

INHIBITION OF SYNTHESIS OF HUMORAL ANTIBODIES UNDER  
THE INFLUENCE OF HYPERBARIC OXYGENATIONL. A. Bokeriya, M. A. Frolova,  
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The action of hyperbaric oxygenation (HBO) on the immune response was studied in mice immunized intraperitoneally with sheep's red blood cells. HBO was found to cause a decrease in the titers of hemagglutinins and hemolysins and a decrease in the number of antibody-forming cells in the spleen.

KEY WORDS: antibody production; hyperbaric oxygenation; immunodepressants.

During hyperbaric oxygenation (HBO) the increased oxygen concentration accelerates the oxidation of phospholipid components of biological membranes [6]. As a result of this, their ionic permeability is substantially changed and the ionic composition of the cytoplasm is disturbed [2]. The effect of the ionic composition of the cytoplasm on the course of the mitotic cycle [4] suggests that, under certain conditions, HBO can act on the functional activity of cells.

The object of this investigation was to study the action of HBO on the immune response. This particular experimental model was chosen because of the fact that cells of the immune system have a number of specific properties, modifications of which can be precisely monitored.

## EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 18-20 g. The animals were immunized intraperitoneally with sheep's red cells (SRBC) in doses of  $1 \cdot 10^7$  and  $1 \cdot 10^8$  SRBC per mouse. The animals were exposed to sessions of HBO [1] under toxic and subtoxic conditions. In the toxic series oxygen was used at a pressure of 3.6 atm until convulsions appeared (the acute form of oxygen poisoning). The duration of saturation was 40-45 min. The subtoxic HBO sessions were carried out at a pressure of 3.0 atm and the duration of saturation was 30 min. In the subtoxic series the animals showed no increase in motor activity and no convulsions.

In the control and experimental groups of animals the hemagglutinin and hemolysin titers were determined on the 5th and 7th days after a single immunization. On the 3rd, 4th, and 5th days after immunization the number of antibody-forming cells (AFC) in the spleens was determined by the method in [7]. The number of peripheral blood cells was counted and the leukocyte formula calculated. Blood for investigation was taken from the retro-orbital vessels.

TABLE 1. Effect of Single HBO Sessions on Hemagglutinin Synthesis on 5th Day After Immunization (M  $\pm$  m)

Scheme of experiments	No. of animals	Reciprocals of antibody titers
Control, immunization with $1 \cdot 10^7$ SRBC	8	$256 \pm 23$
Immunization:		
after HBO at 3.6 atm	8	$150 \pm 12$ $P < 0,05$
after HBO at 3.0 atm	8	$176 \pm 16$ $P < 0,05$
after motor activity for 35 min	8	$240 \pm 22$ $P > 0,05$

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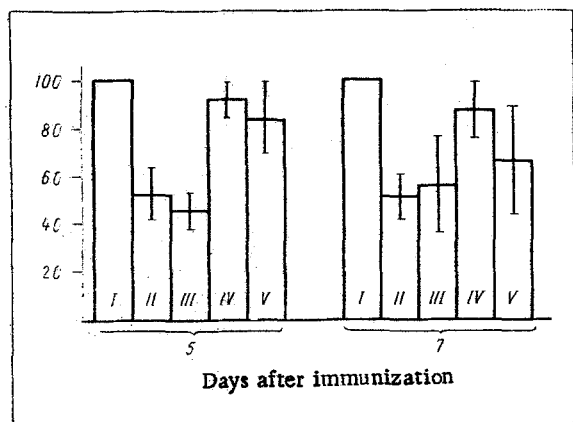


Fig. 1

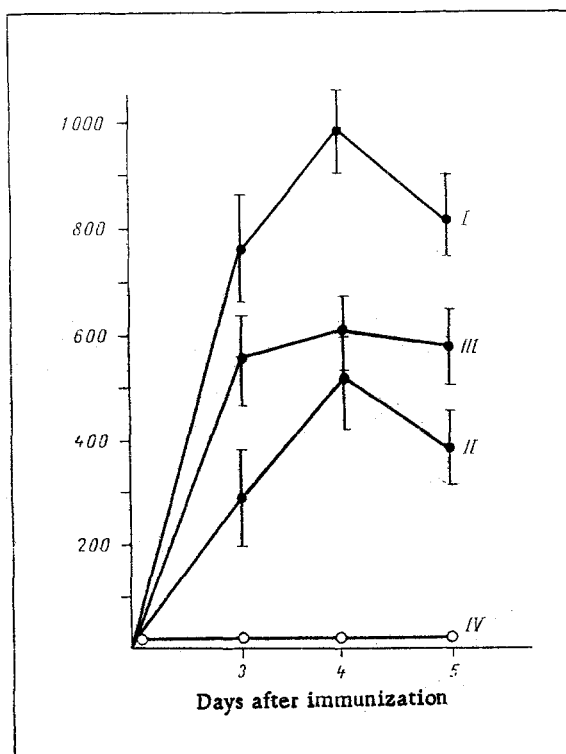


Fig. 2

Fig. 1. Reduction in hemagglutinin titer under the influence of HBO. Ordinate, reciprocals of antibody titers (in % of control). I) Control, immunization with  $1 \cdot 10^7$  SRBC; II) immunization before a single session of HBO; III) immediately after 5 daily HBO sessions; IV) immediately before a single HBO session; V) immediately before 5 daily HBO sessions (25 animals in each group).

Fig. 2. Decrease in number of AFC in spleen and their influence on HBO sessions. Ordinate, number of AFC. I) Control, immunization with  $1 \cdot 10^7$  SRBC; II) immunization after 5 daily HBO sessions; III) immunization before single HBO session; IV) number of AFC in unimmunized animals (eight animals in each group).

The numerical results were subjected to statistical analysis.

#### EXPERIMENTAL RESULTS

The effect of single sessions of HBO under different conditions on synthesis of humoral antibodies was studied in a preliminary series of experiments. To rule out the possible effect of stressors on immunogenesis [3], in the same series experiments were carried out to differentiate between the factors of stress (increased motor activity, convulsions) and the direct effect of an increased oxygen pressure on antibody synthesis. It follows from the results in Table 1 that single sessions of HBO, preceding immunization, caused a statistically significant decrease in the hemagglutinin titer. The absence of any significant effect of stressor action on immunogenesis indicates that the immunodepressant action of HBO was due to its direct effect on the immune system, and was not mediated indirectly through stressor factors. Since HBO under toxic conditions gave a more marked effect than under subtoxic conditions, in subsequent series of experiments the animals were exposed to toxic sessions of HBO.

In order to choose optimal conditions for realization of the immunodepressor effect of HBO experiments were carried out to study different combinations of HBO sessions and immunization. The results of these experiments (Fig. 1) show that antibody synthesis was most effectively inhibited if the HBO sessions were given before immunization. Under these circumstances no significant difference was found between the immunodepressor action of one and of many HBO sessions.

Investigation of the effect of the HBO sessions under toxic conditions on hemolysin synthesis in animals immunized with the same dose of SRBC ( $1 \cdot 10^7$ ) gave results similar to those illustrated in Fig. 1. Investigation of the effect of HBO sessions on the number of AFC in the spleen also showed that the immunodepressor action of HBO sessions is manifested if they are given before immunization (Fig. 2).

The effect of HBO on immunogenesis described above was revealed when the experimental animals were immunized with a comparatively small dose of SRBC ( $1 \cdot 10^7$ ). Since the realization of the immune response depends essentially on the degree of antigenic stimulation, it was decided to investigate the action of HBO on immunogenesis in response to an optimal immunizing dose of antigen. Accordingly the effect of 5 sessions of HBO on the number of AFC was studied in animals immunized with  $1 \cdot 10^8$  SRBC. In this case the effect of HBO was manifested only at the state of a general decrease in the number of AFC: On the 5th day after immunization the number of AFC in the experimental group was reduced compared with the control by  $44 \pm 5\%$  ( $P < 0.05$ ). The results thus showed that the immunodepressor action of HBO is manifested only under certain conditions.

To study the mechanisms of the immunodepressor action of HBO, the effect of HBO sessions on the blood count of unimmunized animals was investigated. A single HBO session under toxic conditions caused a decrease in the peripheral blood leukocyte count by  $46 \pm 6\%$  ( $P < 0.05$ ) and a decrease in the lymphocyte counts by  $71 \pm 7\%$  ( $P < 0.05$ ), followed by restoration of the normal level of these parameters 24 h after the HBO session. After 5 daily HBO sessions there was a stable decrease in the number of blood cells without any clearly defined tendency toward normalization: On the 5th day after the last session the leukocyte count was 22.2% lower ( $P < 0.05$ ) and the lymphocyte count  $39 \pm 4\%$  lower ( $P < 0.05$ ) than initially. It can be concluded from these results that repeated sessions of HBO cause a true decrease in the number of white cells in the peripheral blood, and not simply a redistribution of these cells in the various organs, as has been claimed [5].

Correlation between the immunodepressor action of HBO and the decrease in the total leukocyte and lymphocyte counts in the peripheral blood suggests a definite link between these two effects of hyperbaric oxygen. On the other hand, considering the dependence of the immunodepressor effect of HBO on the experimental conditions, the possibility cannot be ruled out that cells of the immune system differ in their sensitivity to the action of hyperbaric oxygen before and after exposure to the antigenic stimulus.

It was shown that, in principle, immunogenesis can be depressed by the action of HBO, and this opens up two pathways for the development of effective methods of immunodepression.

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