A METHOD OF INVESTIGATING THE FORMATION OF ANTIBODIES BY ISOLATED CELLS

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To study antibody formation it has been proposed to investigate the immunological reactions of isolated solitary cells [1, 4]. The principle is that solitary cells of the lymphatic system of the immunized animal are placed in a minute drop of nutritive fluid, and a study made of the rate of synthesis of antibodies by them. The chief advantages of the method are that the class of cell to which the isolated cells belong may be determined and the rate of formation of antibodies by a single cell may be measured.

Despite the great interest in these methods, until now they have not been repeated, probably because of technical difficulties. In the present communication we describe experiments made to study the formation of antibodies by solitary cells by means of this method. We hope that some of the results will be useful in other biological investigations which utilize micrurgical technique.

The Soviet industry produces the MM-1 micromanipulator. This instrument is very suitable for carrying out all the manipulations involved in the study of the production of antibodies by a single cell. The OI-10 condenser for use with a light or dark field and the MBI-3 microscope may be used in conjunction with the manipulator.

To prepare the glass instruments required for the various micromanipulations Fonbryun [2] has described a special apparatus—the "microforge". No such instrument is produced by the Russian industry. However, it can be assembled out of instruments and parts of laboratory apparatus in accordance with Fonbryun's principle. The general appearance of such an instrument constructed in our institute by the originators Ya. S. Shvartsman and B. M. Broitman is shown in Fig. 1. The "forge" consists of the following main parts: 1) the "furnace"; 2) the "vise", for holding the parts to be treated; 3) microscope with illuminating device, and 4) rheostat.

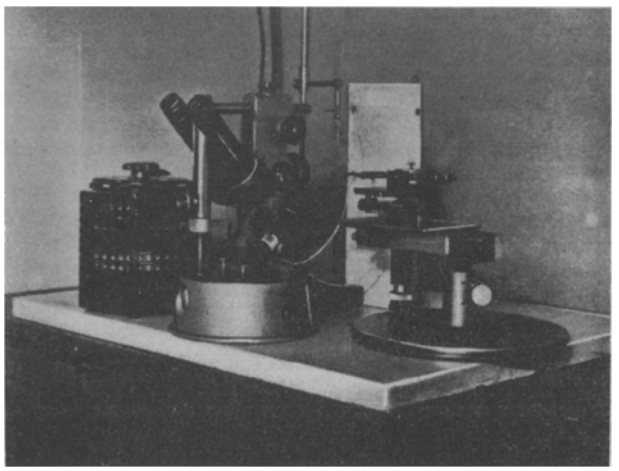

Fig. 1. Arrangement for manufacture of micro-instruments. From left to right: LATR-1 autotransformer, MBS-1 microscope, "vise", "furnace".

To prepare the "furance" we used a left control arm from a MM-1 micromanipulator fixed in a massive metallic disc. On the stand was fixed a heating element consisting of ebonite insulators, metallic holders, and threads. The threads were prepared from pieces of platinum or nichrome of diameter 0.15-0.3 mm. They were used not only as a source of heat but also as the "hammer" for preparation of microdrops, for bending fine pipettes, etc. The control arm enabled movement of the thread in any direction to be achieved.

The "vise" consisted of a bunsen stand to which was fixed a special device made from a metal tube. The sleeve with the set crew, the joint between the

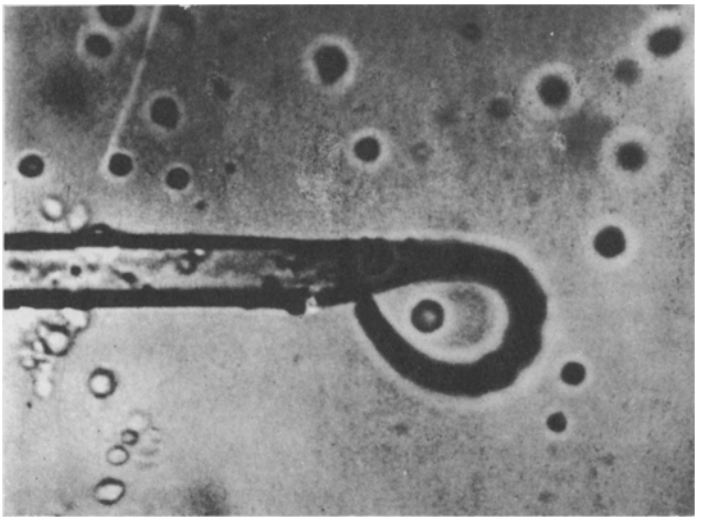

Fig. 2. Collecting a cell from the depot-drop. Magnification 150×.

different portions of the tube and the micro-instrument holder fixed to the end of the tube enabled the part to be moved in any direction. For observations to be made on the operations the MBS-1 microscope was used. A LATR-variable autotransformer was used for heating the thread.

In preparing the instruments we were guided by Fonbryun's recommendations. With some practice on the device all the principal micro-instruments may be prepared. For application of microdrops and for manipulation with cells the "broad" pipettes having an aperture of 50-60 μ are convenient, and for introduction of single bacteria into the drop the "narrow" pipette having a 10-20 μ diameter aperture is suitable. For isolation of cells we used a drop 30-40 μ in diameter and 5-10 μ deep. After each experiment the micro-pipettes were changed. To sterilize the micro-pipettes by dry heat each was fixed with its base in the cork of a test tube. At the end of the experiment the pipettes were immersed in a chromic acid mixture for 24 h, they were then carefully washed in hot tap water, and sterilized by dry heat.

The isolation of single cells and all manipulations with them were conveniently carried out in Fonbryun's grease chamber. To prepare this chamber a square measuring 60 × 60 mm was cut out from a photographic plate from which the emulsion had been washed off. Two glass plates 35 mm long and 5 mm wide were fixed to it by means of hot 3-5% gelatine solution. The height of these strips was 2.5 mm. They were cut out from window glass. The final portion of the chamber consists of a cover slip measuring 45 × 35 mm. The cover slips were carefully washed and boiled three times in distilled water. The chamber was sterilized by dry heat. Before the start of the work the space between the microscope slide and the cover slip was filled with medicinal vaseline.

The technique of the isolation of cells was as follows. From a lymph node we prepared a suspension of cells which we filtered through capron tissue. The cells were washed in a centrifuge at 500-600 revs/min; they were resuspended in nutritive medium, and their concentration was determined in a Goryaev chamber.

To work with the cells we used a nutritive medium of the following composition: normal serum of the species 30%, Kern and Eisen's saline 70% [3]. Of course in the serum there should be no antibodies to the antigens used for immunization. The cellular suspension was diluted to a concentration of $5 \cdot 10^4$ per ml, and was introduced into a "broad" pipette fixed to a hollow holder. The chamber was set up on the platform of a microscope. The manipulator was used to introduce a pipette into the chamber, and 2-3 depot-drops 3-5 mm in diameter were applied to the lower surface of the cover slip. A second pipette was used to introduce a number of microdrops of pure nutritive medium which consisted of large "bath" drops, and experimental and control drops. The volume of the experimental and control drops was approximately 10^{-6} ml.

Isolation of single cells was achieved by means of loops. A loop was introduced into a depot-drop, brought into position above the cell intended for isolation, and slowly lowered. When the loop had descended below the bottom of the drop, the cell could be seen in the small drop of medium surrounded by the loop. By operating the screws of the preparation holder and of the manipulator the loop was introduced into the bath drop. Then the cell

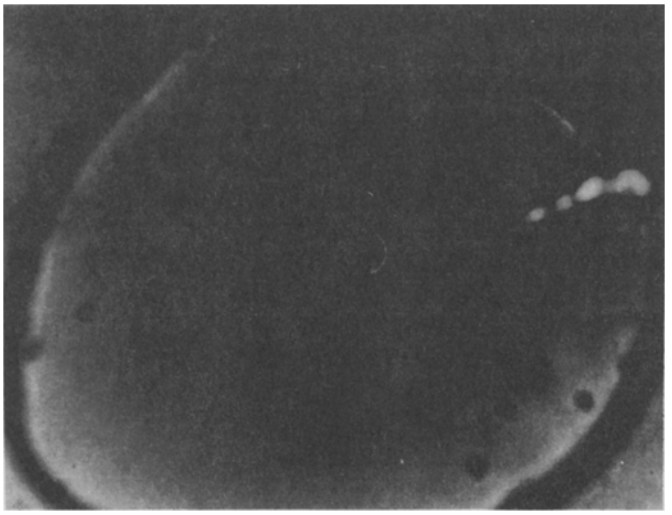

Fig. 3. Single cell isolated under the microscope. Magnification 300×.

left the loop. After the cell had been washed free from traces of antibodies it was transferred into the experimental drop and a drop of washing fluid from the bath drop was introduced into the control. With quite a small amount of practice 20-30 cells could be isolated in 1 h.

Figures 2 and 3 show the different stages in the isolation of a cell from a depot-drop into a microdrop.

After the intended number of cells had been isolated the chamber was incubated in a thermostat, and the next step was to determine the formation of antibodies by the single cells. These manipulations were made on a dark field at a magnification of 225×. To introduce single bacteria or tens of bacteria into the microdrops the "narrow" pipettes were used. A pipette was fixed in a holder; a stiff rubber band connected a syringe to the pipette, and the pipette was then filled with a broth culture of bacteria.

To determine the H-antibodies we used the reactions of immobilization, adhesion, and agglutination, or for determination of the O-antibodies we used adhesion and agglutination. The method of carrying out these reactions and making observations will be described subsequently. By means of this microtechnique we succeeded in studying 527 cells. Of these 289 cells formed antibodies.

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