

EFFECT OF PYROGENAL ON SYNTHESIS
OF HEMAGGLUTININS IN CULTURES OF LUNG,
SPLEEN, AND PARATRACHEAL LYMPH GLAND TISSUES

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When injected into rabbits immunized with sheep's erythrocytes, pyrogenal stimulated hemagglutinin synthesis in tissue cultures of explants of the spleen and paratracheal lymph glands. No activation of antibody synthesis was observed in explants of the lungs. Only if pyrogenal was added to the culture medium was antibody production by lung tissue stimulated slightly in the early periods of cultivation.

Experiments [1, 3-5] have shown that immunogenesis is stimulated by endotoxins of microorganisms and their lipid fractions (pyrogenal, etc.).

The object of this investigation was to study the action of pyrogenal on antibody synthesis in cultures of lung, spleen, and paratracheal lymph gland tissues after application of the antigen by various methods.

EXPERIMENTAL METHOD

Experiments were carried out on 29 rabbits immunized twice at 10-day intervals by injection of sheep's erythrocytes intravenously or into the trachea in a dose of 0.5 ml of a 10% suspensions per kg body weight. Before intravenous or intratracheal immunization, some animals were injected on 5 successive days with pyrogenal in a dose of 20 g/kg body weight. The immunologic activity of explants of the lungs, spleen, and paratracheal lymph glands was investigated in tissue culture 72 h after the second injection of antigen. In some experiments, pyrogenal (2.5 $\mu\text{g}/\text{ml}$) was added to the culture medium. During the study of the dynamics of antibody synthesis, parallel samples were cultivated for 12, 18, 24, 30, and 34 h. The antibodies were determined by the hemagglutination test (HT).

EXPERIMENTAL RESULTS

Intravenous injection of pyrogenal clearly stimulated antibody formation in explants of the lymphoid organs by comparison with the control (organ of rabbits only immunized intravenously). For example, the hemagglutinin titer in the spleen of the experimental rabbits was $1 : 137 \cdot (\surd) 1.41$,* compared with $1 : 58 \cdot (\surd) 1.059$ for the control ($P < 0.001$). Titers of antibodies in the culture fluid of explants of the paratracheal lymph glands were $1 : 28 \cdot (\surd) 1.064$ and $1 : 14 \cdot (\surd) 1.059$, respectively ($P < 0.001$). No stimulation of hemagglutinin formation by the lung explants was observed.

The most active synthesis of hemagglutinins in the intratracheally immunized animals was found in explants of the paratracheal lymph glands ($1 : 94 \cdot (\surd) 1.094$) and lungs ($1 : 23 \cdot (\surd) 1.135$), and less marked synthesis was found in explants of the spleen ($1 : 18 \cdot (\surd) 1.091$). Intravenous injection of pyrogenal stimulated the immune response of the spleen ($1 : 38 \cdot (\surd) 1.180$; $P < 0.001$ compared with the control) and of the lymph glands ($1 : 146 \cdot (\surd) 1.199$; $P < 0.001$), without stimulating the response of long explants ($1 : 25 \cdot (\surd) 1.188$; $P < 0.5$). A similar effect was observed in the experiments when pyrogenal was injected into

*Between $1 : 58 \cdot 1.059$ and $1 : 58/1.059$.

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the trachea (for the lung 1 : 23 · (✓) 1.115, P > 0.5; for the spleen 1 : 40 · (✓) 1.144, P < 0.001; for the lymph gland 1 : 160 · (✓) 1.212, P < 0.001).

To study the possibility of a direct action of pyrogenal on the antibody-producing cells, experiments were carried out in which pyrogenal was added to the culture fluid of the explants of organs taken both from animals receiving pyrogenal in vivo and from control rabbits. The experiments showed that addition of pyrogenal in vitro had no stimulant action on antibody production in the spleen and lymph glands. Characteristic changes in hemagglutinin production were found in explants of the lungs when the dynamics of this process was studied (cultivation of parallel samples for 12, 18, 24, and 34 h). Some stimulation of antibody synthesis was observed in the initial periods of cultivation (18 and 24 h), and this was most marked in explants from animals stimulated with pyrogenal in vivo and receiving added pyrogenal in vitro also. Hence, when added to the culture medium, pyrogenal accelerates the synthesis of hemagglutinins in the lungs without modifying the process quantitatively.

These results are in agreement with those reported previously [4, 6, 7] concerning the activation of the inductive phase of immunogenesis by pyrogenal, through stimulation of the proliferation of plasma cells in the intact organism. The absence of activation of antibody synthesis in the lungs is perhaps evidence that this process is carried out in the lungs by cells other than those of the lymphatic series, and notably by the alveolar macrophages, the action of pyrogens on which is unique [2].

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