# IMMUNOHISTOCHEMICAL INVESTIGATION OF ANTITOXIC IMMUNITY OF MICE IN HYPOXIA

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The development of antitoxic immunity was investigated in mice immunized with tetanus toxoid and kept for 30 days in a pressure chamber at a pressure of 378 mg Hg. The antitoxin content in the blood of the experimental animals and their resistance to tetanus toxin remained normal despite hypoplasia of their lymph glands and a decrease in the number of plasma cells in them. It is postulated that preservation of the normal blood antibody level despite the decrease in the number of antibody-producing cells takes place on account of an increase in the intensity of antibody synthesis in each individual cell.

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A previous investigation [2] showed that an increase in sensitivity to bacterial infection in mice kept for a long period at an atmospheric pressure of 378 mm Hg is due to a decrease in phagocytic activity of the neutrophils and macrophages, antibody production in the experimental animals remaining unchanged. It was concluded from these results that in cases when resistance to infection depends on the formation of antitoxins, keeping animals under hypoxic conditions must influence the state of antitoxic immunity.

In the present investigation the effect of hypoxia was studied on the development of immunity after immunization with tetanus toxoid.

TABLE 1. Content of Antitoxin in Blood Serum of Mice Immunized with Tetanus Toxoid

| Group of mice | Content of tetanus antitoxin (in i.u.) at various times after immunization | | |
|---|---|---|---|
| | 10 Days | 16 Days | 21 Days |
| Control | 0.01 | 1 | ≥1 |
| Experimental | ≥0.01 | 1 | >1 |

TABLE 2. Weight of Regional (Inguinal) Lymph Glands, M ± m

| Time (in days) | | Mean weight of lymph glands in mg | | P |
|---|---|---|---|---|
| After beginning of expt. | After immunization | in control mice | in experimental mice | |
| 19 | 10 | 7.7 ± 1.10 | 8.1 ± 1.10 | > 0.05 |
| 25 | 16 | 8.0 ± 0.70 | 5.5 ± 0.86 | < 0.05 |
| 30 | 21 | 7.9 ± 0.64 | 4.8 ± 0.42 | < 0.01 |

## EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing about 20 g and kept for 30 days in a ventilated pressure chamber under a pressure of 378 mm Hg. On the 9th day from the beginning of the experiment, 0.1 ml (2 units) of absorbed tetanus toxoid was injected subcutaneously into 30 experimental and 30 control mice (the latter kept at normal atmospheric pressure). On the 10th, 16th, and 21st days after immunization the mice were weighed, decapitated, and their blood collected for estimation of the serum antitoxin concentration (10 experimental and 10 control mice at each time). The serum antitoxin concentration was determined by the usual method of titration in mice.*

*Advice on the Method of titration of tetanus antitoxin was obtained from the Department of Sera and Vaccines (Head, Professor F. A. Chertkova) of the L. A. Tarasevich State Control Institute of Biological and Medical Preparations, from Candidate of Medical Sciences E. S. Shain, to whom the authors are grateful.

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Regional (inguinal) lymph glands relative to the site of injection of antigen were taken for immunohistochemical investigation, weighed, fixed in Carnoy's fluid, and embedded in paraffin wax. Sections of the lymph glands were stained with hematoxylin-eosin and with methyl green–pyronine (control with ribonuclease).

To study the state of antitoxic immunity, $ED_{50}$ value of tetanus toxoid also was determined (by the method used at the L. A. Tarasevich State Control Institute) in 90 mice (30 experimental and 60 control). The value of $ED_{50}$ and the number of immunizing units were calculated by Ashmarin's method [1].

## EXPERIMENTAL RESULTS

The antitoxin concentration in the serum of the mice immunized with tetanus toxoid and kept under hypoxic conditions was indistinguishable from that in the control animals (Table 1). Determination of the immunogenicity of the tetanus toxoid likewise revealed no difference in the response of the control and experimental mice: the number of immunizing units (i.u.) was 526 for the former and 500 for the latter. Hence, the resistance of control and experimental mice immunized by different doses of toxoid to tetanus toxin was identical. The blood antibody level of the experimental animals was normal despite hypoplasia of the lymph glands (Table 2) and the distinct decrease in the number of plasma cells in these glands. This may be attributedto an increase in the intensity of antibody synthesis in each individual plasma cell. This hypothesis is based on results indicating an increased intensity of protein synthesis in the excretory cells of the pancreas during hypoxia [4]. The existence of rather larger germ centers in the lymphoid follicles of the lymph glands of the experimental mice than in the controls and the increase in number of phagocytic macrophages in these centers are evidently connected with increased cell destruction (this has been observed by other authors during hypoxia [3]). Hence, although antibody production in mice remains normal during hypoxia, the decreased number of antibody-producing cells indicates certain (still uncompensated) changes in the system responsible for development of humoral immunity.

## LITERATURE CITED


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