Pol. J. Appl. Sci., **2015**, *1*, 97-101

ENVIRONMENTAL INFLUENCES ON THE DYNAMICS OF HEMOGLOBIN DERIVATIVES VARIATION IN HUMAN BLOOD

Olexander I. Bilyya, Taras Z. Grechukha, Kateryna P. Dudoka, Nataliya O. Sybirna

Faculty of Electronics, Physical and Biomedical Department
Ivan Franco National University of Lviv, Lviv, Ukraine

E-mail: bily2011@yandex.ua

Abstract: The article aims at describing environmental influences on the function of oxygen transported by blood. It considers the dynamics of parameters' variation, such as total hemoglobin, individual hemoglobin derivatives and kinetic of oxygen bound by human hemoglobin. Human blood of donors who work at shoes factory was adopted as the basis for scientific enquiry. It was determined to increase the content of constant oxygen saturation, methemoglobin and sulfhemoglobin derivatives, lowering the content of oxyhemoglobin derivatives. The fluctuation of hemoglobin saturation with oxygen correlates with the changes of the hemoglobin content derivatives of human blood.

Key words: blood, hemoglobin derivatives, oxygen capacity, environmental influences

Introduction

The problem of influencing a person's organism by the surrounding factors becomes particularly valid in connection with ejection's of chemical combinations, yields of scraps from the industry, pollution from automobiles and air transport, mechanization and chemicalization of agriculture. It is necessary to provide mixed gases to such junctions, a composition of: CO, hydrogen sulfide, nitric oxides, cyanides, aniline, phenylhydrazine, xylem, toluene and many others. Such a variety of chemical combinations in the surrounding determines the probability of their combined influence on an organism of a person or an animal. In human and animal erythrocytes, the formation of methemoglobin is being passed permanently. It is stipulated by the activity of endogenic oxidizing agents, which translate iron of gem from divalent into threevalent. But the accumulation of methemoglobin in blood does not pass, because simultaneously with its formation, the intensive deoxidization processes take place. Due to this, the level of methemoglobin in blood of the able-bodied people does not exceed 1-1,5%, and only occasionally it can reach the level of 3-6%. The raise of the level of methemoglobin in blood can be interlined with an amplification of production of endogenic methemoglobin formation agents, the violation of methemoglobin biological reduction processes, the occurrence of an abnormal form of – MetHb hemoglobin, and also the activity of exogenous methemoglobin formation agents. The transmutation of some part of hemoglobin in Met-form leads to gem hypoxia. The principal reason for the development of hypoxia in methemoglobin anemia is the decreasing amount of "active" hemoglobin, which is shared by transporting oxygen from lungs to tissues – the drop of oxygen capacity and raised affinity of hemoglobin to oxygen. In the literature, the magnifications of MetHb contents in blood of caprone firm workers are circumscribed [1]. The chemical agents are applied to derive a polyamide fiber, which is a derivative of benzene: as a raw material – caprolactam, heat transfer medium – denial. These materials influence the MetHb formation in blood. In the given publication, we provide the reduced modifications' outcomes of some blood parameters of the major shoe factory workers from the examination that was conducted at the beginning of the 90s.

Parameters for Estimating Blood Transport Properties

In a composite system of regulation supply of oxygen to tissues, hemoglobin plays a significant role. Hemoglobin has unique properties: it binds and returns molecular oxygen and makes the molecular basis for the respiratory function of blood. Hemoglobin, by interacting with oxygen, organizes oxyhemoglobin (HbO) and also reacts with such derivatives as CO, which organizes carboxyhemoglobin (HbCO). Hemoglobin of a person and an animal will experience the influence of oxidizing agents, having an endogenic and exogenous parentage. Therefore, methemoglobin (MetHb) or other nonperishable derivatives of the shape, in particular deoxyhemoglobin (RHb) or sulfhemoglobin (SHb), are not possible to be gained by a person to transport oxygen. Due to this, quantitative and qualitative analyses of hemoglobin shapes derivatives in blood have a relevant value for estimating blood transport properties.

Quantitative and qualitative behavior of hemoglobin in binding and returning oxygen in blood is possible to estimate in parameters affinity of hemoglobin to oxygen and in the blood oxygen capacity. In the process of oxygenation and deoxygenating, the provided property of hemoglobin is determined by many factors and, first of all, by protein's frame: by an aminoacidic composition of a molecule's site, which binds oxygen, the degree of its dissociation on dimmers and hem - hems interaction. Oxygen dissociation of hemoglobin is influenced by the presence of particular junctions. So, the ions $Cl^-, H^+, S_4^{2-}, H_2PO_4^-, HPO_4^{2-}, HCO_3^-$ inhibit an oxygenation. Glutamine, cysteine and acidum ascorbinicum are also decelerated in the process of interlining oxygen by hemoglobin [2]. It should be considered that a primary factor of metabolic monitoring affinity of hemoglobin to oxygen in miscellaneous physiological and even pathological requirements for the majority of backbones equals 2, 3 – dephosphateglycerate – organic phosphate, on destiny which has to be 64% of a common erythrocytes phosphorus. Therefore, when examining the ability of hemoglobin in binding oxygen, it is necessary to take into account the activity of the miscellaneous factors of inerytrocytes medium.

Method of Defining the Derivates of the Hemoglobin Shapes

The spectrophotometric method of determining the content of individual compounds in multi-component solutions is described by the Lambert-Beer law [3, 4]. This method of analysis is ideal because the concentration of component solution is a function of the total quantity of optical density. If the Lambert-Beer law is true for the number of individual compounds in a solution, it is applicable to a mixture of these compounds as well, if they do not interact. If a solution contains i compounds with sufficiently different absorption spectra, it can be analyzed by measuring at least j different wavelengths. These measurements provide n equations of the type and can be described by the following equation:

$$D_j = K_{j1}C_1 l + K_{j2}C_2 l + \ldots + K_{ji}C_i l, \tag{1}$$

where D_j denotes the optical density of the solution, K_{ji} is the molar absorptivity of a component in a solution, C_i is the concentration of components in a solution, and l is the light path length. The values of $C_1, C_2, \ldots, C_i$ can be calculated by solving the set of equations:

$$[C_i] = [K_{ij}]^{-1}[D_j]\frac{1}{l}, \tag{2}$$

Provided that the values of molar absorption component and light path length are know. In equations (2) $[C_i]$ the column matrix is a component concentration, $[K_{ij}^{-1}]$ is the inverse matrix of molar absorptivity of components, $[D_j]$ is the row matrix measure of optical density. The considered method is of limited application for the analysis of multi-component solutions containing varying quantities of absorption compound in chemical and quasichemical interactions between components in a solution. In the quasichemical interaction, in order to perceive the interaction of association and the formation of hydrogen bonds, the molecules should affect their molar absorptivity. Thus, the molar absorptivity coefficient is functionally dependent on the concentration of components. In this case, the directly proportional relationship of optical density and concentration are unavailable. As this takes place, on account of an error in calculations in the coefficients of matrix $[K_{ij}]$ of dimension $n \times n$, the error in determining $[C_i]$ and the concentration will increase n^2.

The suggested method of defining the contents of the hemoglobin shapes derivatives was used in the data examinations and can be found in the ration of optical densities [5]. In the given method, the concentration of components in a solution appears in functioning of the optical densities value rations of the solution components. The central problem of this method was the fact that the construction of matrix arbitrarily designated the matrix normalized average coefficients. Due to this, the calculations are performed as follows. The absorption spectrum of one-component compounds of every multi-component solution from the fixed concentration of C_o in the normalized absorbency coefficient $D_\lambda(C_o)$ at one selected wavelength λ on the absorption spectrum of a one-component solution is deprived of further components. A matrix normalized average coefficients $[H_{ij}]$ are to be constructed in the following way

$$[H_{ij}] = \frac{[D_{ij}]}{D_\lambda(C_0)}. \tag{3}$$

Because for every component of multi-component solution, the following quantity

$$K(C_0) = \frac{D_\lambda(C_0)}{lC_0}, \tag{4}$$

is constant and the determination of elements in the matrix of molar absorptivity $[K_{ij}]$ can be calculated in the following manner

$$[K_{ij}] = K(C_0)[H_{ij}] \tag{5}$$

Calculations of the relative concentration of components in a solution are possible by multiplying the inverse matrix of normalized average coefficients by the row matrix meter of optical density according to the equations

$$[C_i^*] = [H_{ij}]^{-1\cdot}[D_j], \tag{6}$$

$$C_i(\%) = \frac{C_i^*}{\Sigma C_i^*}, \tag{7}$$

where C_i^* stands for the concentration of i-components in a solution calculated on the basis of the normalized average coefficients' matrix, where $C_i(\%)$ is the relative concentration of components. Calculations of the absolute quantity of the component solution concentration are available and known as a relative concentration of components and the absolute quantity of molar absorptivity of every component in the K_{oi} solution at one selected wavelength where the error in calculations of these coefficients on the absorption spectrum of one-component solution is minimum, measured on the D_o optical density on the absorption spectrum of a multi-component solution at this selected wavelength and calculated in the following manner

$$C_i = C_i(\%) \times \frac{D_o}{l \times \Sigma K_{oi} \times C_i(\%)} = C_i(\%) \times C_{tot} \qquad (8)$$

where C_{tot} is defined as the sum of an absolute concentration of the component solution with a particular dimensionality of an absorption index. The following are the matrix aspects of reduced absorption coefficients for the derivative forms of hemoglobin: deoxy-, oxy-, carboxy-, met- and sulf- hemoglobin is presented in [5, 6].

Method of Defining Parameters of Hemoglobin Affinity to Oxygen Capacity of Hemoglobin

The parameter of hemoglobin affinity to oxygen is expressed as $p50$ – where the partial pressure of oxygen in a solution of hemoglobin is half saturated with oxygen. The given parameter was determined by build-up curves of oxygen dissociation of a hemoglobin solution, with the use of the spectrophotometric method [7] modified by Ivanov [8]. The essence of the method lies in examining the modification of an optical density of a hemoglobin solution on a wavelength of 558 nm after tonometry with a stationary value of temperature in ultrathermostat. The cuvette of a tonometer imported 4 ml of a hemoglobin solution with particular concentration. The tonometer was closed, and the air was deflated from it by a vacuum pump. After the final selection, the bubble of gas scavenging the air prolonged the time. We monitored the deoxygenation reaction conducted by spectrophotometric under the ration of optical densities on lengths of waves 540 and 555 nm. For completely deoxygenated hemoglobin, the given parameter equaled 1.24. According to our requirements, a suitable option for examination was the solution with a coefficient of 1.19. Next, the particular amount of CO_2 was inserted into the tonometer and put in ultrathermostat. After ten minutes, it was possible to determine the optical density of a solution at a wavelength of 558 nm. With the help of the system of cocks in the tonometer, started by portion air, the partial pressure of oxygen was extended. After each subsequent addition of air, a curette was tonometered during 10 minutes' period in ultrathermostat, and each time an optical density was determined. The last gauge was created at 100% of hemoglobin oxygenation and before that atmospheric pressure was maintained in a solution. The saturation of hemoglobin with oxygen was calculated under the following formula

$$\%HbO = \frac{D_{RHb} - D_x}{D_{RHb} - D_{HbO}}. \qquad (9)$$

Where:

D_{RHb} – optical density of deoxyhemoglobin,
D_{HbO} – optical density of oxyhemoglobin,
D_x – optical density of a solution of hemoglobin at a given partial pressure.

After the completion of the relevant calculations, a curve of a dissociation of oxyhemoglobin was drawn in a frame.

The content of oxygen in blood that complies with all the requirements, when all hemoglobin turns to oxyhemoglobin, is called the blood oxygen capacity. The given parameter tells that 1 gram of hemoglobin theoretically should bind 1.34 ml of oxygen. In our case, oxygen capacity of blood was calculated with the content of oxyhemoglobin in blood under the formula

$$O_2ct = 1,34 \times C_{HbO} \qquad (10)$$

Where:

C_{HbO} – absolute concentration value of oxyhemoglobin in blood.

Results of Examinations

Average data taken from 152 workers of the shoe factory, represent higher parameters of the examinations' outcomes shown in Fig. 1-6. The analysis of hemoglobin derivative forms of the people shows a considerable modification in the relations between the studied hemoglobin forms. It has been determined that the content of deoxyhemoglobin makes up only a minor part of the total hemoglobin, the magnitude is comparable with assumptions of the method. Due to this, the datum hereinafter started to equal point zero. The relevant value is oxyhemoglobin, because with this derivative form, the process of oxygen delivery to organs and tissues of an organism is interlined. The outcome of examinations (Fig. 1) proves that 74% of the studied HbO- content lies within the limits of 85 - 93% and only 19% of the examined HbO- content that lies within the norms makes 95%. The content of the second principal blood component i.e. carboxyhemoglobin, which determines its transport properties, stays within the norms for the majority of patients. Only in 4% of cases, the given parameter was bigger i.e. from 5 up to 10% HbCO. The greatest modifications can be seen for minor forms of MetHb- and SHb- hemoglobin, which a person does not share in the processes of oxygen transport

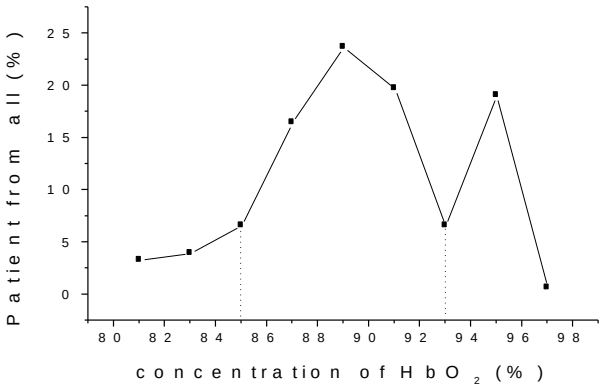

Fig. 1: Average data of oxyhemoglobin content.

in an organism. In normal conditions, the content of the mentioned forms do not exceed 1%. In our examinations for all the patients, the content of MetHb lies within the limits of 2 - 10%, and the content of SHb reaches 6%. As this takes place, for the majority of patients, oxygen capacity of blood is too small, which should in normal conditions make 0,19mls of oxygen on 1ml of blood. The norm of the affinity index of hemoglobin to oxygen for an adult should be 25-29mm Hg. In the examination, this parameter appeared to be higher than normal for the majority of the studied patients.

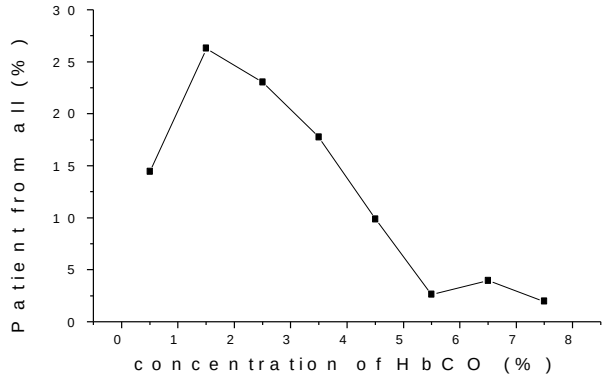

Fig. 2: Average data of carboxyhemoglobin content.

The outcomes prove that the workers of the shoe factory experience the development of hypoxia due to the activity of endogenic methemoglobin formation agents. There exist the observable correlation between a content of minor derivative forms of hemoglobin, affinity of this hemoglobin to oxygen and common oxygen capacity of the total hemoglobin. The rise of MetHb-, SHb- concentration can be explained by the fact that a minor raise of a HbCO- content reduces a raise of hemoglobin affinity to oxygen and a drop of oxygen capacity. Therefore, the detected modifications of hemoglobin parameters can be a relevant reason for the modification of red-ox processes in an organism. Results of examinations indicate that the methodical approach, used

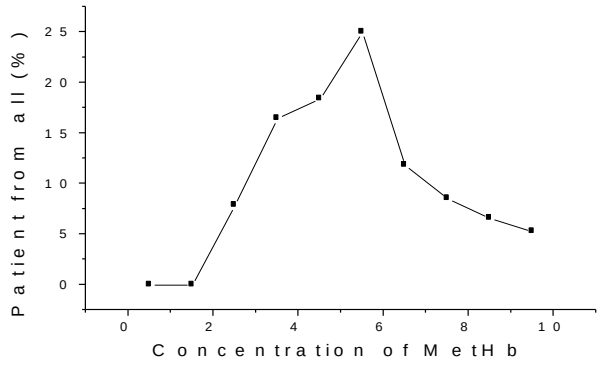

Fig. 3: Average data of methhemoglobin content.

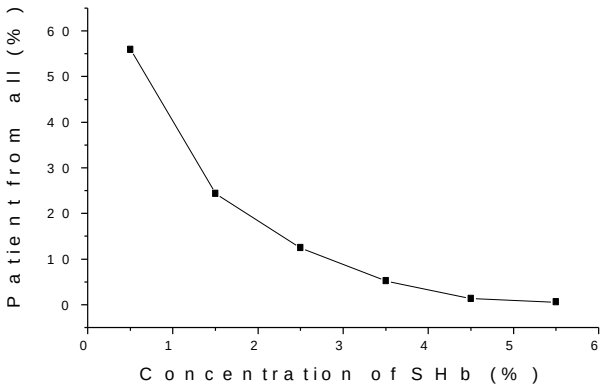

Fig. 4: Average data of sulfhemoglobin content.

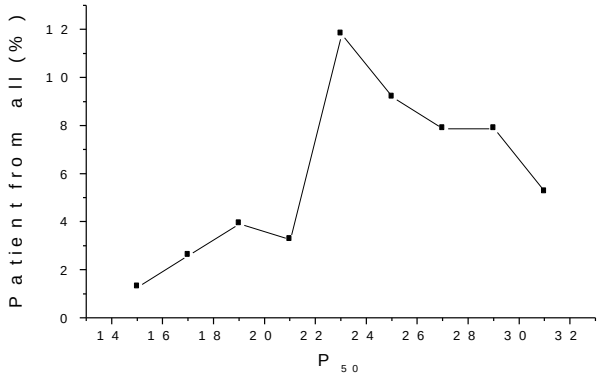

Fig. 5: Average data of oxygen affinity.

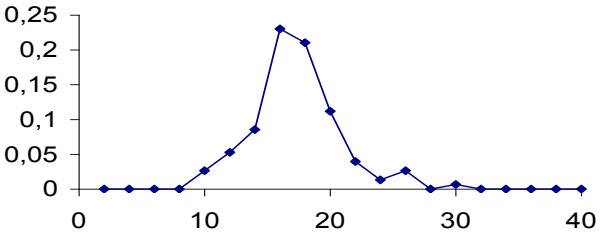

Fig. 6: Content of Oxygen in ml.

in the operation, can be utilized in clinical medicine when

the influence of extreme factors on animal and human organisms can be predicted.

Literature

[1] G.P. Artyuchina, T.V. Muslakova. *In Study of action accumulating of the factors on an organism of the person*, chapter A hemoglobin content, methemoglobin and activity of methemoglobin reductases in a blood of the workers of caprone production, pages 178–181. Medicine, Moscow, 1980.

[2] S.N. Golikov, I.V. Canotckyi, L.A. Tiunov. *Common mechanism of a toxic operation.* Medicine, Moscow, 1986.

[3] A.J. Benrens. Whole-blood hemoglobin determinations a comparisons of methodologies. *American Journal of Clinical Pathology*, 72:904–908, 1979.

[4] B. Geseburg, W.G. Zijlstra A. Zwart, A. Buursa, E.J. van Kampen. A multi -wavelength spectrophotometric method for the simultaneous determination of five hemoglobin derivatives. *Journal of clinical chemistry and clinical biochemistry*, 19:457–463, 1981.

[5] I.H. Yarynovska, O.I. Bilyy. Absorption spectra of sulfhemoglobin derivates of human blood. *Proceedings of SPIE*, 6094:60940G–1– 60940G–6, 2006.

[6] O.I. Bilyy, M.M. Veliky, K.P. Dudok. Original method of concurrent determination of hemoglobin derivatives. *Proceedings of SPIE*, 3926:223–228, 2000.

[7] R. Benesch, G. Macduff, R.E. Benesch. Determination of oxygen equilibrium with a versatile new tonometer. *Analytical Biochemistry*, 11:81–89, 1965.

[8] Yu. G. Ivanov. Modification of spectrophotometric method of definition the oxygen dissociation curves of a hemoglobin. *Bulletin of experimental biology and medicine*, 1:122–123, 1975.