VERSITA

Bull Vet Inst Pulawy 56, 183-188, 2012
DOI: 10.2478/v10213-012-0033-1

DETERMINATION OF ACID-BASE BALANCE DURING THE ACUTE STAGE OF EXPERIMENTAL PULMONARY BAROTRAUMA AND AFTER ITS HYPERBARIC TREATMENT

PIOTR SIERMONTOWSKI, DOROTA KACZERSKA[1], BARTOSZ MORAWIEC[2], AGNIESZKA PEDRYCZ[3], ADAM OLEJNIK[4], ROMUALD OLSZAŃSKI, AND ZBIGNIEW BORATYŃSKI[5]

Department of Maritime and Hyperbaric Medicine,
[2]Department of Epidemiology and Tropical Medicine
Military Institute of Medicine, 81-103 Gdynia, Poland.
[1]Department of Clinical Nutrition, Medical University of Gdansk, 80-210 Gdansk, Poland
[3]Department of Histology and Embryology with Laboratory of Experimental Cytology,
Medical University of Lublin, 20-080 Lublin, Poland
apw4@wp.pl
[4] Department of Underwater Work Technology, Naval Academy, 81-103 Gdynia, Poland
[5]Department of Animal Anatomy and Histology, Faculty of Veterinary Medicine,
University of Life Sciences Lublin, 20-950 Lublin, Poland

Received: January 23, 2012 *Accepted: May 15, 2012*

Abstract

The aim of the presented study was to determine whether pulmonary barotrauma without pneumothorax affects the parameters of acid-base balance. The experiment was conducted on rabbits divided into experimental and control groups. The animals from the experimental group were subjected to compression in the hyperbaric chamber and pulmonary barotrauma was induced using a method developed by authors. The arterial blood was sampled before the experiment, after anaesthesia and intubation, after injury, and after treatment (in the group undergoing hyperbaric therapy). All parameters of acid–base balance were found to be within the reference ranges. Since the biggest changes were observed after anaesthesia and intubation and not after the injury, it was concluded that pulmonary barotrauma had no significant effect on the parameters of acid-base balance.

Key words: rabbit, pulmonary barotrauma, acid-base balance.

Pulmonary barotrauma, the most common life-threatening pathology in divers, is also observed in intensive care and neonatal intensive care units. The ventilator-induced pulmonary barotrauma is commonly accompanied by pneumothorax whereas vascular injuries and gas embolism are frequently observed in divers. According to numerous literature reports, the dramatically decreased respiratory surface, observed in the cases of pneumothorax, exerts significant effects on respiratory efficiency and further on acid-base balance (3). However, there are no studies on the effects of pulmonary barotrauma without pneumothorax on acid-base balance carried out in larger groups of animals.

The aim of the study was to determine whether pulmonary barotrauma without pneumothorax affected pO_2, pCO_2, HCO_3^-, blood pH, and other parameters of acid-base balance. Till now, there are very few articles on the presented subject. Our study, apart from conclusions drawn from the experiments, shows some results (norms) in acid-base balance in blood of control rabbits. It can be useful for other authors in their future studies on acid-base balance in rabbits. This type of results cannot be found in literature and veterinary handbooks.

Material and Methods

The experiment was carried out on 34 German Lop rabbits of both sexes, aged 28-34 weeks, and weighing 2,240-5,250 g.

The experiment was performed in a pressure chamber for small animals, into which the anaesthetised and intubated rabbits were placed (10). The air pressure in the chamber was increased at the rate of 1 ata per min up to the 3 ata. At the peak of inspiration, the airway was mechanically blocked and immediate decompression was initiated at the rate of 3 ata/min. Once the atmospheric pressure was achieved, the animals were removed from the chamber and desintubated. The entire management based on our experimental model imitates the mechanism of pulmonary barotrauma in divers (14).

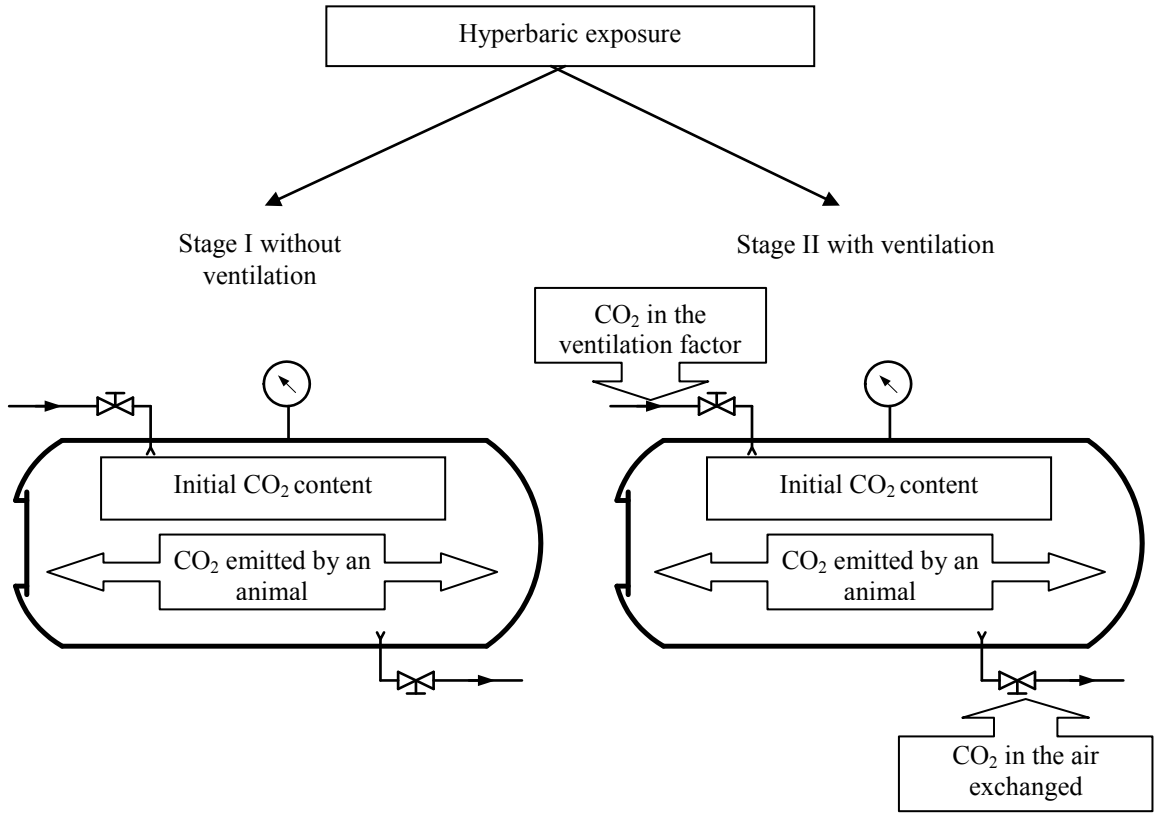


Fig. 1. Hyperbaric exposure divided into two stages - without and with ventilation.

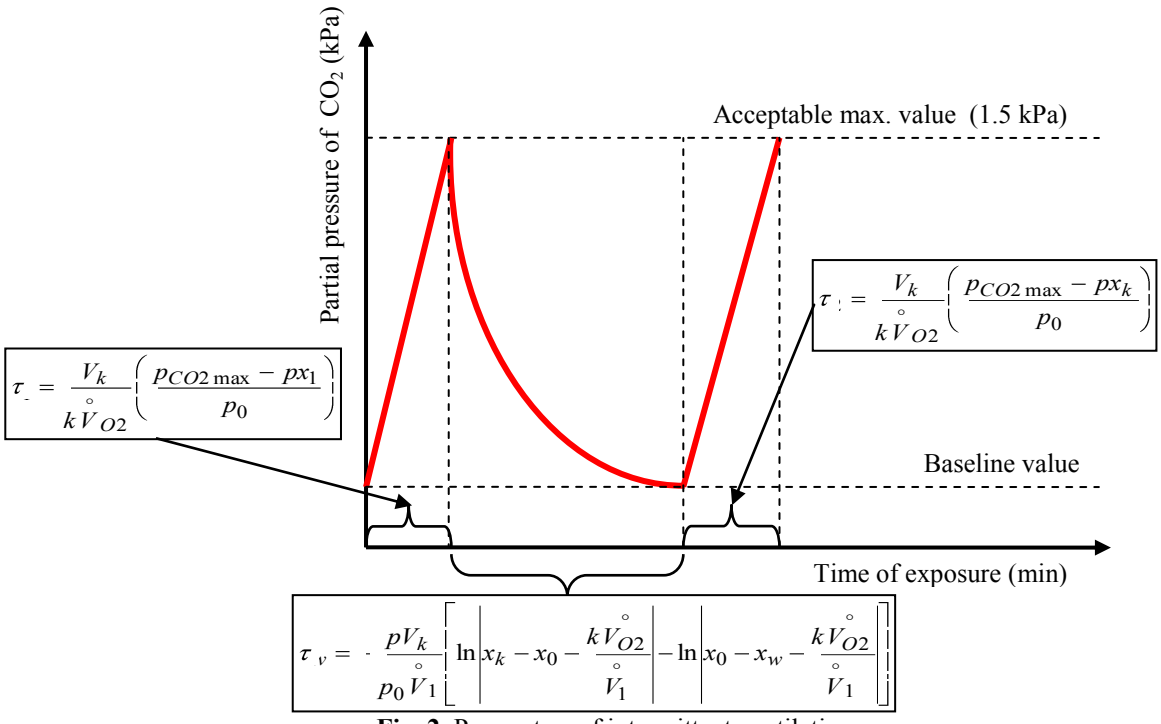


Fig. 2. Parameters of intermittent ventilation.

τ_1 – time to the onset of first ventilation, τ_w – time of ventilation, τ_2 – time to the onset of another ventilation, V_k – decompression chamber volume, p_{CO2max} – max. acceptable partial pressure of carbon dioxide in the chamber, p – working pressure of the chamber, x_1 –molar fraction of CO_2 in the chamber air before exposure, k – number of animals (in the present experiment -1), x_k – molar fraction of CO_2 after completion of ventilation, x_w – molar fraction of CO2 in the ventilation factor, x_0 – molar fraction of CO_2 in the chamber air at the onset of ventilation.

To obtain reliable results, proper ventilation of the chamber had to be provided preventing any changes in the percentage composition of respiratory mixture. Proper ventilation methods should not allow achieving the borderline partial pressure of CO_2, assumed at 1.5 kPa. The intermittent ventilation was used, involving cyclic exchange of respiratory air in the chamber. For this reason, hyperbaric exposure was divided into two stages: without and with ventilation (Fig. 1) (4, 12).

The used method is based on several mathematic formula derived from the relations describing the changes in the molar fraction of carbon dioxide during individual phases of exposure, which enable to determine the studied ventilation parameters (Fig. 2).

The used methods included standard diving and operating procedures carried out during any kind of exposure (4, 12). The time to the first ventilation and the time of ventilation were calculated based on the formula considering the CO_2 content in the chamber before exposure, whereas the time to the next ventilation(s) was calculated using the formula with the CO_2 content after completion of ventilation. Using the tables (9), the level of CO_2 emitted by the animal in the chamber was determined. The CO_2 fraction in the chamber atmosphere was determined based on calculations and measurements before and after exposure. Moreover, the fact that each next decompression station decreases the partial pressure of carbon dioxide was considered.

The exposure to hyperbaric conditions was short (4 min). Therefore, air pressure, including partial pressure of oxygen, was increased over the first 3 min; during the 4th min, the animals did not breathe and the chamber was not ventilated. During hyperbaric therapy, the chamber was ventilated.

The animals of the control group were provided with the same breeding conditions as the experimental animals. The control group was divided into two subgroups: the first one - five animals subjected only to intubation and another one (procedure control) five animals subjected to all experimental procedures performed in the experimental groups except for airway blocking.

The experimental animals with induced barotrauma were also divided into two subgroups:

- subgroup d – 10 animals sacrificed immediately after decompression to atmospheric pressure,
- subgroup dl – 10 animals undergoing post-decompression hyperbaric oxygen therapy according to the modified therapeutic table '6' (Table 1) and then sacrificed.

During the autopsy, the presence or lack of pneumothorax was determined. Moreover, the presence of macroscopic morphological markers of a recent pulmonary barotrauma was confirmed. The results of animals with autopsy-detected pneumothorax were excluded from further analysis (15).

The arterial blood was collected from the auricular artery branch, medially or externally to the auricle, using a 0.6 mm needle to heparinised capillaries (11) before anaesthesia, after intubation, after barotrauma, and after hyperbaric therapy.

Table 1

	Depth (m)										
	50	42	36	30	24	18	15	12	9	6	3
Time (min)	3	1.2	1.2	1.2	1.2	3	3	3	72	12	12

After collecting and mixing, the capillaries were marked and placed in the refrigerator at 4°C. After the experiment, gasometric measurements were performed in each animal; pH, partial pressure of carbon dioxide (pCO_2), and partial pressure of oxygen (pO_2) were determined. The remaining parameters of acid-base balance, *i.e.* actual and standard concentrations of bicarbonates ($aHCO_3$ and $sHCO_3$, respectively), base deficit-excess (BDE), concentration of total carbon dioxide (tCO_2), were calculated by an automated analyser. The determinations were carried out in a rkz analyser (Corning 288, Ciba-Corning) using suitable reference electrodes: an Ag/AgCl electrode for pH determinations (16), a Severinghaus electrode for pCO_2 determinations (13), and Pl-cathode and Ag-anode for pO_2 determinations for electrolysis intensity. The actual concentration of bicarbonates was calculated by the analyser using the following formula:

$$HCO_3 = 0.031 \cdot p_{CO2} \cdot 10^{(pH-6.1)} \quad (1)$$

Whereas, the standard concentration was calculated according to the formula:

$$HCO_3 = (24.5 + 0.9A + (4 - 0.9A)) \cdot \frac{(2.65 + 0.3Hb)}{1000} \quad (2)$$

where

$$A = (3E - 0.2Hb) \cdot \frac{(100 - 0_{2SAT})}{100} \quad (3)$$

and total CO_2 concentration according to:

$$t_{CO2} = [0.031 \cdot p_{CO2}) + HCO_3] \quad (4)$$

The quality of results was checked using standard samples of gases from Ciba-Corning. The precision of determinations was described as the coefficient of variation CV%, and the values obtained were CV% 3.6–4.9 for the analysed parameters.

The results were collected in tables and statistically analysed using the analysis of variance.

Results

The findings enabled to assess the effects of the individual stages of the experiment, particularly of barotrauma, on acid-base balance.

The level of blood pH after the experiment was slightly, yet statistically significantly increased (Fig. 3) and was within the level of confidence for the baseline pH. The results in all groups were within reference values for rabbits (6, 8) and similar species (11). The highest pH increase was observed immediately after intubation.

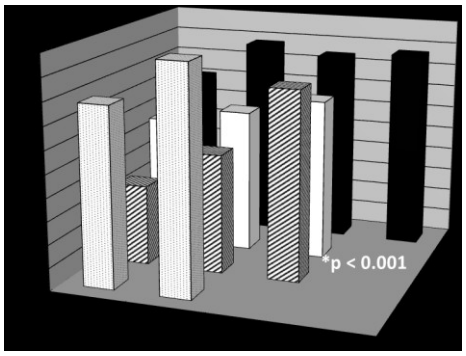


Fig. 3. Changes in pH.

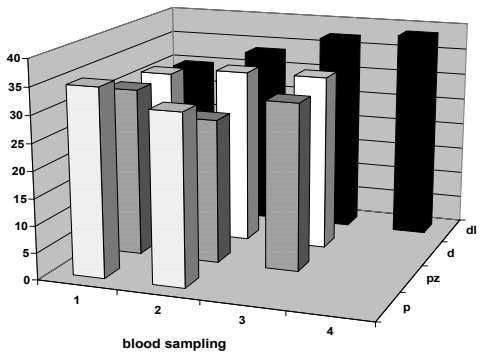


Fig. 4. Changes in partial pressure of CO_2.

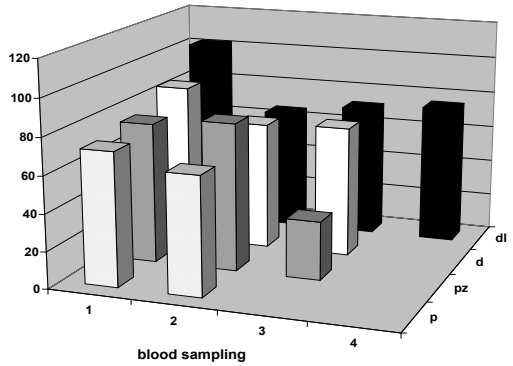


Fig. 5. Changes in partial pressure of O_2.

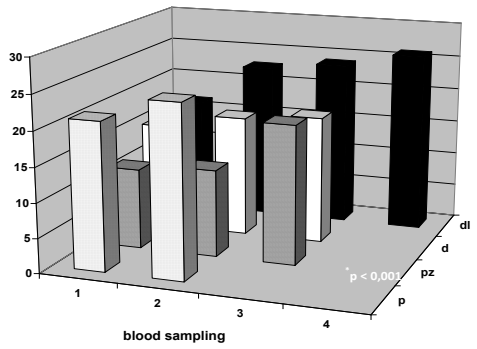


Fig. 6. Changes in actual concentration of bicarbonates.

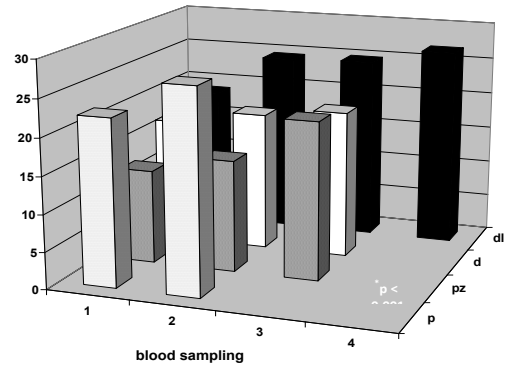


Fig. 7. Changes in standard concentration of bicarbonates.

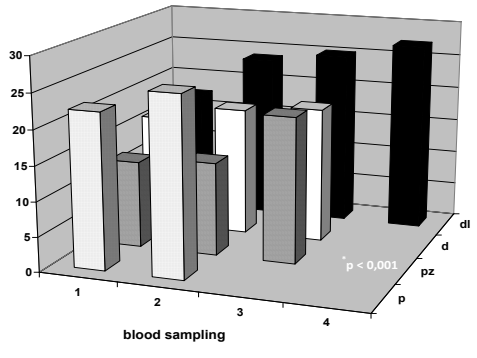


Fig. 8. Changes in total concentration of CO_2.

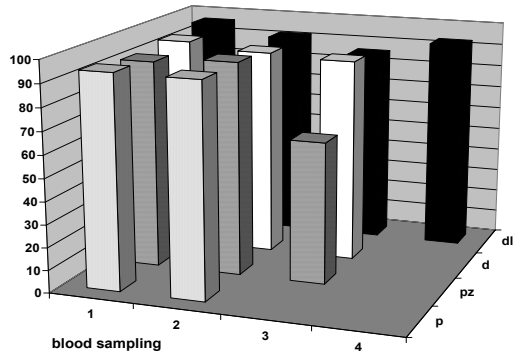


Fig. 9. Changes in O_2 saturation.

Moreover, partial pressure of carbon dioxide was found to be elevated, yet within the reference ranges (Fig. 4), whereas, partial pressure of oxygen was decreased (Fig. 5).

During the experiment, both the actual (Fig. 6) and standard concentration of bicarbonates were statistically significantly higher (Fig. 7); however, they were still within the reference values).

Similarly to partial pressure, the total concentration of carbon dioxide statistically significantly increased (Fig. 8).

Moreover, oxygen saturation was slightly increased, yet the changes were not statistically significant (Fig. 9).

Discussion

In the bicarbonate buffering system, carbon dioxide (CO_2) acts as an acid and bicarbonates (HCO_3^-) as a base (1, 2, 5). Since in all rabbits from the experimental groups the concentration of HCO_3^- was elevated after barotrauma, it can be assumed that this factor causes an increase in blood pH.

In subgroups d and dl after anaesthesia and intubation, gasometric changes were observed, *i.e.* a decreased oxygen pressure and increased carbon dioxide pressure. This was most likely associated with hypoventilation caused by anaesthesia without the control of respiratory parameters (respiration rate, oxygen saturation) and with intubation followed by short airway blocking, which led to a momentary lack of pulmonary ventilation and inability to remove the excess of CO_2 from the body. By tending to restore the balance, the homeostatic system induced secondary CO_2 turnover (7), *i.e.* CO_2 and H_2O combined to form the carbonic acid (H_2CO_3) under the higher pressure; after decompression and return to normobaric conditions, weak H_2CO_3 was decomposed to H^+ and HCO_3^- ions.

Intubation followed by airway block also caused a decrease in partial oxygen pressure, leading to anoxia and a decrease in oxygen saturation. However, the findings demonstrated that after the experiment, partial O_2 pressure slightly increased.

After induced barotrauma, no statistically significant changes in gasometric parameters were observed in the experimental group. Hence, we can conclude that lung injuries, other than pneumothorax, do not significantly affect the partial pressure of oxygen and carbon dioxide. Hypoventilation with carbon dioxide retention should lead to respiratory acidosis (5, 7). During the individual stages of the experiment, pH did not change significantly whereas increased concentration of bicarbonate ions after anaesthesia and intubation evidenced metabolic compensation for hypoventilation-induced respiratory acidosis. This is particularly visible in animals under longer observation, treated for barotrauma, which is connected with slower action of compensation metabolic mechanisms in response to rapid respiratory changes.

It is worth analysing why the pressure of gases and concentration of bicarbonate ions were not normalised during hyperbaric therapy with proper chamber ventilation. Was it associated with the depressive effects of drugs on respiratory parameters, parenchymal damage, or CNS changes caused by hypoxia and hypercapnia? Both hypercapnia observed after intubation and experimental parenchymal injury should result in accelerated respiration with carbon dioxide elimination, yet the pressure of this gas remained within normal limits and was only slightly different from the pre-barotrauma values.

The findings demonstrated that barotrauma, whose morphologic markers were found during autopsy macroscopically and microscopically, did not markedly affect acid-base balance. Moreover, the results showed indirectly that even extensive and multiple barotrauma did not affect significantly the respiratory efficiency.

In conclusion, pulmonary barotrauma, unless accompanied by pneumothorax, does not have significant effects on acid-base balance. Laboratory acid-base balance tests are not useful for the diagnosis of pulmonary barotrauma uncomplicated with pneumothorax. The changes in gasometric parameters observed during the experiment were related to anaesthesia, intubation, and airway block.

References

1. Adrogue H.J., Madias N.E.: Management of life-treatening acid-base disorders. N Engl J Med 1998, **338**, 26-34, 107–111.
2. Barzago M.M., Bortolotti A., Omarini D., Aramayona J.J., Bonati M.: Monitoring of blood gas parameters and acid-base balance of pregnant and non-pregnant rabbits (*Oryctolagus cuniculus*) in routine experimental conditions. Lab Anim 1992, **26**, 73-79.
3. Brongel L., Duda K.: Mnogie i wielonarządowe obrażenia ciała. PZWL Warszawa, 2001, pp. 217–219.
4. Charchalis A., Olejnik A: Doświadczalna weryfikacja modelu wentylacji atmosfery komory dekompresyjnej podczas powietrznych ekspozycji hiperbarycznych. Pol Hyperbaric Res 2004, **1**, 43–49.
5. Harabin A.L., Farhi L.E.: Blood-gas transport in awake rabbits exposed to normobaric hyperoxia. Undersea Biomed Res 1987, **14**, 133-147.
6. Hillyer E.V.: Pet rabbits. The veterinary clinics of North America. Small Anim Pract 1994**, 24,** 25-65.

7. Jin W., Jiang J., Wang X., Zhu X., Wang G., Song Y., Bai C.: Continuous intra-arterial blood pH monitoring in rabbits with acid-base disorders. Respir Physiol Neurobiol 2011, **177,** 183–188.
8. Jones R.T.: Normal values for some biochemical constituents in rabbits. Lab Anim 1975, **9,** 143-147.
9. Lumb W.V.: Small Animal Anesthesia. Lea & Febiger. Philadelphia 1963, pp. 40-41.
10. Mack P.O., Ng T.H., Song I.C.: Anaesthesia for animals in experimental research. Ann Acad Med Singapore 1994, **23,** 470-474.
11. Nicpoń J., Sławuta P., Nicpoń J., Noszczyk–Nowak A.: Hematological, biochemical, and acid-base equilibrium parameters of the European brown hare. Medycyna Wet 2007, **63**, 1239-1241.
12. Olejnik A.: Metodyka wentylacji atmosfery komory dekompresyjnej podczas powietrznych ekspozycji hiperbarycznych: Ed. PTMiTH, Gdynia 2007, pp. 78-102 and 120–123.
13. Severinghaus J., Bradley A.: Electrodes for blood pO2 and pCO2 determination. J Appl Physiol 1968, **13,** 515–520.
14. Siermontowski P., Kozłowski W., Olszański R., Pedrycz A.: The significance of an experimental model of pulmonary barotrauma in examining diving accidents. Pol Hyperbaric Res 2012, **1,** 6–24.
15. Siermontowski P., Kozłowski W., Olszański R., Wojcieszek P.: Macroscopic and microscopic changes in the exponents of experimental pulmonary barotrauma. Pol Przegl Med Lotn 2001, **1**, 17–25.
16. Sorensen S.: Enzyms studien: II uber die messung and die bedeutung der wasserstoffionenkonzentration bei enzymatischen prozessen. Biochemistry 1909, **12,** 131–132.