

# EFFECT OF INTERMITTENT ADAPTATION TO HYPOXIA ON IMMUNOBIOLOGICAL REACTIVITY

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During gradual adaptation to hypoxia, some decrease in the phagocytic activity of the neutrophils, a decrease in antibody production, and a decrease in the number of antibody-containing cells in the lymph glands, together with hypoplasia of the lymph glands, develop in mice. These changes are less marked than during continuous adaptation to hypoxia.

Since intermittent adaptation to hypoxia is an effective method of increasing the resistance of animals and man to the action of several extremal factors, including hypoxia itself [2], it was decided to study the immunological reactivity of animals during intermittent adaptation to hypoxia and at the end of training, because of the conflicting and scanty nature of the published data on this problem [3, 9, 10].

## EXPERIMENTAL METHOD

Experiments were carried out on 150 male mice weighing about 20 g and subdivided into two equal groups, experimental and control. The experimental mice were kept for 6 h daily for 1 month in a ventilated pressure chamber, in which the pressure was 405 mm Hg, corresponding to an altitude of 5000 m. The control group of mice was kept in an atmosphere with normal oxygen concentration. Immunological reactivity of the mice was investigated during intermittent adaptation to hypoxia, using animals receiving subcutaneous injections of 40  $\mu$ g typhoid Vi-antigen into the inguinal region on the 20th day of the experiment. The mice were sacrificed on the 24th, 26th, and 29th day of the experiment (7 experimental and 7 control animals at each time). The immunological reactivity of the mice was studied after the end of intermittent adaptation to hypoxia, using animals immunized by two subcutaneous injections of alcohol-treated typhoid vaccine ( $4 \times 10^8$  bacterial cells) or bovine serum albumin (BSA) in a dose of 200  $\mu$ g, into the inguinal region; the first injection of antigens was given on the 7th day of the experiment and the second injection 7 days after the end of intermittent adaptation to hypoxia. The mice immunized with typhoid vaccine were sacrificed 4, 7, and 10 days (10 experimental and 10 control animals at each time), and the mice immunized with BSA 5, 9, and 14 days (7 experimental and 7 control animals at each time) after re-immunization. The body weight, the weight of the regional lymph glands, relative to the site of injection of the antigen (inguinal), the phagocytic activity of the blood neutrophils [1] and peritoneal macrophages [12], and the titers of Vi- or O-antibodies, by the passive hemagglutination method [7], were determined for the mice receiving Vi-antigen or typhoid vaccine. The inguinal lymph glands were fixed in Carnoy's fluid and embedded in paraffin wax. Serial sections were stained with hematoxylin-eosin and methyl green-pyronine (ribonuclease control). The content of antibody-forming cells was determined in the lymph glands of mice immunized with BSA by means of the indirect immunofluorescence method [11]. The numerical results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

The state of the mice remained good throughout the experiment, the gain in weight was the same in both the control and experimental groups of mice, and no deaths occurred in either group.

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TABLE 1. Titers of Vi-Antibodies in Blood of Mice Immunized with Vi-Antigen during Intermittent Adaptation to Hypoxia

Time from beginning of experiment (in days)	Time after immunization (in days)	Titer of antibodies		n	P
		control	expt.		
24	4	42,66 $\times$ 0,12	13,18 $\times$ 0,09	12	<0,01
26	6	39,81 $\times$ 0,13	15,14 $\times$ 0,06	12	<0,02
29	9	60,26 $\times$ 0,06	24,55 $\times$ 0,06	12	<0,001

**Note.** Statistical analysis of results of antibody titration carried out by the usual method [8].

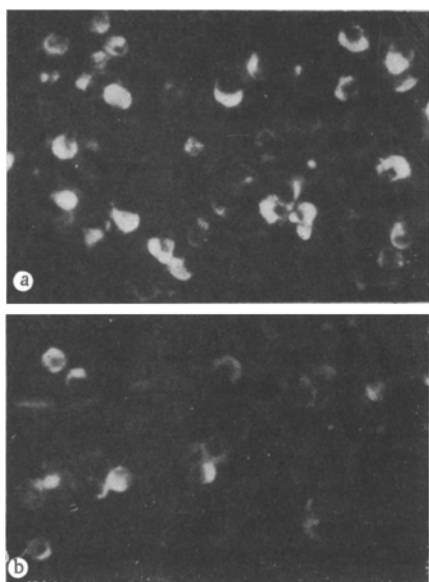


Fig. 1. Decrease in number of antibody-forming cells in lymph gland of a mouse adapted gradually to hypoxia (b) compared with control (a). Indirect Coons' method, 200 $\times$ .

which antibodies were detected by the immunofluorescence method was small compared with the total number of plasma cells. In 5 of the 7 mice of the experimental group sacrificed on the 5th day after reimmunization, the number of antibody-forming cells in the lymph glands was slightly below the number in the animals of the control group (Fig. 1). This was reflected primarily in a decrease in the number of foci of these cells in the medullary cords of the lymph glands, and also by a decrease in their number in each separate focus; in the control and experimental mice sacrificed on the 9th and 14th days after the second injection of BSA, no difference could be found in the content of antibody-forming cells in the lymph glands.

Hence, these results indicate that during intermittent adaptation to hypoxia, changes develop in the mice and, in particular, in their immunocompetent organs. These changes, although less marked, are analogous to those taking place during continuous adaptation to hypoxia [4, 5, 6], and they indicate depression of the immunobiological reactivity of the animal; some of these changes (hypoplasia of the lymph glands) persist for approximately two weeks after the end of intermittent adaptation to hypoxia.

During intermittent adaptation to hypoxia and after its end, no significant changes were found in the phagocytic activity of the neutrophils and macrophages, and only in the mice sacrificed on the 24th and 29th day of the experiment was a tendency observed for the phagocytic activity of the neutrophils to decline; there was a slight decrease in the number of phagocytic cells, but no change in their ingestive power.

Production of Vi-antibodies in the experimental group of mice, sacrificed on the 24th, 26th, and 29th days of the experiment, as Table 1 shows, was reduced compared with that in the mice of the control group. This decrease took place against the background of hypoplasia of the regional lymph glands relative to the site of injection of the antigen. A lower weight of the lymph glands in the mice of the experimental group was observed both during intermittent adaptation to hypoxia and after its end, and although the difference was statistically significant only on the 24th day of the experiment and on the 17th day after its end, there was a definite tendency for the weight of the lymph glands to diminish in the mice of the experimental group. Hypoplasia of the lymph glands, as the results of immunomorphological analysis showed, was due to a decrease in the number of elements of the plasma-cell series in the medullary cords of the lymph glands and, in particular, to a decrease in the number of lymphocytes in the cortical layer of the lymph glands, reflected in a decrease in width of the cortex, a decrease in the size of the follicles and their pale centers, and a decrease in the number of lymphoblasts and prolymphocytes in these centers. "Catarrh of the sinuses" also was less marked in the lymph glands of the experimental group of mice than in the control animals.

Despite the persistent hypoplasia of the lymph glands, the titers of O-antibodies in the mice of the experimental group were indistinguishable from those in animals of the control group 11, 14, and 17 days after the end of intermittent adaptation to hypoxia.

Immunofluorescence analysis of the lymph glands of the mice immunized with BSA showed that antibodies were present in mature and immature cells of the plasma series, which usually were arranged in groups of 5-20 in the medullary cords of the lymph glands. The number of cells in

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