

ASPECTS OF THE CELL POPULATION IN THE SKIN OF THE GUINEA PIG EXPOSED TO HYPERBARIC-HYPEROXIC ENVIRONMENT

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Summary. Knowing that in hyperbaric-hyperoxic environment, the production of free oxygen radicals is increased, with the direct implications on cellular metabolism, our study follows the influence of the hyperbaric environment, a very complex organ that is exposed to directly to this environment. We used a group of 10 guinea pigs split into to lots (test and control lots). The test lot was exposed for consecutive days to an ambient pressure of 6 ATM for a period of 30 min. After 50 days both groups was given lethal doses of pentobarbital, afterwards skin was harvested and fixed into Bouin's fluid and formaline, than paraffin embedded. The slides stained with Haematoxylin-Eosin and trichromic Van Gieson. The epidermal fields examined by us revealed no significant variations of nuclear number, though the mean saturation of nuclei was significantly greater in test animals than in the control group, while the nuclear area fraction was higher in the control group. The melanin load of the epidermal cells was increased in the control animals. The presence of intraepidermal vacuoles was identified, their amount being almost double in test animals than in the control group.

Key words: hyperbaric, hyperoxic, environment.

INTRODUCTION

The entrance of the human being into hydrospace for heuristic reasons (the exploration of the silent world), but especially for economic and military reasons means to expose of the human body to aggressions of complex and intense stress.

The study of complex-adjustment response of answer of the human body to greater depths is a major tissue of hyperbaric medicine. Being aware of the fact that under conditions of hyperbarism hyperoxic a great quantity of oxygen free radicals with direct implications in to cellular metabolism, and the hyperbaric conditions determine the long term stimulation of the sympathetic-adrenaline system, our survey approaches the study of the influence of these environmental conditions upon a very complex organ from morpho-functional point of view – we refer to the tegument, exposed directly to hyperoxic hyperbaric conditions [2].

At the cellular level, the main goal of the free radicals is represented by the membranes of the cellular organites; at their level there are producing precocious or late modifications. Although the general structure of the cell is the same for a while, the modification of certain parameters of the nucleus and of some

cytoplasmatic elements can be of use in evaluating the general level of activity of the cell [4, 7]. For this purpose we considered some parameters:

- the color saturation of nucleuses;
- the ratio between the nuclear surface and epidermal surface;
- the number of nucleus on microscopic field.

We have accomplished the dynamic and the morphology of the nucleuses because these represent an indirect code of the metabolic activity in a tissue. We have also carried out morph-metric studies regarding the assessment of some functions of the tegument, like the process of melanogenesis.

This thing was accomplished through the ratio between area occupied by the melanin pigment inside the cell's cytoplasm and the total epidermal area. Establishing the ratio between the vacuole and the total epidermal area monitored some of the involutive processes, which appear in tegument, as vacuolization of the epidermal cells. At dermal level we took into consideration the cellular and tissue parameters, which we compared to the parameters studied in the experimental lot.

MATERIAL AND METHOD

The material used in our experiment consisted of a lot of 10 guinea pigs with short fur (English kind), of 5–6 weeks and weight of 190–280 g. This lot, the witness lot, was unexposed to the hyperoxic hyperbaric conditions and the lot no. 2, the test lot, was exposed to hyperbarism. The weight average in the witness lot was of 242 g, and in the test lot 252 g difference being insignificant.

The test lot was submitted to chronic hyperbarism, carried out through unitary sinking at a depth of 50 m, corresponding to an absolute pressure of 6 atmospheres during 50 consecutive days. At this depth, the guinea pigs lived 30 minutes, after that following a decompression phase, using a method of professional divers. The breathing mixture used was air. All through the experiment, the animals of the test lot and witness lot cohabitated (except the diving period).

After 50 days, the guinea pigs were anesthetized and sacrificed. There were sampled fragments of tegument from the thorax, underbelly and ear areas, which were processed in compliance with classic techniques of histology. The histological preparations obtained were examined on the NIKON E600 microscope, and then transferred to a computer with a SONY video camera. The images obtained were processed using the LUCIA G3.52 soft of image analysis. By calibrating the color sill, we obtained "binorized" images (in which conventionally for black "0" is allocated and "1" for white). Based on these images, binorized, the LUCIA G3.52 program used the matricidal calculation, being able to measure accurately the parameters required by the user. There have been studied 100-contiguitaly microscopic fields, during examination moving the section as for carrying out the leucocytes formula.

RESULTS

Examining the histological preparations obtained, it is to be remarked the general aspect of the epidermis, which does not cover particular shapes, except profound areas in which it is to be seen many cellular vacuolization at the test lot, compared to the witness lot, as well as the presence of a increased quantity of melanin in the basic layer and in the profound cover of the thorny layer in the witness lot compared to test lot. The study of the cellular parameters at epidermis level takes into account the proposed parameters:

- Nucleus and epidermis area;
- The number of the nuclei on microscopic field;
- The middle color saturation;
- Quantity of melanin;
- Vacuolization.

NUCLEAR AND EPIDERMIS AREAS

The significant decrease of the nuclear area through chromatin condensation reveals a specific aspect of cellular apoptosis. The chromatin arranged under the shape of dense weights, with clear contour, is disposed under the nuclear membrane. The shape of the nucleus appears with modifications, and the contour of the cells appears to be increased and contorted. The epidermis cells, being in continuous movement and stratification toward the keratin superficial cell, the apoptosis offers a simple mechanism to eliminate the cells, which reach abnormal places during development (Figure 1, Table 1).

The statistic significance in the test lot for this parameter was $P(T < t) = 0.005181$ (significant significance).

NUMBER OF THE FIELD NUCLEUSES

By accomplishing the significance test (the Student – Fisher test) upon the number of nucleuses in field, there are not significant modifications between the two lots (lot T and lot M).

Table 1
Statistic significance of the nuclear and epidermis areas

	Control	Test
Mediate	85.3	77.5
Standard deviation	211.1222	680.2778
Obs.	10	10
t Start	0.826149	
P (T<t)	0.211288	
t critical	1.7611309	

MIDDLE COLOR SATURATION

Taking into account the 4th parameter, the middle color saturation, we detect significant differences between the witness and the test lot $P(T < t) = 1.41E-09$ (Figure 2, Table 2). The interpretation is made upon the contrast between the new-apoptosis nucleuses from neighbourhood and their color saturation, in contrast with the nucleuses of apoptosis cells colored in brown (more hypo-chromatic).

Table 2
Accomplishing the significance test (the Student – Fisher test)

	Control	Test
Mediate	45.151	73.074
Standard deviation	23.53039	38.46467
Obs.	10	
t Start	-11.2146	
P(T<=t)	1.41E-09	
t critical	1.739606	

QUANTITY OF MELANIN (OCCUPIED AREA / TOTAL EPIDERMIS AREA)

In order to assess the parameters, which reflect some of the physiological activities as melanogenesis, we took into consideration the area occupied by the melanin pigment out of the total epidermis area, both at the witness and the test lot. By accomplishing the significance test of this parameter, we find major differences in the test lot against the witness lot $P(T < t) = 0.0018677$ (Figure 3).

VACUOLAR AREA / TOTAL EPIDERMIS AREA

In order to evaluate these involutive processes, which appear in tegument at the level of epidermis, in the animals submitted hyperbar environments such as vacuolization of the epidermis cells, we accomplished the ratio between vacuole and the total epidermis area. The significance test for this parameter reveals a significant value $P(T < t) = 0.029873$. The vacuolization of epidermal cells either in the profound layers, but also in the superficial ones accompanied by the condensation of the chromatin represents another characteristic feature of cellular apoptosis (Figure 4).

HISTOLOGICAL AND HISTOMETRICAL MODIFICATIONS AT DERMAL LEVEL

In order to survey these modifications, we took into consideration the same parameters: the number of the dermal cells by estimating the cellular nucleuses, the nuclear diameter, the ratio volume and area (Table 3). In the witness lot, the

number of the nucleuses was of 4.337, to against 5.982 in the test lot. The nuclear diameter in the witness lot was of 4.337, and in the test lot this was of 5.279. The nuclear volume in the witness lot was of 35334, comparing to 45304 in the test lot.

Table 3

Statistic significance of the parameters: the number of the dermal cells by estimating the cellular nucleuses, the nuclear diameter, the ratio volume and area

Control					Test				
	<i>Derm</i>					<i>Derm</i>			
	Nc.	Diam.	Vol.	AF		Nc.	Diam.	Vol.	AF
1	2.37	4.35	38.11	3.5	1	6.36	5.28	46.78	6.33
2	3.04	4.17	41.9	4.25	2	5.48	4.49	43.12	5.98
3	3.28	4.26	37.25	4.56	3	6.87	6.03	69.63	7.25
4	3.57	4.55	32.27	5.36	4	6.06	5.57	56.29	6.26
5	3.84	4.27	36.95	5.78	5	5.14	5.76	52.78	6.12
6	3.45	4.66	34.58	4.29	6	6.35	5.35	55.28	6.87
7	3.65	4.15	32.71	4.35	7	6.16	5.24	44.98	6.25
8	2.89	4.12	31.05	3.89	8	5.47	4.76	43.56	5.46
9	3.22	4.55	35.77	4.27	9	5.66	5.08	44.27	5.76
10	3.89	4.29	32.75	5.29	10	6.29	5.23	44.86	6.87
Mediate	3.32	4.337	35.334	4.554	Mediate	5.982	5.279	45.304	6.315

The area of the fraction was measured at 4.554 in the witness lot, comparing to 6.315 in test lot. Accomplishing the statistic signification of the dermal cells, we find out that for this parameter the differences are major $P(T < t) = 4.68E-0.6$ (0.00000468). For estimating the capillary dynamics, during exposure to hyperbaric oxygen, we have analysed the capillary dynamics and structure at dermal level and we also found out great differences.

Thus, the capillary average was of 0.874/microscopic field at the witness lot compared to 1.481 / microscopic field in the test lot. The statistic variation accomplished by the same method revealed very important differences in the dermal capillaries $P(T < t) = 1.3E-0.5$ (0.0000013). Analysing the fibril structure at dermal level, we notice a significant decrease of the elastic fibres in the test lot. We find out that the average of the elastic fibres in the test lot is of 0.076731 compared to 0.09851 of the witness lot. There were noticed many fragmented elastic fibres, or even point particles in the test lot as against the witness lot.

DISCUSSIONS

Assessing the nuclear and epidermis areas in the field, we find out major modifications. Our results through evaluating this parameter constitute undoubtedly morphological signs of apoptosis (8, 11) (amplified by the hyperbaric stress). The environment of the second universe (the sub aquatic environment) is a

non physiological for a human being and represents a real aggression against body. The hyperbaric issue ultimately limits itself to the responsive and adjusting of the body. The different aspect of the heterochromatin is used as a diagnosis in evaluating the general level of the cell's activity. A cell, which enters again in the cellular cycle from G0, converts a great part of heterochromatin into euchromatin, while in a degenerate cell in which the transcription reaches a dead point; most of the chromatin is condensed in weights of heterochromatin [5].

The decreasing capacity of melanin capture by the epidermis cells could have important implications in the epidermis photo-immunology with the development of the non allergic skin diseases, by affecting the immune surveillance system. Disturbances of the same system can lead to local and biochemical immunological modifications, being at the same time a factor supporting the development of the dermal pathology. The shock states and the hyperbaric oxygen imply lysosomal injuries, which lead to cellular and tissue destructions. The recent researches offer explanations about the mechanisms of self-keeping through injuries of the lysosomal membranes.

Due to the fact that after decompression and the pulling off to surface of the guinea pigs we did not find out special modifications in dry hyperbar and in different breathing mixtures used upon the dermal sensitivity, the tactile discrimination did not show essential modifications, except the sill for tactile and painful stimuli. We consider this is the result of the fact that the hyperbar environment does not visibly affect the inter-epidermis free nervous terminations.

The hyperbaric stress strongly influences the second element of the tegument, the derma. We consider these modifications to be the consequence of a direct action of the increased pressure and/or indirect of reflex neuro-umoral mechanisms, which carry out a new distribution of the sanguine, circulating volume in which there seems to be a certain hierarchy in various territories [3].

We consider that the quantitative modification of the number of capillary in the test lot is the consequence of dermal vasodilatation and opening of new capillary or shunting arterio-venal, as well as the reduced perspiration and transpiration. Once the capillary bed increases, the mid dermal temperature also increases. The measurements showed that both rectal and dermal temperatures had small values in the morning and they increased during the daytime. The thermal homeostasis in the hyperbaric environment is still an undecided issue because there are also other researches, which underlined variation between the decreasing and increasing of the dermal temperature, reversing the diurnal rhythm. Once the capillary layer grows, at dermal level we see other injuries of the capillary wall, placed at endothelium and also modifications of the capillary diameter.

The hyperbaric stress strongly influences the cardiovascular system, causing important modifications, which in their turn put within operation adapting over stressing mechanisms, trying to keep the cardiovascular functions into normal parameters [1, 6]. We think that this morphological modification at the elastic

fibres is the consequence of the growth of the free radicals with noxious influence upon the elastic fibres. In the experimental hyperbarism, important modifications of the metabolism of proteins, glucides, and lipids were seen. It was noticed that the hyperbar oxygen determines, for the beginning, either directly or indirectly, on umoral or nervous way, an easy peripheral constriction of veins. In time, exposed to hyperbar conditions, the cardiovascular system passes through adapting modifications, which does not represent a limitative factor of performing effort in hyperbarian environment. The creation of free oxygen radicals is and remains a continuous threat for the cells and tissues in which the blood flow was established after a interrupted period. During the interrupting or decreasing of the blood flow, the intracellular mechanisms that control the producing of oxygen free radicals, as well as the counter oxidizer systems are disturbed. When the blood flow comes into normal, the cell is suddenly confronted with an extra number of oxygen free radicals, the hypoxic modifications representing the most important causes of tissue injuries [9, 10].

CONCLUSIONS

Our results through evaluating the parameters (nucleus and epidermis area, the number of the nucleuses on microscopic field, the middle color saturation, quantity of melanin vacuolization) constitute undoubtedly morphological signs of apoptosis (amplified by the hyperbar stress). The environment of the second universe (the sub aquatic environment) is a non physiological for a human being and represents a real aggression for body. At the cellular level, the main goal of the free radicals is represented by the membranes of the cellular organites; at their level there are producing precocious or late modifications. Although the general structure of the cell is the same for a while, the modification of certain parameters of the nucleus and of some cytoplasmatic elements can use in evaluating the general level of activity of the cell.

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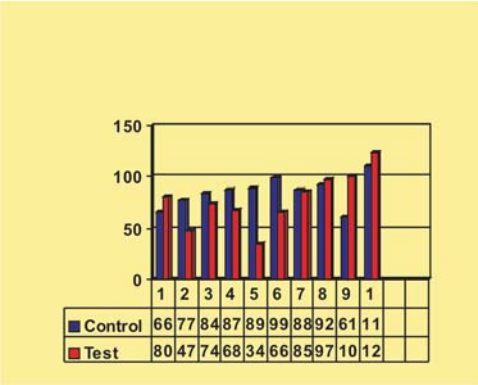


Figure 1 – Nucleus and epidermis area (test and control lots)

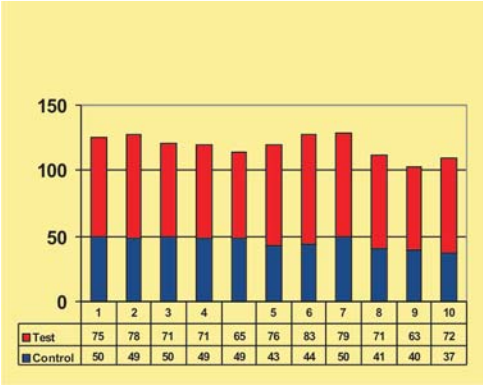


Figure 2 – Middle color saturation (test and control lots)

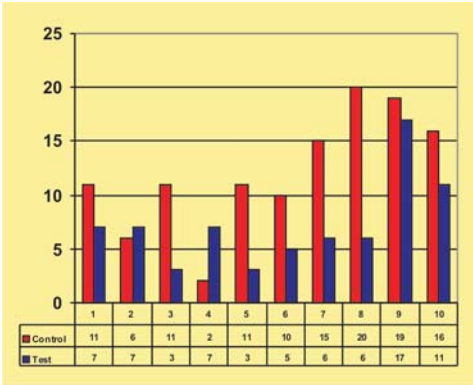


Figure 3 – Quantity of melanin - occupied area / total epidermis area (test and control lots)

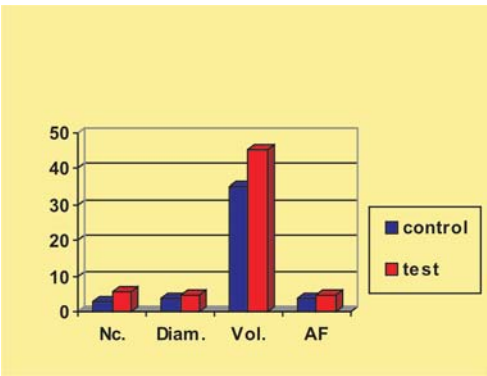


Figure 4 – Dermal level, comparison between cellular and experimental parametrics