EXPERIENCE IN THE USE OF THE PLAQUE METHOD FOR RECORDING INDIVIDUAL CELLS FORMING ANTIBODIES

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The urgency of studying immunogenesis at the "cellular level" is presently beyond question. However, the uniqueness and complexity of this problem makes its solution difficult with respect to the method. Therefore, it seemed to us expedient to master the method of Jerne and Nordin [2] which, in spite of its relative simplicity, can help to solve certain immunological problems.

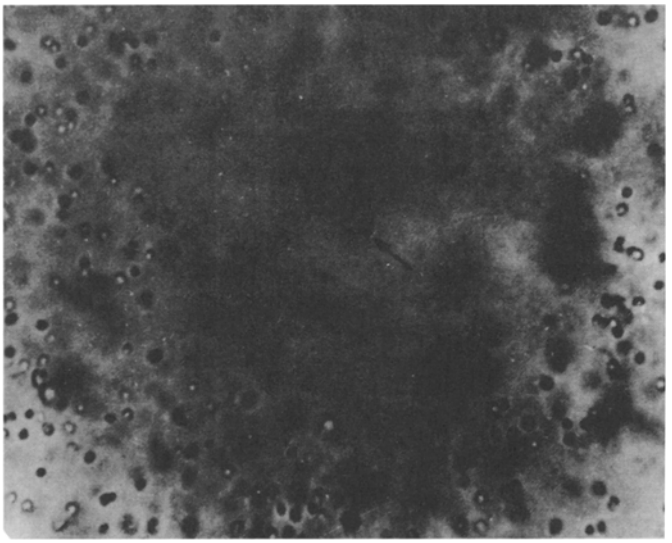
EXPERIMENTAL METHOD

We used a modified version of the method described by the indicated authors.

Rabbits were immunized three fold with sheep erythrocytes at 7-day intervals, injecting each time an average of $5 \cdot 10^9$ erythrocytes under the skin of the foot of one of the hind legs. Seven days after the third injection we removed the subpatellar lymph nodes of both extremities (control and experiment). As a control in some of the experiments we used the lymph nodes of rabbits immunized with tetanus antitoxin.

In order to extract the cells from the lymph nodes we first tried the method of enzymatic treatment of tissue pieces with a 3% solution of trypsin, which did not yield clear results, probably because of the injurious effect of trypsin on the cell wall.

Satisfactory results were noted upon treating ground tissue of the lymph nodes in Earl's solution, where isolated cells in a sufficient quantity were obtained. In place of the Difco medium we used our own agar treated with acetone and thrice distilled water [1].



"Plaque" under the microscope. In the center is a cell of the lymph node, the producer of hemolysins.

Into petri dishes with 1.4% agar we poured a mixture consisting of 2 ml of 0.7% agar, 200 million sheep erythrocytes, and 1 million cells of the lymph nodes, after which the vessels were placed in a thermostat at 37° for 1 h. Then onto the surface of the agar we poured the complement (serum of guinea pig in a dilution of 1 : 5) and the vessels were again incubated in the thermostat for 15 min.

As a result of these manipulations a greater or lesser quantity of small "plaques" glowing on a pinkbackground, similar to "pin pricks," and clearly seen with a loupe, formed on the dishes. Under the microscope the "plaques" were areas with complete or almost complete lysis of the erythrocytes. In the center of the "plaque" was seen a cell of the lymph node, which was evidently the producer of hemolysins (see figure).

Such "plaques" were not noted on the control dishes with the cells of the lymph node or node extracted on the side of the injection of the tetanus antitoxin.

The described method can be of promise in experiments with immunization of animals not only with erythrocytes, but also with other antigens. In this case, as the authors of the method noted, it is possible to use erythrocytes sensitized by the corresponding antigen.

LITERATURE CITED

1. S. G. Dzagurov, G. A. Safonof, G. A. Ivanova, et al., Vopr. virusol., 5, (1960), p. 632.
2. N. K. Jerne and A. A. Nordin, Science, 140, (1963), p. 405.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
