LEVELS OF PROTEIN FRACTIONS IN DIVER'S BLOOD SERUM

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ABSTRACT

The study consisted in an analysis of the blood plasma of 72 divers aged between 21 and 23 years with paper electrophoresis method. The examined protein fractions reached the following values: albumins 61.7%, α1 globulins 26%, α2 globulins 6%, β globulins 9.5% γ globulins 20.2%. The obtained values were within physiological norm, with the most significant deviations observed in group I – beginner divers. The authors link this observation with a possible impact of an emotional factor and momentary disorders in hormonal regulation.
Key words: diver, diving, blood proteins, protein electrophoresis, paper electrophoresis.

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INTRODUCTION

The objective of our work consisted in providing an answer to the question of whether an increased atmospheric pressure is harmful for the human organism and whether its impact may be measured by examining levels of protein fractions in the blood serum. Until very recently little was known about changes occurring in organisms subjected to high pressures, and the possibility of certain changes tended to be rejected upfront. The works by Dolatowski and others [55] on the circulatory and respiratory systems in divers confirmed the existence of changes that had been previously observed. However, initial works on the image on peripheral blood by the same authors indicated the existence of changes that had not been anticipated in former studies [55 p.247]. Being encouraged by these studies, we have become involved in an endeavour that may constitute an important contribution in the explanation of a mechanism behind the changes occurring in the pattern of proteins in the blood serum, as well as the pathomechanism related to the impact of high pressures.

METHODOLOGY

We have examined a group of 72 healthy men, trainee-divers or divers with the diving experience of one to two years, aged between 21-23 years, equally nourished (the daily calorific value of the food of 5707 kcal), and provided with the same living conditions. We shall not submit a detailed list of the administered food stuffs, as it has been shown that the diet has only minimal impact on the total level of proteins in the blood serum and interrelations between the fractions examined with the paper electrophoresis method [Szajna, 44 Pedrazzini]. The blood samples were taken from the divers' cubital vein immediately after diving so as to ensure that at the time the blood was drawn they had been static in a sitting position for the shortest possible time.–. 3 ml of blood was drawn into a dry syringe, which was a sufficient amount for the conducted examination. Next, it was attempted to transport the blood, in the shortest possible time, to a laboratory, where it remained in a temperature of +10°C until the following day. The said blood storage temperature was chosen on purpose, due to the fact that preserving blood in lower temperatures (refrigerated) is presumed to induce an increase in gamma-globulins and a reduction in alpha- and beta-globulins [13, Gross], although not all researchers tend to agree with this allegation [23]. Haemolysed blood serum was rejected. The blood serum used in the study was collected with a pipette from above the clot and centrifuged for 30 minutes. The electrophoresis was performed in an apparatus comprising a vinidur chamber containing water and a line current rectifier [construction details - see bibliography it. 19]. 6 strips with the dimensions of 32x3 cm were prepared simultaneously with the use of Whatman paper no. 1. Before providing the blood serum, the paper strips were moistened with buffer, whereas excess liquid was dried with clean paper. The buffer used in moistening of the strips was veronal buffer with pH=8.6 and ionic strength of mi=0.1, whereas borate buffer with ionic strength equal to 0. 05 [Kowalczyk] was used to fill the chambers. The blood serum was placed on the strips with the use of micropipettes in the quantity of 0.01 ml, linearly, leaving free edges with the width of ca. 0.05 cm, so that the quicker movement on strip edges would not disrupt the electrophoresis.

The spot for blood serum placement, the "starting spot", was defined 3cm from the middle part of the strip towards the cathode. The current was switched on 30 minutes after placing the strips in the chamber and closing it, with the purpose of enabling an even moistening of the paper and saturating the chamber with water vapour. The voltage of 0.3 mA was applied per each cm of the strip's width, which with 6 such strips and the

consideration of the resistance of the "U" pipe connector reached in the approx. mA; the voltage amounted to 300 V (according to the voltmeter placed on the rectifier), whereas the actual voltage measured on each strip reached 25 V, i.e. 10V per each cm of the strip. The time of the electrophoresis was 6 hours. The specified conditions facilitated the obtainment of strips with good fraction distribution. The strips were dried in a temperature of 140°C for approx. 10 minutes, stained with Azokarmin B for ca. 1 hour and discoloured in two 10% solutions of acetic acid. After drying the strips at room temperature they were cut into segments with regard to the visible fractions, eluated in5 ml (albumins in 10 ml) of 0.1 N NaOH for a period of 30 minutes (shaken with the electric shaker), and after another 30 minutes the result was read with a Pulfrich photometer with the use of an S 53 filter. The assumed extinction sum was 100 which served as a reference in calculating the percentage of protein fractions in particular measurements.

The total protein level in the blood serum was determined with Phillips and van Slyke's specific gravity method. The test is not characterised by high accuracy, yet it sufficed for the assumed objective consisting in capturing the differences of decimal fractions of a gram per 100 ml of the blood serum, with an error within 10-15% [57]. The percentage share of particular fractions may be calculated as an absolute content in grams-percents; however, this would require determination of total protein in the blood serum with one of the quantitative methods (Klejdahl's method, biuret test).

RESULTS

Paper electrophoresis is a simple, available and a rather precise method. Still, it needs to be realised that certain difficulties may accumulate if we require electrophoresis to provide us with results that would give an insight into the so far unrecognised ratios of particular protein fractions in the blood serum. As it was rightly observed by *Azerad* [a quote from 46[S1]] "paper electrophoresis is a thousand times cheaper than free electrophoresis, however it does not mean it is a thousand times better". Paper electrophoresis provides repeatable, however not comparable results. In order to make them so, all laboratories would have to use precisely the same reagents and ensure the same test conditions. The lack of any proper results having materialised from a conference held in 1957 in Belgium (within an international scientific session) regarding paper electrophoresis equipment standardisation [Wender, 49] demonstrating how difficult this issue is.

Tab. 1

Protein fraction values (own studies).

A. Norm.
Number of cases 10
Total protein value 7.5g%
Albumin/globulin ratio 1.5

	Protein fractions				
	Albumins	Globulins			
		alfa 1	alfa 2	beta	gamma
Arithmetic mean	62,0	2,3	6,5	9,3	19,8
Max	65,7	2,8	7,8	11,6	24,6
Min.	58,6	1,8	4,8	7,3	13,6
Sigma	2,7	0,36	0,99	0,37	3,1
S	0,84	0,11	0,30	0,12	0,96

cont Tab. 1

B. Divers
Number of cases 71
Total protein value 7.6g%
Albumin/globulin ratio 1.6

	Protein fractions				
	Albumins	Globulins			
		alfa 1	alfa 2	beta	gamma
Arithmetic mean	61,7	2,6	6,0	9,5	20,2
Maksim.	71,2	5,1	10,6	13,6	27,3
Minim.	49,1	0,9	2,4	5,2	13,5
Sigma	6,0	1,2	1,9	2,0	3,5
S	0,71	0,14	0,22	0,24	0,41

The difficulties concerned with quantitative evaluation within the electrophoresis of proteins included a phenomenon involving an albumin loss on the way towards the anode [Merklen] with the applied corrections [Hardwicke] placing it below the error value and being quite inconvenient to use in practice. Numerous authors on this topic have emphasised the observation that the -various affinities of the dye with albumins and globulins do not seem to be factors which have a significant effect on the result, and so according to Magas [60] such a difference for. bromophenol blue reaches ca. 5%. The results obtainedby different laboratories which use their own modifications may not be compared. Each laboratory should work out its own norms and evaluate the received gel electrophoresis only on their basis.In order to work out such values we have conducted an electrophoresis test on the blood serum of 10 clinically healthy men, non-divers, of the same age as the group subjected to the quoted examination, with similar living conditions and diet. The obtained results were as follows (see also table 1, A): albumins 62.0%, alpha 1 2.3%, alpha 2 6.5%, beta 9.3% and gamma globulins 19.8%. The norm values were within the broad range of data available in the related literature (see table 2). We are not entirely familiarised with the material on whose basis the said norms were worked out however, so consequently even a rough comparison would be risky. As we were not responsible for producing the data which determined what a “norm” is, we felt it right to presume that the majority of those norms could have been applied to people of a different age, sex and environment, examined in a different time and conditions, whereas our research could provide a certain insight into the values of protein fraction and the blood serum of a uniform group of subjects:

Tab. 2

Protein fractions norms as quoted from literature according to Ruszkowski [39], Gross and Wrońska [12], and own results.

Authors	Protein fractions				
	Albumins	alfa 1	alfa 2	beta	gamma
1. Antonini	55,7	4,0	7,9	12,6	19,5
2. Moncke	61,8	4,2	7,6	10,3	16,1
3. Korver	57,8	4,5	8,2	9,6	19,9
4. Pluckthun	59,0	4,2	8,0	10,6	18,2
5. Satoskar	58,4	4,3	6,5	10,4	22,0
6. Levin	62,4	3,4	7,2	14,3	12,7
7. Bodart	61,9	4,2	8,0	11,3	14,6
8. Grassmann	61,3	4,1	8,1	11,0	15,5
9. Geinitz	60,4	3,9	7,5	11,3	16,8
10. Fine	60,2	3,3	7,6	12,4	16,5
11. Opplt, Kutacek	58,0	4,0	8,0	10,0	20,0
12. Dole	63,2	4,9	7,5	12,7	11,6
13. Reiner	56,8	15,9		12,8	14,4
14. Bogdanikowa	67,0	5,7		8,9	18,4
15. Bogdanowicz	61,9	4,2	8,0	11,3	14,6
16. Ostrowski, Mikucki	58,8	4,8		11,0	25,3
17. Ruszkowski	59,1	4,1	7,4	10,0	19,4
18. Gross, Wrońska	64,0	3,9	7,0	9,4	15,7
19. Normy własne	62,0	2,3	6,5	9,3	19,8

The average results of the examined group of 72 divers are as follows (see also table 1 B): albumins 61.7%, alpha 1 2.6%, alpha 2 6.0%, beta 9.5% and gamma globulins 20.2%. Table 1 specifies the values of the arithmetic mean, maximum and minimum, the values of sigma and standard error. In statistical calculations it would be more convenient to discuss a group of 100 cases; however, due to technical reasons, we were able to gather only a group of 72 subjects.

The comparison of values from both groups (norm-divers) indicates that the tripled remainder error value was smaller than the remainder of arithmetic means, and consequently the remainder of values of the obtained results was not statistically significant. The examined material was divided into 3 groups, depending on the depth of diving; however, the obtained results were not subject to statistical analysis, since the differences between particular groups were even smaller than before.

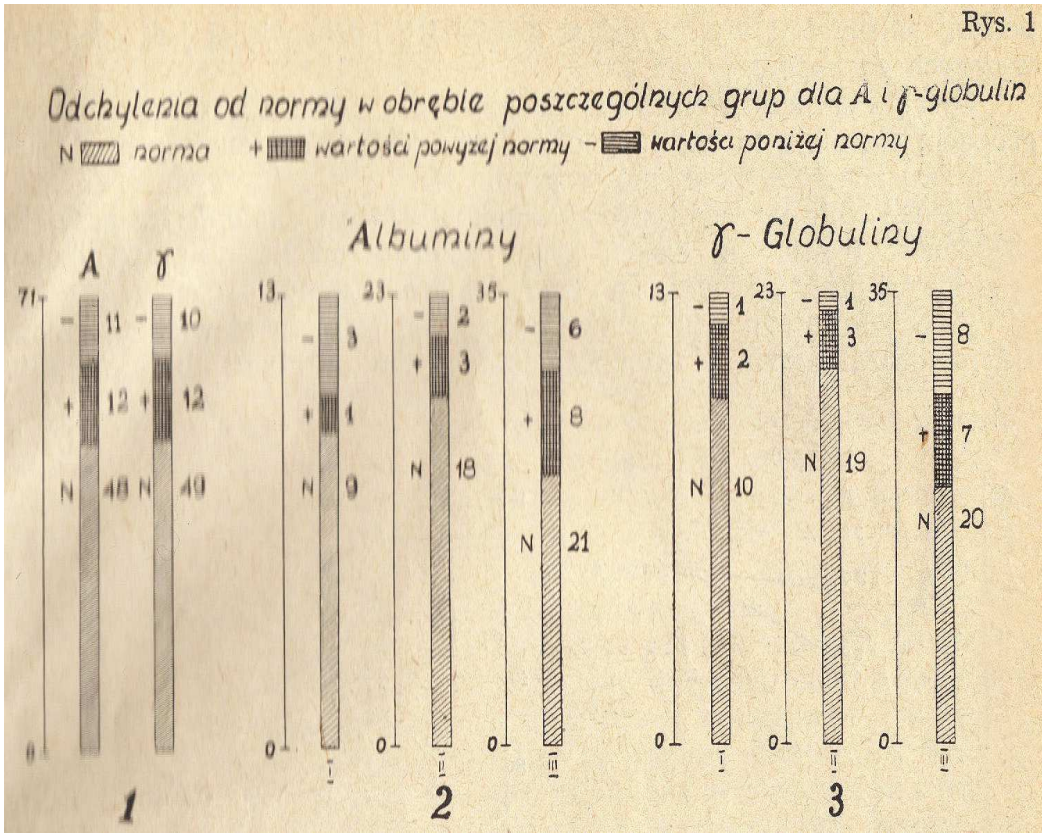

Fig. 1. Deviations from the norm within particular groups for alpha and beta globulins. N norm, + values above the norm, - values below the norm.

Group I (13 cases): albumins 59.9%, alpha 1 2.6%, alpha 2 6.6%, beta 10.5%, gamma globulins 20.4%.

Group II (23 cases): albumins 62.1%, alpha 1 2.9%, alpha 2 5.7%, beta 9.1%, gamma globulins 20.2%.

Group III (35 cases): albumins 62.6%, alpha 1 2.4%, alpha 2 5.8%, beta 9.3%, gamma globulins 19.9%.

The division into the above groups was justified with physiological factors, according to which diving is categorised as medium-deep (up to 40 m) - group I, deep (up to 60 m) - group II, deep-sea diving (below 60 m) - group III.

The fraction values of particular groups did not reveal any significant deviations and were within the norm. Nonetheless, it seems to be worthwhile emphasising the observed growth and decrease tendencies within particular fractions in different groups. And so, in group I the albumin values were slightly lower and the globulin values higher than in the other groups. Group II was not particularly characteristic in this regard, whereas in group III the values of albumins and gamma globulins returned to the value close to the norm. However, the said changes were insignificant and could serve only as an approximate value. Fig. 1 was prepared in order to depict the changes, showing the values being within the norm (symbol *N*), higher values (symbol +) and lower than the norm (symbol -) represented with bars. Numbers placed to right of the bars indicate the number of persons with particular values in a given group. The bar representing the norm (N) contains the majority of repeating cases within a given group being within the limit of one sigma, for instance, for albumins 55-65%, and similar for gamma globulins.

Number 1 indicates the mean results with regard to the albumin and gamma globulin levels in the entire group of subjects. It shows that the number of results being

within the norm with regard to both fractions is nearly the same and the observed deviations are of the same nature.

Number 2 was used to indicate the character of albumin deviations in particular groups (I, II, III). In group I we see that the number of cases with a value being below the norm is higher than in group III which is characterised by a greater number of deviations towards values exceeding the norm.

Number 3 marked the nature of globulin deviations in particular groups, however the general tendency was different: the number of negative deviations in group I was significantly lower than in group III.

The thin line placed to the left of the bars provides a scale adopted as a unity (different for each group depending on the number of cases) in relation to which deviations from the norm were calculated.

We wish to emphasise that here also group-specific differences were only slight and that the aim was to provide mere characterisation of an inclination towards "in plus" or "in minus" deviations.

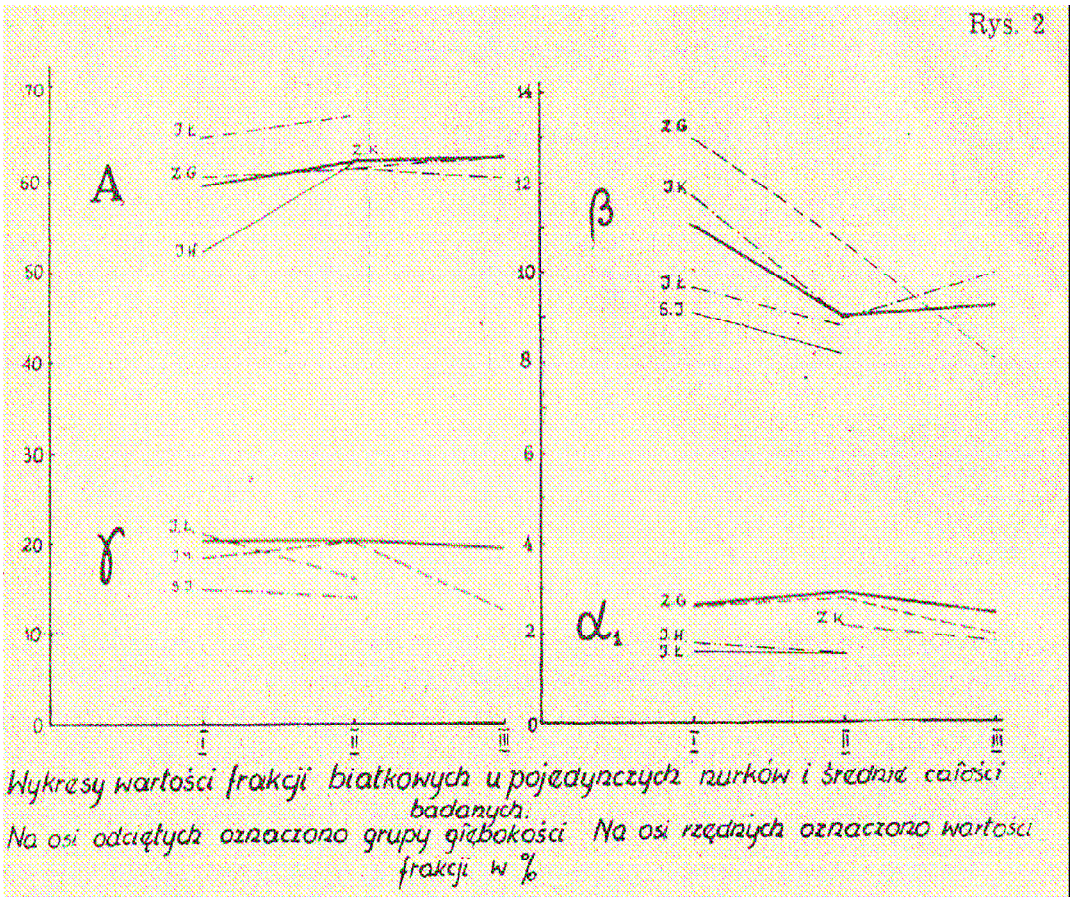

Fig. 2. Charts representing protein fractions in individual divers and mean values of the entire group of subjects. The axis of abscissae indicates depth classification, whereas the axis of ordinates presents fraction values expressed in %.

In the case of several divers the examination was repeated two, three or four times (the mean value is provided for examination regarding the same depth) depending on the depth. The results are presented in fig. 2. The axis of abscissae indicates depth classification, whereas the axis of ordinates presents fraction values expressed in %. Dashed lines indicate fraction values for particular divers, whereas the bold line indicates mean fraction value of all the subjects within a given group. The convergence of these two

curves (mean value and individual diver values) may not be indicated to the same extent for all fractions, nevertheless it confirms the tendency to deviate, which was discussed in relation to fig. 1; alpha 2 fractions, whose discrepancy was significant, had not been depicted here.

DISCUSSION

What should be particularly emphasised is a number of extraneous factors affecting protein levels in the blood serum. Vertical position (mainly at 54° to horizontal position) causes a larger concentration of plasma proteins due to an increased ultrafiltration [51, 53].

A similar effect is noted in the process of drawing blood with an applied pressure on the vein; however, both these factors do not result in visible quantitative changes of the blood serum proteins [30, 53].

The obtained results allow one to confirm that the examined blood serum showed the correct values with regard to protein fractions and total protein. The calculated mean values were within norms, although a part of them indicated slight deviations. The study concerned clinically healthy men, tested within a periodic medical check -up and before each descent. It involved taking measurements of the divers' pressure and pulse as well as interviewing them with regard to their comfort. Only divers meeting particular requirements were cleared for diving. It is obvious that the result of an electrophoretic test depended on a number of stimuli influencing the divers, such as emotions, an increased atmospheric pressure, water temperature or their health condition. It was not possible to isolate all of the above factors; however, we will devote more attention to the divers' health condition. Some of the examined subjects experienced periodic nasopharyngitis, and two divers had a reported dental caries. Research results in these cases did not include characteristic values, however they did involve a clear tendency to indicate increased gamma globulins and decreased albumins, which is a typical reaction of the(above the allowable systolic pressure of 140 mm Hg) more often constituted an accompany ing factor together with an accelerated pulse, which frequently disqualifies divers to go under water. As it results from the quoted observations, the above changes in divers from groups II and III were much rarer. Group I encompassed beginner divers, with the least experience in working under water and the shortest training. We have reason to assume that the observed slight deviations in the protein spectrum constituted function disorders of unaccustomed organisms and involuntary reactions to emotional stimuli related to the nature of the performed work.

The pattern behind the blood serum proteins is extremely labile and changes of that type may level out easily, unless they are connected with a more permanent cause, such as the one mentioned above in the case of the diver J.M. Today we know that an organism may react to emotional stimuli with changes in the protein fraction in the blood serum.

The pathomechanism of those changes is an open matter: what is possible is a direct impact of an increased atmospheric pressure on an organism not adjusted to the provided working conditions or a release of an increased quantity of hormones, e.g. adrenaline. Żydowo demonstrated in his work the effect of adrenaline on the growth of the blood serum protein level; however, he did not study the level of protein fractions [53]. In the groups of qualified divers (group II and III) changes in the arterial blood pressure and pulse were rare. Also in these groups, slight deviations in the fraction level tended to reverse, and in comparison with group I reach the values that were closest to the norm in group III. A lack of changes in greater depths may be explained with a relatively short stay

under water and the presence of only minor emotional stimuli connected with the work being performed by qualified divers.

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