

CHANGES OF ELECTROPHORETICAL FRACTIONS IN SIMULTANEOUS EXPOSURE TO GAMMA RADIATION AND HYPERBARISM

MONICA VASILE¹, OVIDIU TEREN¹, VICTOR CIUPINA², GRIGORE TURCU³

¹ Faculty of Medicine, "Ovidius" University of Constanta, Constanta, Romania,
monica@medcon.ro, ovidu@medcon.ro

² Faculty of Physics, "Ovidius" University of Constanta, Constanta, Romania

³ Faculty of Physics, University of Bucharest, Department of Biophysics, Bucharest-Magurele,
Romania

(Received July 2, 2008)

Abstract. Hyperbaric exposure gives rise to an increase of oxygen free radicals concentration in living organisms. Meanwhile the exposure of the organism to gamma radiations, accidentally or during radiological treatment provides the same effects. Nowadays studies describe treatment methods, where treatment with radiation is followed by hyperbaric therapy with an increase concentration of oxygen. Our study presents the cumulative effects of hyperbaric exposure and therapeutical gamma irradiation. The results we got from both exposures evidence that the cumulative exposure to those two factors gives rise to a significant change in electrophoretical fractions: significant damage of alpha and beta proteins.

Key words: free radicals, irradiation, hyperbarism.

1. INTRODUCTION

Hyperbarism, defined as an exposure of the organism to high pressure and professional irradiation and/or therapeutical, plays an important role in industry and medicine. Treatment by hyperbaric oxygen therapy of tegument and organic damages due to radiotherapy treatment of oncological phenomena's is an usual practice. Meanwhile the diver's exposure during the working process is commonly met in marine installation defectoscopy [11].

The two effects hyperbaric exposure and gamma irradiations have been investigated separately and the cumulative effect has not been taken into account. Both processes provide an increase in free radicals of oxygen in organisms which can conduce to alterations (damages) of plasmatic proteins [6, 7, 8].

The effect of oxygen free radicals over proteins is described in the special literature [5, 9, 12, 13, 14].

Direct measurements of the presence of oxygen free radicals *in vivo* experiments are very difficult due to the very short life time of the free radicals complex. Only indirect measurements are possible by analysis of changes induced by those radicals at cell and tissue level [10].

The alteration of plasmatic proteins is caused by a wide category of factors among which free radicals are present as well [3, 5, 13].

Our study starts on the hypothesis that if maintaining the other parameters constant we can only determine the cumulative effect of oxygen free radicals under hyperbaric exposure and irradiation.

2. MATERIAL AND METHOD

The experimental study has been carried out on adult Wistar rats, male and female, with ages between 13 and 15 months, and body weight between 170 and 250 g. The animals, provided by the Biobasis of the Faculty of Biology of Ovidius University of Constantza, have been divided into 4 samples for study:

- The witness sample set (1) – formed of 4 rats, 2 male and 2 female, of 18 months of age, and weight between 200–250 g for each individual ;
- The experimental sample set (2) – consisting of rats of the same age (3 females and 1 male) have been subjected to internal irradiation by subcutaneous injection with radioactive Tc-99m after which they were immersed in a hyperbaric chamber at 6 ATA, respectively a depth of 50 msw (meters sea water) using a decompression schedule similar to that used by professional divers;
- The experimental sample set (3) – made of 4 animals (males) of 12 month of age was immersed at 6 ATA following the same decompression schedule as we used for the experimental sample set (3), the diving being made for 5 successive days as in case of sample sets (2) ;
- The experimental sample set (4) – was made of 4 animals (female) of 18 month of age, and they were irradiated with Tc-99m, by subcutaneous injection, similar to the second experimental sample set.

The animals were submitted to hyperbaric chamber for 10 days, at the same day time between 14 and 15:30 pm for 1h and 30 min to secure unitary diving. The animals did not receive food and water during the diving period. A similar working protocol was used as that of professional divers in The Diving Center.

We made the internal irradiation each day, for 10 days in the morning, between 9 and 9:30. To each individual sample was delivered 10mCi Tc-99m (technetium).

Tc-99m was prepared before injection by less than half an hour. The preparation was made with technetium generator. The injected solution activity was measured instantly before injection with a scintillation detector.

3. SERUM PROTEIN ELECTROPHORESIS

In alkaline media, most proteins are negatively charged and move to anode under the electrical field action, while their mobility is proportional to their net charge. Once the separation complete, the proteins are fixed and quantitatively assessed by densitometry.

In Fig.1 it is seen serum protein electrophoresis for the subjects of witness sample set and in Table 1 are represented protein fractions of the witness sample set.

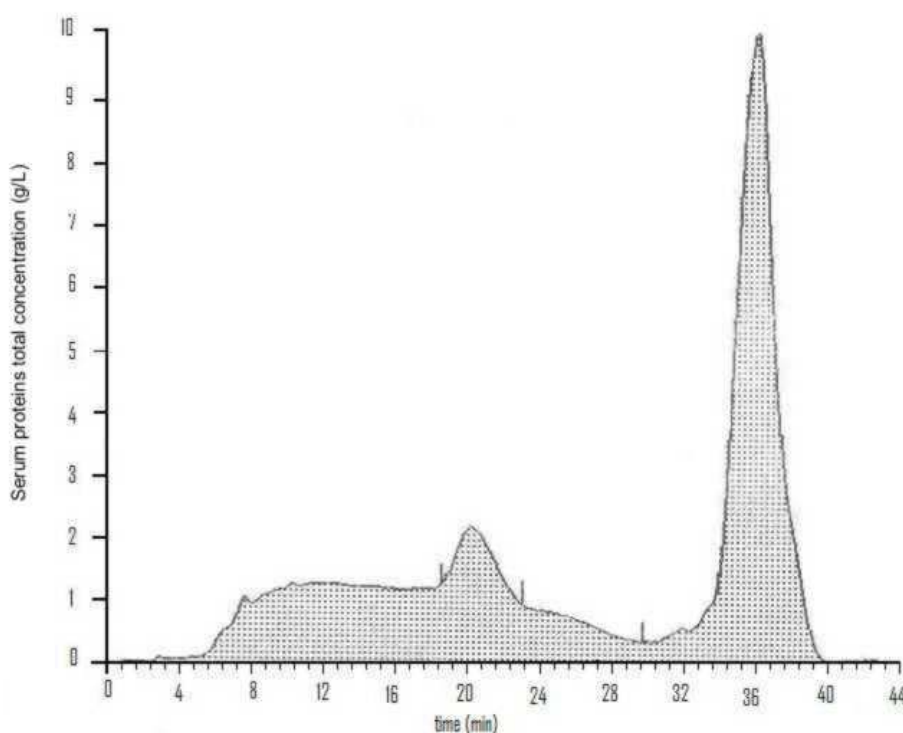


Fig.1 – Serum protein electrophoresis of subjects in witness sample.

Table 1

Electrophoresis fractions values of witness sample set

No.	Concentration of total serum proteins (g/L)	γ -globulin (%)	β -globulin (%)	α_1 (%)	α_2 (%)	Albumin (%)
1.	22.15	26.54	13.62	8.35	4.70	46.79
2.	29.96	3.62	17.07	18.37	10.99	49.95
3.	21.61	12.76	18.83	8.78	4.15	55.48
4.	23.25	15.02	15.08	6.39	5.26	58.25

The increase values of protein fractions α_1 , β and γ in hyperbaric exposure and internal irradiation are related to the inflammatory process produced by the lymphokine production. Synthesis of lymphokine is stimulated by the presence of free radicals. Due to cumulative stress (hyperbaric and irradiation) the destructive phenomena-damages of protein fractions α_1 , β and γ are very high. Degradation of albumin is much higher than its synthesis. For sample set the increase of albumin fragmentation is correlated with the radiosensitivity and with stimulation factor for oxygen free radicals appearance as it is seen in Fig. 2 and Table 2.

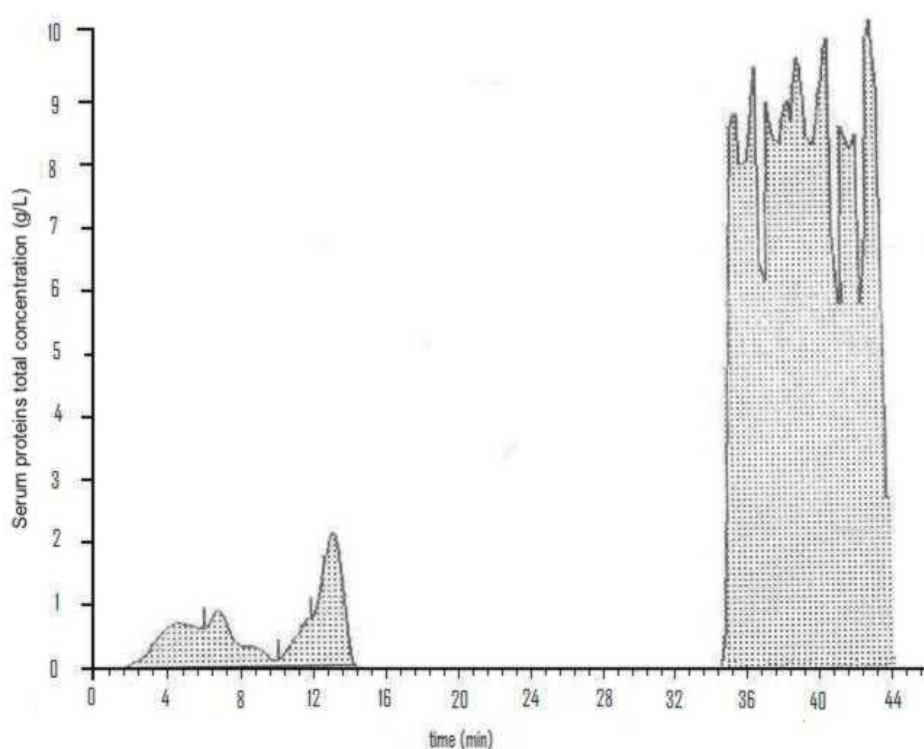


Fig. 2 – Serum protein electrophoresis for subjects in experimental sample (2) subjected to irradiation and hyperbaric conditions.

Table 2

Electrophoresis fraction values for the sample set irradiated and dived

No.	Concentration of total serum proteins (g/L)	γ -globulin (%)	β -globulin (%)	α_1 (%)	α_2 (%)	Albumin (%)
5.	33.09	2.63	2.40	1.03	69.75	24.19
6.	37.03	3.64	7.44	11.13	31.69	46.09
7.	32.15	10.58	17.60	7.15	25.25	39.41
	44,49	6,61	1,35	6,32	34,65	51,07

It is seen from Fig.3 that the increase of total plasmatic proteins as well as their fractions (α_1 , β , γ). These increases are due to hyperbaric exposure in correlation with the limphokine production (Table 3) in the presence of free radicals of oxygen [1, 4].

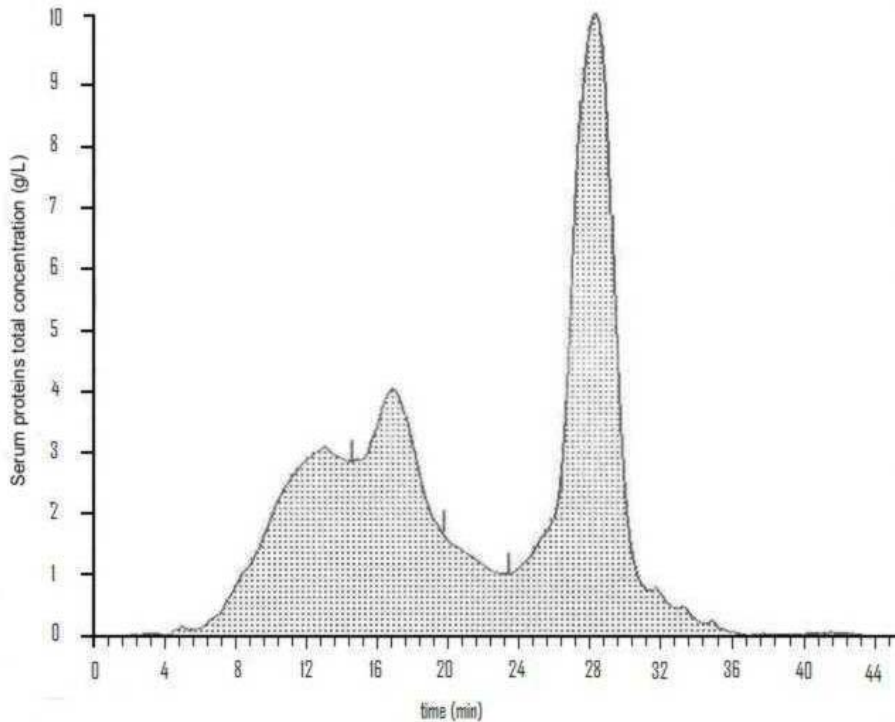


Fig. 3 – Serum protein electrophoresis for experimental sample (3) submitted to acute hyperbarism.

Table 3

Fraction values for total serum proteins electrophoresis for the dived experimental sample set

No.	Concentration of total serum proteins (g/L)	γ -globulin (%)	β -globulin (%)	α_1 (%)	α_2 (%)	Albumin (%)
9.	43.54	23.03	22.34	6.65	6.37	41.61
10.	41.37	16.56	23.24	7.99	9.36	42.85
11.	37.83	13.96	8.50	20.57	15.33	41.64
12.	77.47	19.80	24.17	9.29	12.91	33.83

In the case of protein metabolism the effects of internal irradiation and hyperbaric exposure are superimposed (Fig. 4). The increases of fractions (creatinine, α_1 , β , γ) or the decreases of fractions (urea, α_2) [2, 3] depend on the producing mechanism and the release mechanism (Table 4).

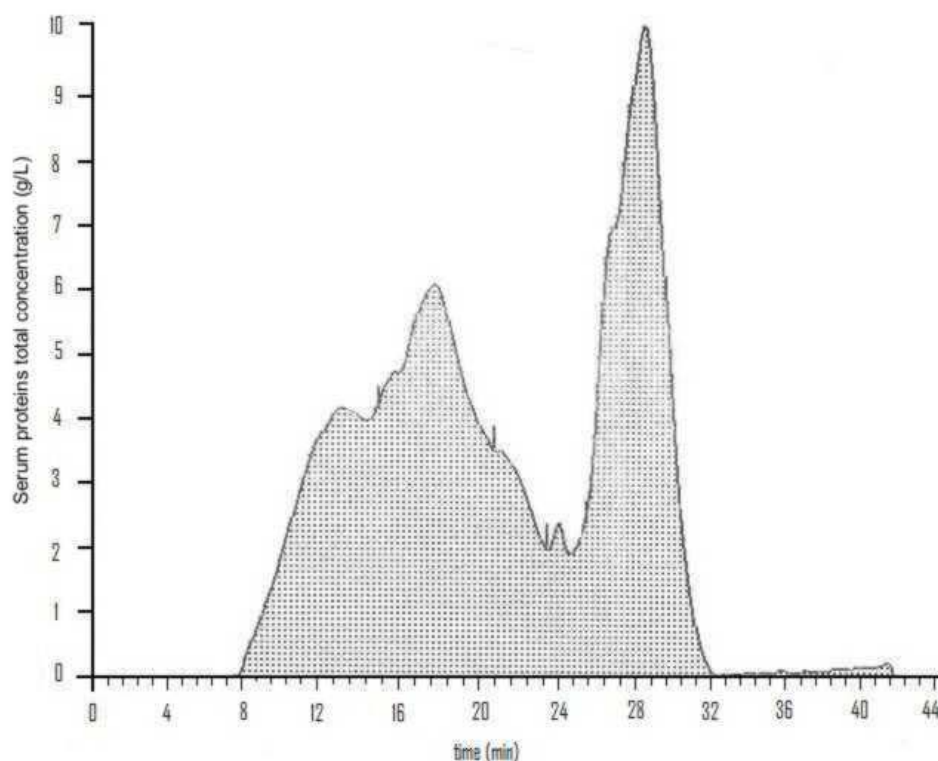


Fig. 4 – Serum protein electrophoresis for subjects of the experimental sample (4) submitted to irradiation.

Table 4

Electrophoresis fraction values for total serum protein for subjects submitted to irradiation

No.	Concentration of total serum proteins(g/L)	γ -globulin (%)	β -globulin (%)	α_1 (%)	α_2 (%)	Albumin (%)
13.	36.54	20.42	29.81	8.25	4.57	36.95
14.	46.06	11.76	15.14	24.71	18.62	29.76
15.	59.34	10.14	18.97	17.69	23.23	29.97
16.	50.54	19.73	21.79	9.95	12.10	36.43

4. CONCLUSIONS

Protein metabolism is not studied enough, most of the research being focused on hyperbaric effects of a single protein of medical concrete interest (mainly referring to the hyperbaric treatment used in order to decrease the effects of thermic burns and irradiation in oncologic therapy).

Irradiation affects protein metabolism mostly protein degradation, even through modification of their structure, which provides their initiation of enzymatic oxidation and by breaking the peptides bond leading to their elimination.

Total serum proteins, are, diagnostically, of a relative importance in assessing the state of health of the organism, their increase appearing especially in inflammatory processes and tissue dysfunctions (Table 5).

Despite the other investigated parameters, determination of total serum proteins conduced to significant statistical difference among all 3 sample studied sets, (2) irradiated and dived, (3) dived, respectively (4) irradiated and (1) the sample set. Meanwhile comparison of between sample sets marks significant differences only as regards sample set (4) irradiated and experimental sample set (2) irradiated and dived.

Tabel 5

The results of statistic tests for the obtained resulta of total serum proteins

Total serum proteins (g/L)	Witness sample	Irradiated and dived sample	Dived sample	Irradiated sample
Val. Medie	24.2425	36.69	50.0525	48.12
Dev. Std.	3.87227	5.61329	18.4292	9.48832
t Stat		-3.65064	-2.74113	-4.65991
P(T<=t) one-tail		0.00737	0.03564	0.0048
t Stat			-1.38722	-2.07358
P(T<=t) one-tail			0.11883	0.04641
t Stat				-0.18646
P(T<=t) one-tail				0.43058

The result are converging to the idea that the hyperbaric as well as internal irradiation triggers metabolic changes at cell/tissue level, due to the stimulation of hepatic synthesis of proteins as a compensatory mechanism for cellular protein damages under the action of free radicals.

REFERENCES

1. Aitken, Buckingham, Richardson, Gardiner & Irvine, *Impact of a deep saturation dive on semen quality*. International Journal of Andrology, **23**, 2, 116–120 (2008).
2. S. Rossi, M. Gallati, L. ROSA, A. Marini, F.T. Viera, M. Maestri, P. Dionigi, *Effect of hyperbarism on radiofrequency ablation outcom.*, AJR Am J Roentgenol., **189**, 876–82 (2007).

3. K.C. Tseng, B.S. Sheu, L.C. Lee, H.M. Tsai, N.T. Chiu, Y.C. Dai, *Application of technetium-99m-labeled human serum albumin scan to assist surgical treatment of protein-losing enteropathy in Cronkhite-Canada syndrome: report of a case*. *Dis Colon Rectum*, **48**, 4,870–3 (2005).
4. J.M. Jeong, M.K. Hong, J. Lee, M. Son, Y. So, D.S. Lee, J.K. Chung, M.C. Lee, *99mTc-neolactosylated human serum albumin for imaging and hepatic asialoglycoprotein receptor*, *Bioconjug Chem.*, **15**, 4, 850–5 (2004).
5. O. Teren, *Biophysical and biochemical changes of the human body in hyperbarism*, Ph.D. Thesis, University of Bucharest, Faculty of Physics, 2004.
6. M. Vasile, O. Teren, I. Ion, N.D. Ceamitru, *Modeling of 99mTc absorption in tissues*, *Annals of Ovidius University of Constantza*, **8**, 2003.
7. Helen B. Stone, William H. McBride, C. Norman Coleman, *Meeting report, Modifying normal tissue damage postirradiation report of a workshop sponsored by radiation research program, National Cancer Institute, Bethesda, Maryland, Radiation Research*, **157**, 204–223 (2002).
8. T. Negru, G. Liliș, N.D. Ceamitru, V. Alexandrescu, *Dynamic study of oxyhemoglobin in traumatic shock*. *Romanian journal of physiology: physiological sciences / Academia de Științe Medicale*, 1999, Jan-Jun.
9. G. Badiu, N.D. Ceamitru, A. Petru, *Hyperbaric Physiology and Physiopathology*, Foundation “Andrei Șaguna” Press, Constanța, 1997.
10. N.D. Ceamitru, G. Badiu, A. Petru, *The Effect of Hyperbaric Pressure (20 ATA and Heliox Respiratory Mixture) on the Erythrocytes Aggregability and Deformability – High Pressure Biology and Medicine*, University of Rochester Press, 1997, pp. 288–292.
11. B. Broussolle (coord.), *Physiologie et Medecine de la Plongee*, Ellipses, Paris, 1992.
12. J. Kieffer, *Biological radiation effects*, Lüderitz&Bauer, Berlin, 1990.
13. J.E. Coggle, *Biological Effects of Radiation*, Taylor & Francis LTd, London, 1983.
14. G. Turcu, *Biochemistry- Bioenergetics*, University of Bucharest Press, 1984.