SPECTROPHOTOMETRIC STUDY OF THE HEAVY METAL EFFECTS IN ANIMAL BLOOD

STUDIU SPECTROFOTOMETRIC AL EFECTELOR METALELOR GRELE ÎN SÂNGELE ANIMAL

CAZACU Ana [1]***, SPÂNU Mihaela***[1]***, OANCEA Servilia***[1]***, BODALE I.***[1*]
*Corresponding author e-mail: ilie.bodale@uaiasi.ro

Abstract. *In order to obtain information on the alteration of blood structure in the presence of heavy metals, mercury and lead, the modification of the absorption maxima of oxyhemoglobin was analyzed. Blood samples were collected from three healthy animal species: cows, horses and dogs. For the samples, 20 ml of 5% $HgCl_2$ and $Pb(NO_3)_2$ solution and 0.5 ml of blood from each species have been prepared. The obtained experimental results have shown that heavy metals affect certain biophysical characteristics of hemoglobin in the blood of the studied animals. The strongest effect has been obtained in the case of mercury, which is the most toxic heavy metal; for the horse blood sample at the wavelength of 410 nm, the absorption measurement gave an error, indicating that the sample was severely degraded.*
Key words: spectrophotometric study, heavy metals, oxyhemoglobin

Rezumat. *În vederea obținerii de informații privind alterarea structurii sângelui în prezența metalelor grele, mercur şi plumb, s-a analizat modificarea maximelor de absorbție ale oxihemoglobinei. Probele de sânge au fost prelevate de la trei specii de animale sănătoase: vacă, cal şi câine. Pentru probele de studiat s-a folosit 20 ml soluție de $HgCl_2$ şi $Pb(NO_3)_2$ de concentrație 5% şi 0,5 ml sânge de la fiecare specie în parte. Rezultatele experimentale obținute au arătat că metalele grele afectează anumite caracteristici biofizice ale hemoglobinei din sângele animalelor studiate. Cel mai puternic efect a fost obținut în cazul mercurului, care este şi cel mai toxic metal greu; pentru proba de sânge de cal, la lungimea de undă de 410 nm, măsurarea absorbanței a dat eroare, ceea ce arată că proba era puternic degradată.*
Cuvinte cheie: studiu spectrofotometric, metale grele, oxihemoglobină

INTRODUCTION

The effect of heavy metals on health is one of the many aspects studied, given that environmental pollution affects the food of humans and all living creatures. One of the methods by which this effect can be highlighted is based on blood tests. The study of blood biophysical parameters in various animal species in relation to their normal state and pathological conditions is among the most recent areas studied in the world. It was observed that the properties of blood differ from one species to another, although the blood structure is relatively similar. A different behaviour of the studied animal blood was also observed in

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

the case of the heavy metal action on oxyhemoglobin, especially in the case of mercury that is the most toxic heavy metal. In this paper, the effect of two heavy metals on hemoglobin of some domestic animals is spectrophotometrically analyzed. Heavy metals are difficult to remove from the environment and, unlike other pollutants, cannot be biologically or chemically degraded, being essentially indestructible. Mercury and lead are toxic elements whose essence for the body has not yet been proven. Introduced in the body by ingestion or inhalation, in sufficient quantities, have toxic effects. These effects are produced when the biochemical reactions are altered. It seems, however, that any element, in a certain amount, becomes toxic (Neathery and Miller, 1975). As heavy metals are so widely used, they have a real potential hazard to the health of animals and humans (Gwaltney-Brant, 2013). Lead and mercury are considered to be two of the most toxic elements for living organisms. Mercury intoxications in animals are commonly encountered in practice. This is explained by the fact that mercury is among the polluting elements that dispute its primacy with lead and cadmium (Kummrow *et al*, 2007). The spectrophotometric analysis allows the detection of a very small amount of toxic substance in blood (Rapa and Oancea, 2006).

MATERIAL AND METHOD

Blood samples were taken from three healthy animal species at the Faculty of Veterinary Medicine, Iasi. Samples were collected from the jugular vein of the cow and horse, and from the cephalic vein of the dog. Blood was collected in tubes containing EDTA as anticoagulant to prevent clotting of blood taken out of the body. The anticoagulant used was chosen so that it does not alter, by its action, the morphology and structure of the cells in the blood. For dilutions were used blood samples from horse, cow and dog; distilled water; solutions of mercury chloride ($HgCl_2$) and lead nitrate $Pb(NO_3)_2$. Three sets of samples of three dilutions were prepared. Control samples contain 20 ml of distilled water to which were added 0.5 ml of blood from each species. For the samples, 20 ml of 5% $HgCl_2$ or 5% $Pb(NO_3)_2$ solution and 0.5 ml of blood from each species have been prepared. Spectrophotometric measurements were performed using a T70 UV-VIS Spectrophotometer from PG Instruments Ltd. with a spectral range between 190 and 1100 nm.

RESULTS AND DISCUSSIONS

Spectrophotometric analysis of the hemoglobin spectrum, both simple and in combination with heavy metals, allows highlighting the changes in hemoglobin caused by heavy metals. Four hemoglobin derivatives are known: oxyhemoglobin (HbO_2), carboxyhemoglobin (HbCO), hemoglobin and methemoglobin. The spectrum is different depending on these hemoglobin derivatives. Hemoglobin has an absorption band in the green, with the maximum at 555 nm. If the hemoglobin molecule binds to oxygen, then oxyhemoglobin is formed and the spectrum is different. Oxihemoglobin has two absorption bands, one in the yellow range and one in the green. Carboxyhemoglobin has a similar spectrum to

oxyhemoglobin, but the bands are shifted toward lower wavelengths. Methemoglobin presents a red absorption band with a peak at 633 nm.

In order to show the absorption maxima of oxyhemoglobin (since we worked with blood in the presence of air, oxihemoglobin was obtained), the spectrum of oxyhemoglobin in UV (0 - 400 nm) and VIS (400 - 700) nm was recorded. A dilution of 10 ml of distilled water and 1 ml of blood was used to prepare control samples and to obtain the graph from figure 1.

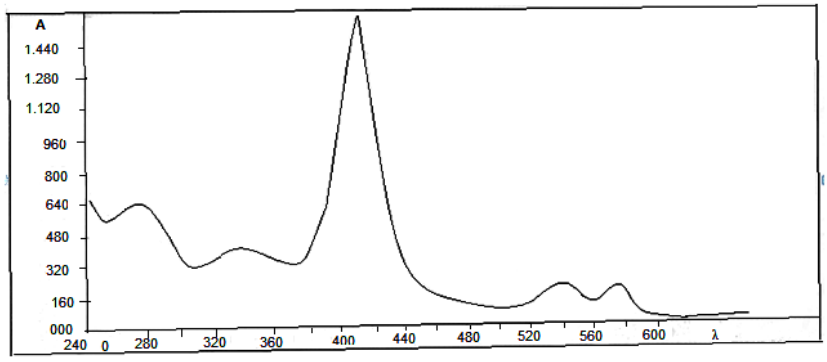

Fig. 1 The absorption spectrum of oxyhemoglobin in the 240 - 600 nm range

Figure 1 shows that in the visible range, the oxyhemoglobin absorption maxima are at 410 nm, 540 nm and 577 nm, in accordance with specialty literature (Galaris *et al,* 1995). For this reason, absorption measurements of oxyhemoglobin to analyze the effect of heavy metals were carried out at these wavelength values, and also at values close to these. Table 1 shows the absorbance values of the control samples at different wavelengths.

Table 1

The absorbance values for control samples

Control sample	Wavelength (nm)						
	408	**410**	**412**	**420**	**430**	**450**	**540**
Cow	2.719	2.819	2.738	2.794	2.839	2.468	2.188
Horse	2.901	3.116	2.903	2.983	3.044	3.027	2.663
Dog	3.005	3.481	2.983	3.017	3.067	2.991	2.681

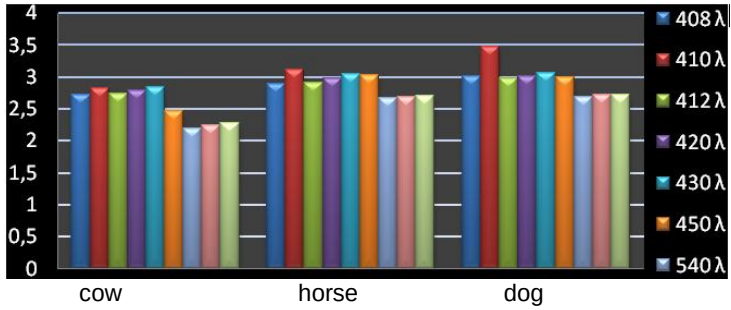

Fig. 2 Dependence of absorption on the wavelength of control samples

As can be seen from table 1 and figure 2, the maximum absorbance value for the blood of cow is at 430 nm, and for the blood of horse and dog at 410 nm.

After diluting the blood samples with mercuric chloride, absorbance measurements were performed (table 2). Shortly after the dilutions, a rapid degradation of the samples was observed.

Table 2

The absorbance values for samples containing mercury

Sample	Wavelength (nm)						
	408	410	412	420	430	450	540
Cow+Hg	2.147	3.993	1.924	1.622	1.413	1.194	3.009
Horse+Hg	3.307	-	3.258	3.291	3.351	3.252	3.922
Dog+Hg	3.174	5.432	3.153	3.229	3.301	3.367	4.093

In the case of samples containing mercury, the maximum absorbance for cow blood sample is at 410 nm, for horse blood sample at 540 nm (at 410 nm the device could not record the value), and for dog blood sample at 410 nm (fig. 3).

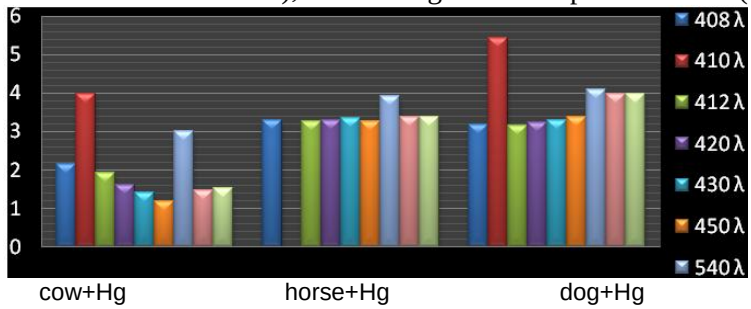

Fig. 3 Dependence of absorption on the wavelength of samples with mercury

The appearance of blood samples with mercury was visibly changed. Samples were less transparent, with deposits on the bottom of the cuvette. The most affected was the horse blood sample, for which the spectrophotometer could not measure any value at the wavelength of 410 nm. Fig. 4 shows the difference between blood control samples and the ones containing mercury, for horse.

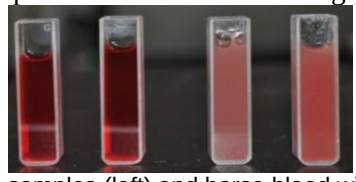

Fig. 4 Control horse blood samples (left) and horse blood with mercury samples (right)

The recorded measurements for blood samples containing lead are presented in table 3. Analyzing the data, it is observed that the maximum absorbance for the cow blood sample with lead nitrate is at the wavelength of 430 nm. In the case of horse blood sample, the maximum value recorded was at 540 nm. For the dog blood sample containing lead, the maximum absorbance was recorded at 430 nm. In figure 5 are the data for samples with lead.

Table 3

The absorbance values for samples containing lead

Sample	Wavelength (nm)						
	408	410	412	420	430	450	540
Cow+Pb	2.601	2.745	2.655	2.735	2.794	2.739	2.765
Horse+Pb	2.716	2.248	2.756	2.817	2.869	2.953	3.341
Dog+Pb	2.399	2.401	2.412	2.445	2.461	2.455	2.426

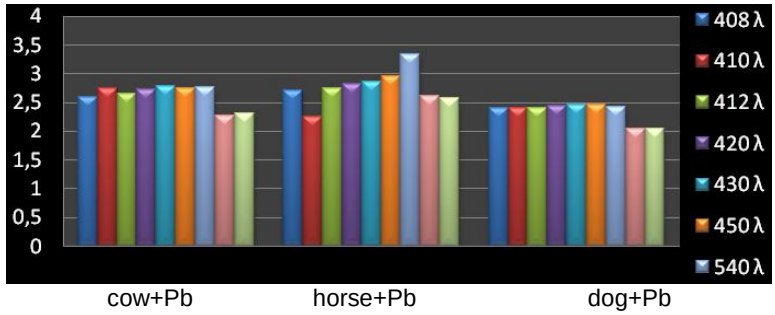

Fig. 5 Dependence of absorption on the wavelength of samples with lead

The comparison of the absorbance values for the two heavy metals in the case of blood collected from horse can be seen in figure 6a.

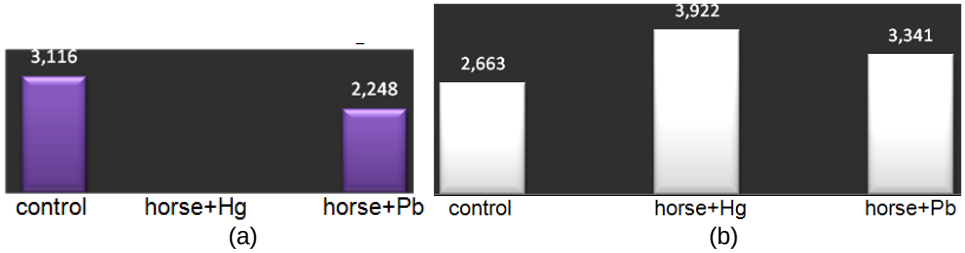

Fig. 6 Absorbance values of oxihemoglobin in horse blood at 410 nm (a) and 540 nm (b)

The absorbance value for horse blood at 540 nm is higher for the samples with mercury and lead than for the control. The sample with mercury has the highest absorbance (fig. 6b).

Regarding the modification of the oxyhemoglobin spectrum for the dog blood samples, at 540 nm, the highest absorbance was recorded for the mercury sample and the lowest value for the lead sample (fig. 7).

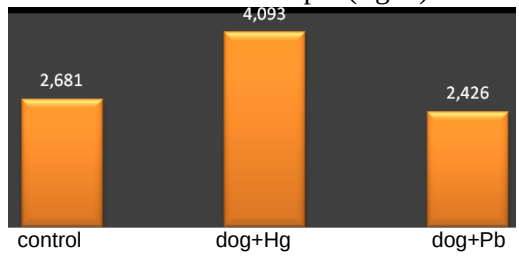

Fig. 7 Absorbance values of oxihemoglobin in dog blood at 540 nm

The weaker effect of lead can be explained by the fact that Pb is a heavy metal less toxic than Hg. Moreover, the blood has been collected on EDTA and this is a specific antidote for Pb. EDTA forms with lead a chelate which is subsequently excreted by the kidneys (Solcan and Chiriac, 2005).

CONCLUSIONS

Experimental results have shown that heavy metals affect certain biophysical characteristics of hemoglobin in the blood of the studied animals.

From the UV-VIS spectra of oxyhemoglobin was determined that its absorption maxima are at 410, 430 and 540 nm. The strongest effect has been obtained for mercury, which is the most toxic heavy metal. These results are consistent with those obtained by other researchers who claim that heavy metal attaches to the hemoglobin molecule in the blood, altering its structure. The modification produced by mercury was observed immediately, the sample changing its transparency. For the horse blood sample containing mercury, at 410 nm, the absorbance measurement gave an error, indicating that the sample was severely degraded. For the oxyhemoglobin absorption maximum at 540 nm, the absorbance of samples containing mercury is higher than the control, for all three animals studied. Furthermore, the values are higher in the case of samples with mercury than the ones with lead for all three species. The strong effect of mercury can be explained by the bonding of mercury to sulfide bonds (thiol) in the hemoglobin molecule. As for the effect of lead, although it is also a very toxic heavy metal, the effects are weaker.

The effects of heavy metals increase over time, suggesting that the heavy metal action is cumulative.

Acknowledgments: This work was supported by the CNCS-UEFISCDI, PN-III-P1-1.1-TE-2016-2336 project.

REFERENCES

1. **Galaris D., Yova D., Korantzopoulos P., Barbounaki S., Koutsouris D., 1995** - *Effects of ascorbic acid on tert-butyl hydroperoxide-induced deformability decrease in human erytrocytes*, Clinical Hemorheology and Microcirculation, 15(1), pp. 107-120.
2. **Kummrow F., Silva F., Kuno R., Souza A., Oliveira P.**, **2007** - *Biomonitoring method for the simultaneous determination of cadmium and lead in whole blood by electrothermal atomic absorption spectrometry for assessment of environmental exposure*, Talanta, 75(1), pp. 246-252.
3. **Neathery W., Miller J.**, **1975** - *Metabolism and Toxicity of Cadmium, Mercury, and Lead in Animals: A Review*, Journal of Dairy Science, 58(12), pp. 1767-1781.
4. **Rapa A., Oancea S.**, **2006** - *Hemoreologie comparată*, Ed. Tehnopress, ISBN 13 978-973-702-404-6.
5. **Gwaltney-Brant S.**, **2013** - *Heavy Metals* in Haschek and Rousseaux's Handbook of Toxicologic Pathology, Third Edition, Elsevier, ISBN 978-0-12-415759-0.
6. **Solcan Gh., Beşchea Chiriac S.I.**, **2005** - *Toxicologie veterinara*, Ed. Tehnopress, ISBN 9737021193.