on the basis of the complex of the standard criteria. At 100 percent of patients the habit to smoking tobacco is revealed. The index of smoking person has made > 200 at 67% of patients of 1-st group and at 73% of the patients including in 2-nd group. In 45% of patients are marked professional harm (the experience of underground work on collieries over 10 years, work on

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woodworking enterprises, cement works), atmospheric pollution (inhabitants of Kazakhstan industrial cities Temirtau, Karaganda, Balkhash have made 79% surveyed).

Basic clinical displays of COPD were cough with sputum and a short wind. Cough was marked during all day, less often only at night. The quantity of sputum was small, outside of aggravations its character was mucous. At 54% of patients elimination of sputum was occured after long cough. All patients carried out cytologic research of sputum which found out presence of alveolar epithelial cells, elastic fibres, siderophages. It was found out a plenty of the leukocytes submitted basically neutrophiles at 54% of patients of 1 group and at 61% of patients of 2 group during an aggravation. It was interpretated as the evidence of pyo-inflammatory process in mucous of bronchial tree.

Patients were divided into 5 groups. 29 patients with COPD, moderate severity mixed form (emphysematous and bronchial), exacerbation, respiratory insufficiency of grade 2 were included in first group. 21 patients with COPD, severity mixed form (emphysematous and bronchial), exacerbation, respiratory insufficiency of grade 2 were included in second group. 35 patients with COPD, moderate severity bronchial form, exacerbation, respiratory insufficiency of grade 2 were included in 3-hd group. 23 patients with COPD, severity bronchial form, exacerbation, respiratory insufficiency of grade 2 were included in 4-th group. The fifth group (n=12) represented patients with very severe bronchial form of COPD. The sixth group consisted of 32 healthy subjects.

Venous blood (3.0 ml) was drawn from patients and healthy ones into a heparinized syringe; blood was centrifuged (2000 g, 10 min, 4°C). The plasma and buffy coat were removed. The erythrocytes were washed (3×) in ice-cold phosphate buffered saline with following centrifugation (2000 g, 10 min, 4°C).

Registration of the RBC charge balance was performed in the conditions of the termal stimulation (heating) following to E.A. Gromov *et al*. protocol [3]. The principle of registration of the charge balance of red blood cells is based on the change of pH of erythrocytes (Δ pH) at the termal stimulation.

For research of charge balance of blood the device was constructed (innovative patent of the Republic of Kazakhstan N 25629) and software was developed.

Washed erythrocytes were diluted by physiological solution having a room temperature, in the ratio 1: 6 (1 part of erythrocytes, 6 parts of physiological solution). The suspension was stirred gently and was poured in the cuvette of device. Program of measurement was launched. The recording began at the heating of cuvette till 35°C.

Registration of RBC thermograms was carried out in two modes. In the first case change of pH in the range of temperatures from 37 to 58°C recorded. In the second case change of pH recorded at a constant temperature 58°C for 20 minutes.

Two domains were used for optimization of analysis. The first domain was in the range of temperatures from 37 to 58°C. The first reference point of pH registration was at temperature of 37°C, the second reference point was at 58°C.

The various variants of Δ pH changing could be observed. This parameter could increase (+ ΔpH) or decrease (-ΔpH). The second domain represented the Δ pH at a constant temperature (58°C) after 20 minutes of incubation.

RESULTS

The typical thermograms of erythrocytes of healthy volunteers are presented on the Figures 1-4. As a result of the data revealed the presence of two subtypes of RBC thermograms.

Figure 1 shows the first subtype of the thermogram of erythrocytes. In the temperature range from 37 to 58°C parabolic dependence of ΔpH change was fixed. Maximum ΔpH (to 0.034) was observed at 41,6 °C. The transition point into the negative region of ΔpH was at 46.8°C and to 58°C ΔpH had reached the value - 0.17.

Figure 2 shows the second subtype of RBC thermogram. Parabolic dependence of ΔpH change was saved in the temperature range from 37 to 58°C. Maximum of ΔpH, equal to 0.088 was observed at 42,8°C. The transition point into the negative region of ΔpH was at 50.9°C. Upon heating to 58°C ΔpH had the value - 0.12.

During the incubation of erythrocytes at the temperature 58°C for 20 minutes in all the examined healthy volunteers the RBC thermograms had two segments: reduction of ΔpH and slow consequent increase.

The quantitative characteristics of ΔpH allowed to divide the RBC thermograms into two subtypes (Fig. 3 and 4).

In 57% of RBC thermograms the negative values of ΔpH relative to the point of incubation starting lying in the range from -0.119 to -0.188 with average value -0.146. Increase of ΔpH was recorded between 8 and 11 minutes of incubation. In this case, the increase of ΔpH was clearly expressed (Figure 3).

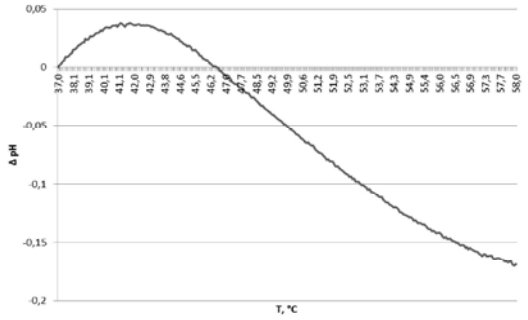

Fig. 1: The RBC thermogram (first subtype) of healthy person (range of temperature change from 37°C to 58°C).

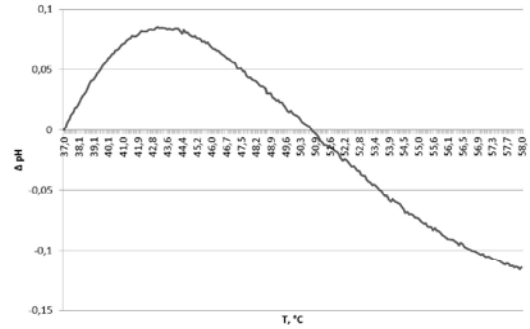

Fig. 2: The RBC thermogram (second subtype) of healthy person (the range of temperature change from 37°C to 58°C).

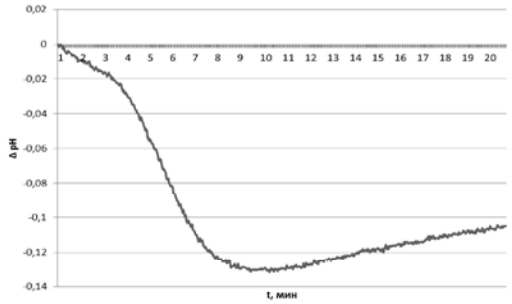

Fig. 3: The RBC thermogram (first subtype) of healthy person (incubation at 58 °C for 20 minutes).

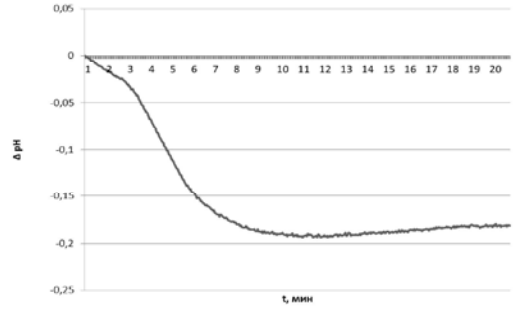

Fig. 4: The RBC thermogram (second subtype) of healthy person (incubation at 58 °C for 20 minutes).

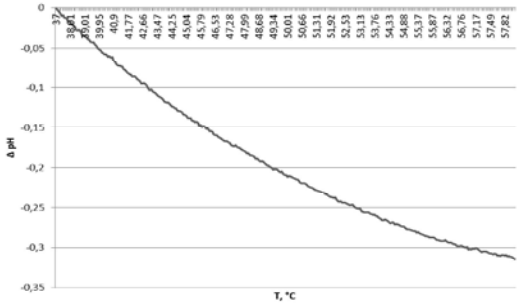

Fig. 5: The RBC thermogram in patient with COPD of mixed type, moderate (range of temperature change from 37°C to 58°C).

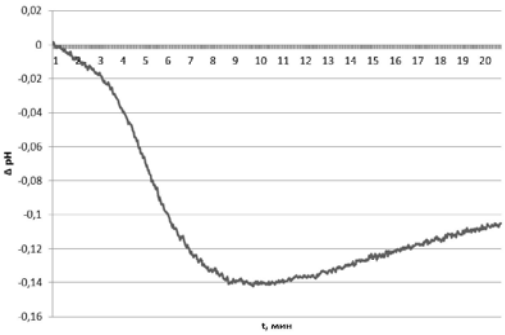

Fig. 6: The RBC thermogram in patient with COPD of the mixed type, moderate (incubation at 58°C for 20 minutes).

In 43% of cases the RBC thermograms had negative value of ΔpH -0,156 (spread from -0.135 to -0.194). Increase of ΔpH value was detected between 9 and 12 minutes of incubation. ΔpH increase was not clearly expressed, as in the previous subtype (Figure 4).

The registration of RBC thermograms in COPD patients (mixed type, moderate) was conducted (Figures 5 and 6). At the heating of erythrocyte suspension from 37°C to 58°C at 100% of patients of this group ΔpH change had the appearance of an uniformly decreasing curve (Figure 5). The negative values of ΔpH compared to starting point in 37°C ranged from -0.293 to -0.367 with average value -0.329.

During the incubation of red blood cells at 58°C for 20 minutes at the 100% of patients with COPD of mixed type, moderate, the thermograms of RBC suspension had a similar type: ΔpH reduction with tendency to increase (Figure 6). The negative values of ΔpH laid in the range from -0.129 to -0.161 with average value -0.146. The trend to increasing of ΔpH was recorded between 9 and 11 minutes of incubation.

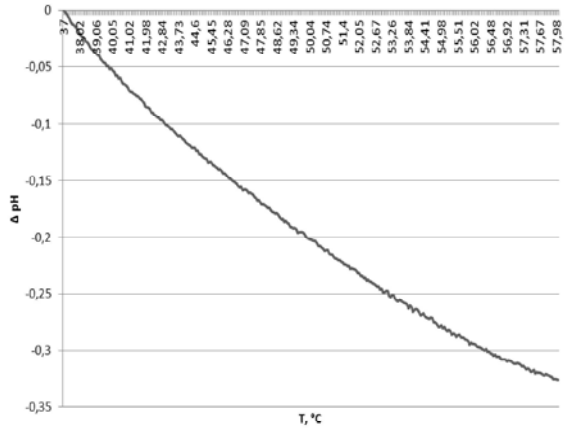

Fig. 7: The RBC thermogram in patient with COPD of mixed type, severe (range of temperature change from 37°C to 58°C).

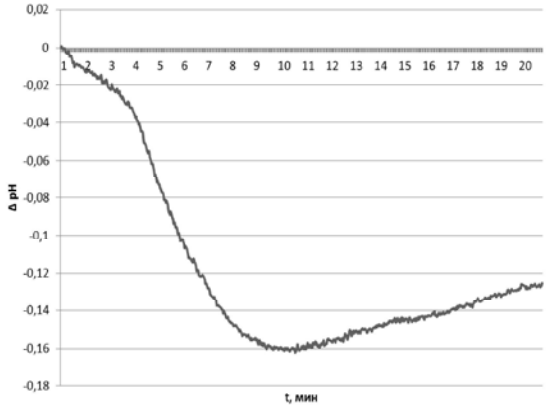

Fig. 8: The RBC thermogram (first subtype) in patient with COPD mixed type, severe (incubation at 58°C for 20 minutes).

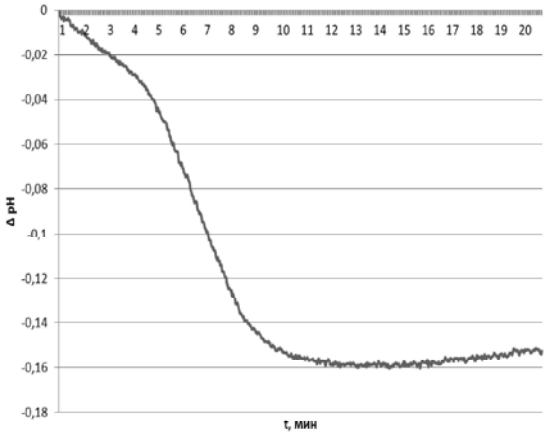

Fig. 9: The RBC thermogram (second subtype) of patient with COPD of mixed type, severe degree (incubation at 58°C for 20 minutes).

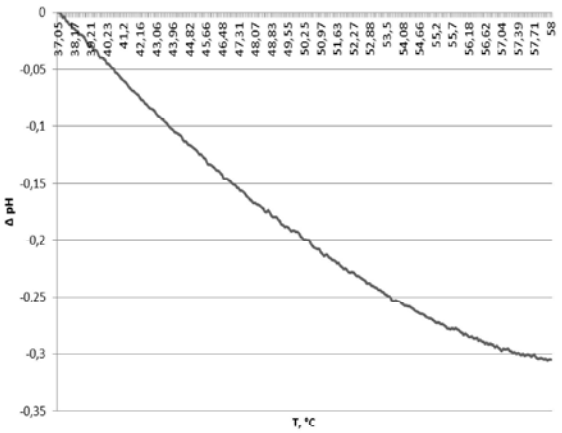

Fig. 10: The RBC thermogram of patient with COPD of bronchial type, moderate severity (range of temperature change from 37°C to 58°C).

Figures 7-9 depicted the RBC thermograms of patients with COPD of mixed type of severe degree.

100% of patients of this group had the same type of ΔpH change during incubation of erythrocyte suspension from 37°C to 58°C, which was described by monotonically decreasing curve (Figure 7). The maximum negative values of ΔpH compared to starting point (37°C) laid in the range from -0.248 to -0.440 with average value -0.330.

In the time of incubation of erythrocytes in patients with COPD of mixed type, severe degree, at 58°C for 20 minutes, the dependence of change for ΔpH from time was characterized by the presence of two types of curves.

The thermograms of 63% of patients of this group recorded a decrease of ΔpH followed by an increasing (Figure 8). The maximum negative values of ΔpH relatively to the point of beginning of incubation are in the range from -0.150 to -0.195 with average value -0.163. The trend to the increasing of ΔpH was recorded between 9 and 10 minutes of incubation.

At 37% of patients with COPD mixed type, severe degree the trend to increasing of ΔpH was not observed. After declining the curve flattened out and ΔpH practically unchanged. The negative values of ΔpH varied from -0.160 to -0.167, the average value of ΔpH was -0.163 (Fig. 9).

The RBC thermograms of patients with COPD bronchial type, moderate severity were depicted on the figures 10-12. In all patients of this group ΔpH dependence from the temperature had the form of a monotonically decreasing curve (Figure 10). Values of ΔpH according to starting point (37°C) were varied in the range of -0.252 to -0.375, average value was -0.316.

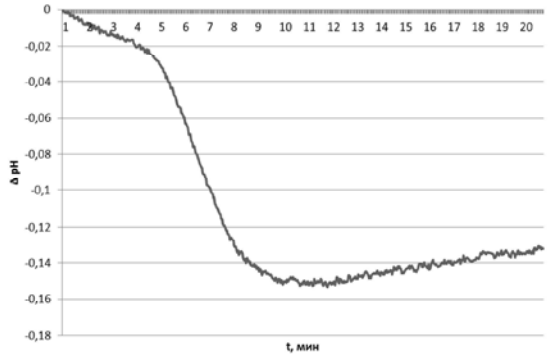

Fig. 11: The RBC thermogram (first subtype) in patient with COPD bronchial type, moderate severity (incubation at 58°C for 20 minutes).

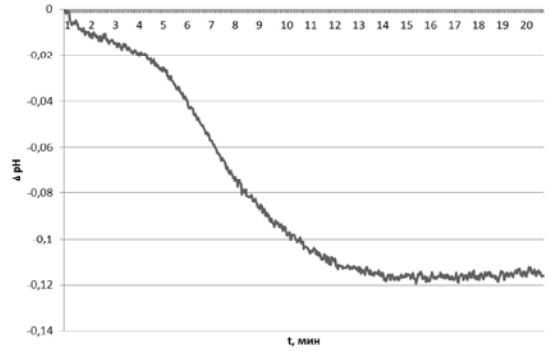

Fig. 12: The RBC thermogram (second subtype) in patient with COPD of bronchial type of moderate severity (incubation at 58°C for 20 minutes).

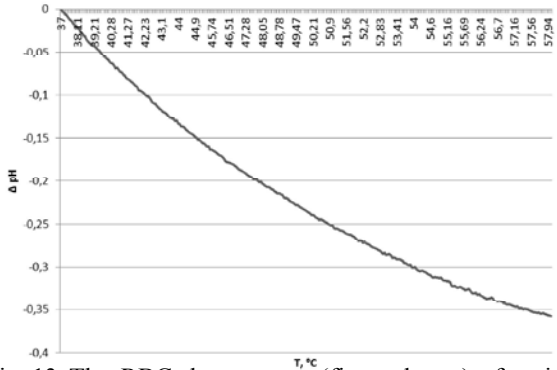

Fig. 13: The RBC thermogram (first subtype) of patient with severe COPD of bronchial type (range of temperature change from 37°C to 58 °C).

In the time of incubation of erythrocytes at 58°C for 20 minutes two types of thermograms were observed. The first type of RBC thermogram was observed at 78% of patients. The ΔpH consistently decreased from 10 up to 13 minutes of incubation (Figure 11). The values of Δ pH changes were in the range of -0.105 to -0.154 with average value -0.136.

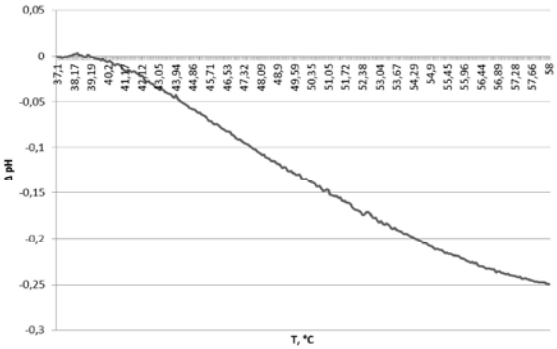

Fig. 14: The RBC thermogram (second subtype) of patient with severe COPD of bronchial type (range of temperature change from 37 °C to 58 °C).

The second type of RBC thermogram was observed at 22% of patients (Figure 12). The peculiarity of this thermogram type was to have the achievement a “plateau” after 5 - 7 minutes of RBC incubation. ΔpH values varied from -0.119 to -0.177, the average value was -0.148.

The thermograms of red blood cells in patients with severe COPD of bronchial type were showed on the figures 13-15.

The analysis of the thermograms allowed to identify two variants of RBC answers to heating. 80% of RBC thermograms reflected uniformly decreasing ΔpH in depending on temperature from 37°C to 58°C (Figure 13). The negative values of ΔpH were in the range from -0.291 to -0.451, average value was -0.358.

Slight increase of ΔpH (0.004) fixed at the 20% thermograms in the temperature range from 37 to 39°C, followed by a transition to negative value (average value was -0.249).

During the incubation of red blood cells at 58°C for 20 minutes one type of curve was fixed (Figure 15). The negative values of Δ pH relatively to the point of starting of incubation varied from -0.114 to -0.204, average value was -0.150. From 10 up to 13 minutes of incubation starting of ΔpH increasing was recorded.

Figure 16 depicted the thermograms of red blood cells of patients with very severe COPD. Change in ΔpH of the erythrocyte suspension, depending on the temperature was characterized by two main types of curves. In 75% we observed uniformly decreasing curves (Figure 16). The negative values of ΔpH relatively to the point of starting of incubation varied from -0.221 to -0.365, the average value of ΔpH was -0.305.

Parabolic dependence of ΔpH depending on the temperature was observed at the 25% (Figure 17). The average value of ΔpH was 0.022 (variation from 0.020

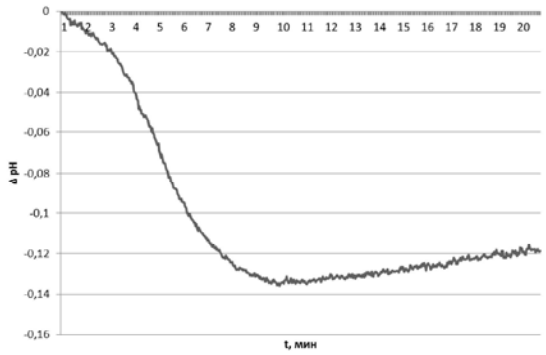

Fig. 15: The RBC thermogram of patient with COPD of bronchial type, severe (incubation at 58°C for 20 minutes).

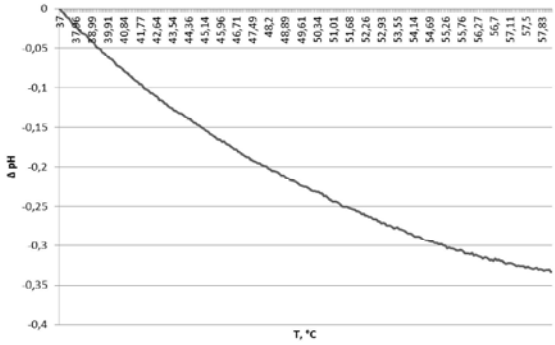

Fig. 16: The RBC thermogram (first subtype) of patients with very severe COPD (range of temperature change from 37°C to 58°C).

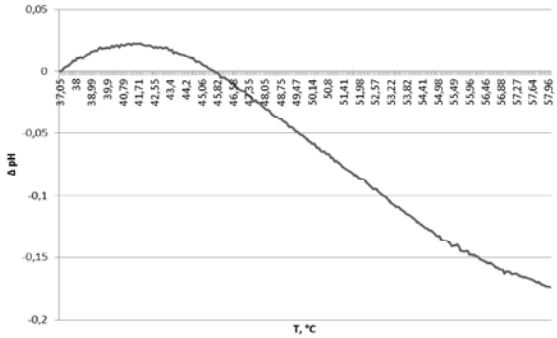

Fig. 17: The RBC thermogram (second subtype) of patient with very severe COPD (range of temperature change from 37°C to 58°C).

till 0.023) in the comparison with ΔpH at temperature of 37°C. The temperature of transition in the negative region was in the range of 45 - 46°C. The average value of ΔpH was - 0.172 (variation from - 0.171 to - 0.174).

The dependence of ΔpH change from the incubation time at 58°C was characterized by three subtypes of curves (Fig. 18-22). The first type was found at 57%.

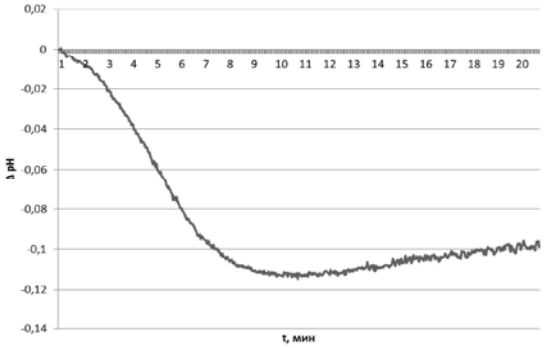

Fig. 18: The RBC thermogram (first subtype) of patient with very severe COPD (incubation at 58°C for 20 minutes).

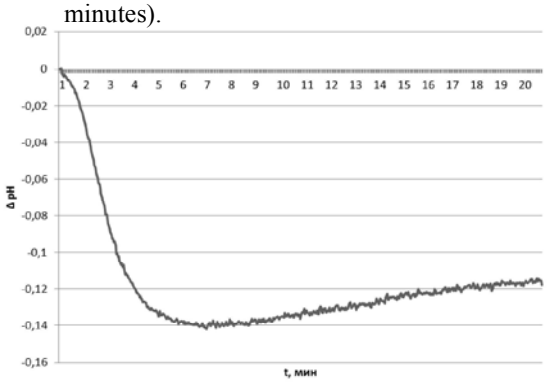

Fig. 19: The RBC thermogram (second subtype) of patients with very severe COPD (incubation at 58°C for 20 minutes).

In the early stages of incubation the change of ΔpH continued to decrease and reached the average value -0.134 (range from -0.103 to -0.177). Light tendency to increasing of ΔpH was fixed on 10-12 minutes of incubation (Fig. 18).

In 29% of cases in the group of patients with very severe COPD were observed variations of types of RBC thermograms. In the early stages of incubation the changes of ΔpH decreased and reached the mean value -0.132 (range from -0.124 to -0.140). Tendency to increasing of ΔpH fixed on the 7-9 minutes of incubation (Fig. 19).

In 12% of cases in the group of patients with very severe COPD we observed the different velocity of ΔpH decreasing. Light tendency to increasing of ΔpH was fixed on 14-15 minutes of incubation (Fig. 20).

One patient of this group had severe degree COPD developed after fibro-cavernous tuberculosis. The RBC thermogram of this patient recorded at the incubation of suspension at 58°C for 20 minutes, had individual type not observed at the other patients before (Figure 21).

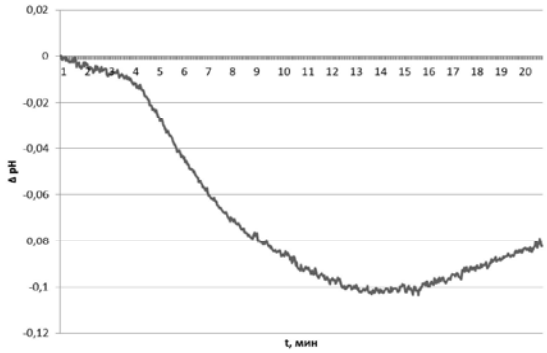

Fig. 20: The RBC thermogram (third type) of patients with very severe COPD (incubation at 58°C for 20 minutes).

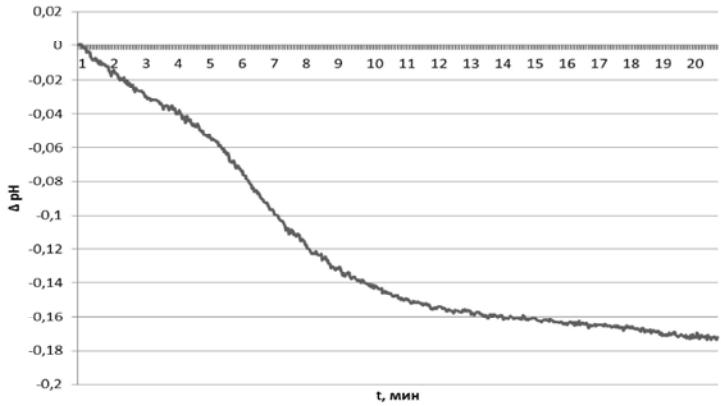

Fig. 21: The RBC thermogram of patient with very severe COPD (incubation at 58°C for 20 minutes).

Such monotonically decreasing curve had "stepped" look. The maximum change of the pH relatively to the starting point is -0.174.

Therefore, the results received showed the existence of differences in the relaxation rate of protons and ionic groups, determining the change of pH at the thermo induction of erythrocytes of patients with COPD with different clinical forms and severity.

DISCUSSION

In the normal conditions, the erythrocytes carry a negative surface charge. The magnitude of the integral charge depends on the qualitative and quantitative characteristics of the lipid component of membranes and sialic acids [4]. Also the erythrocyte charge is determined by the state of their metabolic processes.

The heating of erythrocytes is characterized by formation of lipid pores [5], infringement of lipid asymmetry particularly at the activation of lipid peroxidation. Formation of carbonyl derivatives in erythrocyte membranes can influence also on the integrated charge of erythrocytes [6].

At the thermoinduction of erythrocytes in patients with COPD in the range of 37 - 58°C a prevalent type of thermograms was found: a monotonically decreasing dependence. The negative value of Δ pH associated with increased release of protons. Proton source in this case may be the oxidation of hemoglobin [7], excessive formation of lactate, infringement of regeneration of adenine nucleotides.

In our opinion, different types of thermograms recorded during the incubation of erythrocytes for 20 minutes at 58°C, determined primarily by the state of the anion exchangers. Results demonstrating the reduction of function of AE1 anion exchanger in erythrocytes of patients with progression of COPD could be considered like evidence in favor of this assumption [8].

We supposed that the different time and points of transition demonstrated the disorder of membrane proteins and its conjunction with cytoskeleton components. Taken together it determines the ability of erythrocytes to regulate their volume. Charge of erythrocytes also largely determines their stability, ability to deformation and ability to reversible aggregation [9].

CONCLUSION

Thus, the trend of infringements of RBC charge balances depending on clinical type and severe of COPD was set by our research. The results obtained demonstrated special features of RBC charge balances alteration among patients with similar type and severity of COPD. The future investigation will be provided to explain mechanisms, determining RBC charge balances changing and its possible significance for diagnosis and prognosis of COPD progression.

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