

# INDUCTION OF IMMUNOLOGICAL TOLERANCE IN ADULT RATS BY REPEATED INJECTION OF XENOGENETIC SPLEEN CELLS

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UDC 612.017.1-06:[612.6.02:612.41

The tolerance created by injecting foreign immunologically competent cells into newborn animals or embryos is frequently complicated by the development of homologous disease [1, 2, 7, 8]. The most promising and practically important problem is therefore the creation of immunological tolerance in adult animals. Investigations during recent years have shown that repeated injections of homologous donors' cells can cause a state of tolerance in adult mice [5]. By means of repeated injections it is possible to induce a state of tolerance even to heterologous antigens. The prolonged administration to rabbits of the stroma of erythrocytes of the sheep [9] and mouse [3], of brucellas [6], or of heterologous serum proteins [4] led to considerable inhibition of antibody production in the animals.

The object of the present investigation was to study the possibility of creating immunological tolerance in rats to antigens of a closely related species of animals—mice—by prolonged and repeated injections of mouse spleen cells into rats.

## EXPERIMENTAL METHOD

Thirty female rats of the Wistar line initially weighing 50 g were repeatedly immunized with a freshly prepared suspension of spleen cells from male mice of line C3H weighing 20-22 g.

Immunization was carried out by intraperitoneal injection of 0.6 ml of the cell suspension (39-41 million cells) three times a week at intervals of 1-2 days.

Starting with the 4th day, blood was taken periodically at weekly intervals from 10 experimental rats (numbers 1-10) and at intervals of 1-4 weeks from the other 20 animals. Blood was taken from the tail into capillary tubes 2 mm in diameter and 11-12 cm in length. The blood clot which formed was extracted with a stilet, and the serum was sealed in the capillary tubes with plasticine and kept in a refrigerator until it was used in the agglutination reaction.

The presence of agglutinins against the erythrocytes of mice of line C3H was determined in the rat serum (serial double dilutions starting with 1:2).

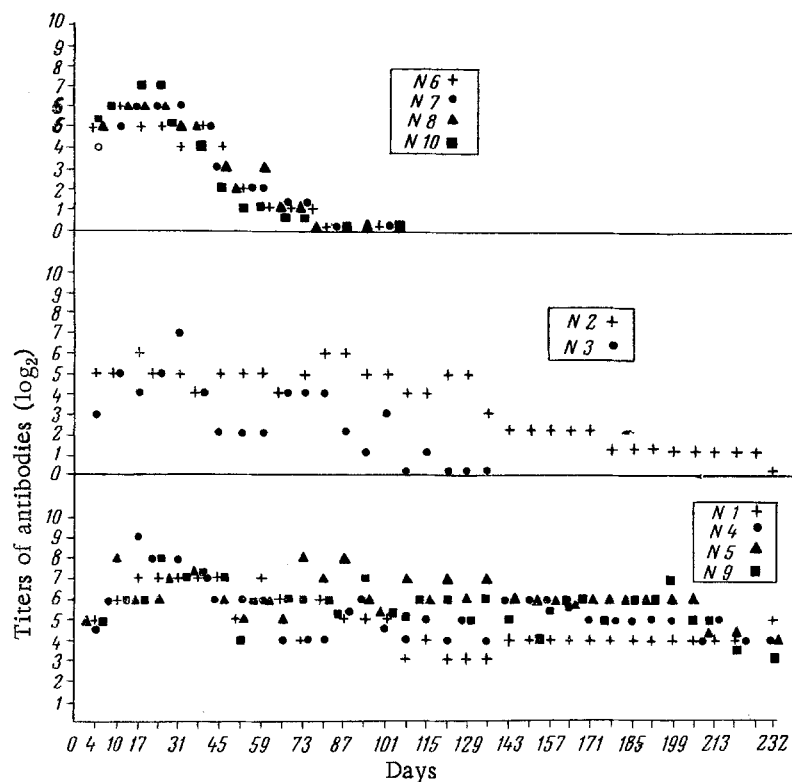
The serum of intact, unimmunized rats was tested for the presence of normal agglutinins against the erythrocytes of mice of this line (initial dilution 1:2).

## EXPERIMENTAL RESULTS

No normal antibodies agglutinating mouse erythrocytes were found in the serum of the intact rats.

The first injections of mouse spleen cells led to the appearance of agglutinins in the blood of the experimental animals. In all the rats on the 4th day antibodies were detected in the serum in dilutions of 1:8-1:32. Later the titer of the antibodies increased and reached a maximum on the 17th day; the titers remained at this level until the 38th day (17 injections of antigen, antibodies found in dilutions of 1:64-1:128). The character of the curves of antibody production subsequently varied. Three types of reaction could be distinguished (see figure).

In rats Nos. 2 and 3 the titers of antibodies fell more slowly after reaching their maximum. In the 1:2 dilution of serum from rat No. 3 no antibodies were found on the 122nd day of immunization (53 injections of antigen), and in rat No. 2 on the 232nd day of immunization (100 injections of antigen).



Dynamics of changes in titer of agglutinins to erythrocytes of mice of line C3H in rats of Wistar line after repeated immunization with spleen cells from mice of line C3H.

In rats Nos. 1, 4, 5, and 9 the titers of antibodies fell only very slight after reaching their maximum, and subsequently the curves resembled a plateau at the level of dilution 1:16-1:64.

In four of the 20 animals in group 2 no antibodies were found in a dilution of 1:2 by the 121st day of immunization, in 3 rabbits—by the 140th, and in 3 by the 232nd day; in the remaining 10 rabbits antibodies were found by the 232nd day in dilutions of 1:16-1:32.

Hence, in 16 of 30 rats of the Wistar line, as a result of prolonged and repeated intraperitoneal injections of spleen cells of mice of line CH3, the antibody formation developing earlier was replaced by a state of immunological tolerance. This was not accompanied by any significant disturbances in the condition of the rats. No deaths took place among the experimental animals, they gained in weight normally, and they showed no anaphylactic reactions.

The results obtained show that a state of immunological tolerance may be induced in adult animals by prolonged and repeated injections of antigen in doses which, if injected once or twice for immunization, give rise to a primary and secondary response in ordinary conditions.

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