PHASE-CONTRAST MICROSCOPY OF ANTIGEN – ANTIBODY AGGREGATES DURING HEATING OF THE TEST MEDIUM FROM A POINT SOURCE

V. V. Kamenskaya

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The possibility of recording antigen–antibody complexes as a precipitate or agglutinate was demonstrated by phase-contrast microscopy using a point source of heat (thermistor). Tests were carried out using albumin and bone tissue as antigens.

Many observations made during phase-contrast microscopy of biological fluids have revealed an interesting situation. Almost transparent objects such as large aggregates of complexes formed during the precipitation or agglutination reaction, if present in low concentration, occur relatively rarely in the field of vision on microscopic examination, and even by careful study it is difficult for the investigator to judge some of their properties.

Existing methods of investigation of dispersed systems by phase-contrast microscopy enable particles of a medium in a very thin layer of liquid to be observed, but the depth of focus of the region examined is small. The experimental conditions are substantially modified if a microthermistor, with the function of a heating electrode, is inserted into the test medium.

Focusing the microelectrode inserted into the test medium and application of a current of 20 mV create the conditions for concentration of suspended particles in the microregion of the heating electrode and, at the same time, in the field of vision of the microscope. The electrical circuit of the apparatus used for heating the liquid with a point source using a type MT-54 microthermistor designed by V. G. Karmanov (a semiconductor element hermetically sealed in a glass capillary tube) is illustrated in Fig. 1. The power of the current is such that a temperature gradient is created between the thermistor and the test medium so that the temperature of the fluid in the immediate neighborhood of the heating electrode is raised by 3-4°C. However, the local change of temperature produces no significant heating of a whole volume of the test medium.

When a current is passed through the microthermistor it is heated and becomes a source of heat. The temperature gradient produced determines the difference between the densities of neighboring layers of fluid. A region of lowered density and lowered pressure is created in the microregion of the thermistor. Light particles attracted by convection currents are displaced into the region of lowered pressure, as a result of which an increased concentration of particles of the dispersed phase is created in the microregion of the thermistor. The fact that the phenomenon observed in numerous experiments is thermal in nature is confirmed by an experiment with a heated steel wire, introduced into the cell instead of the microthermistor.

By regulating the degree of heating of the microthermistor by the current the concentration of particles in the observed region can be altered, their rate of movement can be controlled, and particles can be examined when oriented in different ways.

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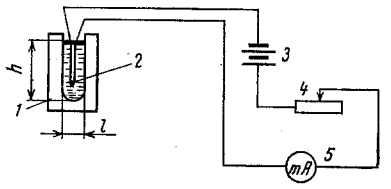

Fig. 1

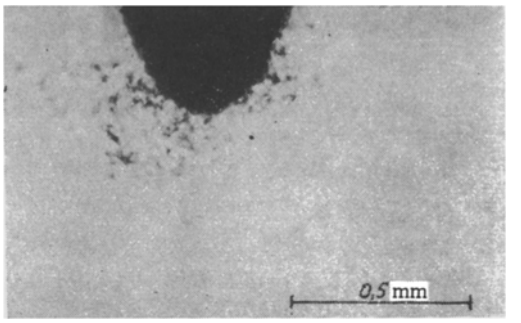

Fig. 2

Fig. 1. Diagram showing connections of the heating electrode: 1) cell containing test fluid; 2) microthermistor (heating electrode); 3) dc source; 4) control resistor; 5) milliammeter.

Fig. 2. Photomicrograph of thermistor surface with aggregation of precipitate complexes adsorbed on it (175×).

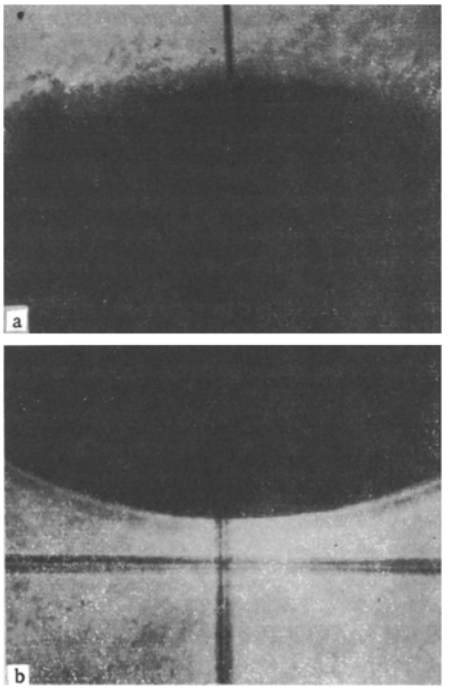

Fig. 3. Photomicrograph of aggregates of complexes arising during antigen–antibody reaction: a) heating electrode switched on; b) switched off.

A type MP-3 microscope, fitted with phase-contrast attachment, was used. The design of the microscope is such that objects can be examined with the stage of the microscope in a near-vertical position. Special cells with internal measurements of the chamber h = 18 mm, 1 = 4 mm, and d = 2.9 mm, made from transparent plastic, their frontal surfaces protected with coverslips, were made for the investigations.

The method of investigation of immunological reactions by phase-contrast microscopy with heating from a point source was tested on the reaction between serum albumin and rabbit antibodies against serum albumin, a reaction which has been well studied and described quantitatively.

The experiments were carried out as follows. Rabbit serum was diluted 1:5 and the antigen 1:20, 1:40, 1:100, 1:1000, and 1:10,000 with physiological saline (original concentration 1 mg/ml). The sera of two rabbits, one of which was immunized with serum albumin while the other was unimmunized (control), were used in the experiments. The diluted sera and antigen were poured into the cell in volumes of 0.1-0.2 ml, and the cell was fixed on the stage of the microscope with special spring clips in the near-vertical position. The microthermistor was lowered into the cell containing the test medium and fixed in its center. Next, as pointed out above, the microscope was focused for phase-contrast examination of the test fluid, during which time a constant current of up to 2 mW was applied to the microelectrode. Parallel with phase-contrast microscopy, the ring-precipitation test was carried out. The greatest titer of antibodies in this reaction was 1:1000.

Phase-contrast microscopy of the process of formation of the antigen–antibody complex using a point source of heat in the test medium enabled the presence of large complexes of precipitate to be recorded much sooner than by the serological test in a tube, and the highest titer was not less than the titer of the precipitation reaction while the recording of the observed effect was superior in its clarity. The time of appearance of the first signs of the reaction varied from 2 to 20 min depending on the dilution.

A photomicrograph of aggregates of the complexes formed by the antigen–antibody reaction is given in Fig. 3.

After the current had been switched on,individual formations with a granular or reticular structure appeared in the microregion of the heating electrode (Fig. 3a). Against the background of gradually increasing viscosity of the medium, an increase was observed in the number of particles of precipitate which, moving in the medium against the forces of gravity, on meeting an obstacle (the microthermistor) were partly held up and deposited on the glass surface of the electrode. The large aggregates in some cases had a clearly defined discrete structure. Some large aggregates of complexes measured 0.05 mm in diameter, whereas the particles composing them were an order of magnitude smaller. Control examination using albumin and the serum of an unimmunized rabbit, diluted in the same way, yielded no aggregates, thus confirming the specificity of the picture observed before. During phase-contrast microscopy of the test media without the use of a point source of heat the experimental conditions were much worse. Although in this case individual aggregates of antigen–antibody complexes (experimental series) were recorded, this concentration in the field of vision was so low that the investigation was ineffective (Fig. 3b).

Antigens of bone tissue were also investigated by phase-contrast microscopy.

It is generally known that bone is a difficult object for immunological investigations, which is why relatively few studies have been made in this field. Attempts by investigators to seek new and more sensitive methods of detecting the antigen–antibody reaction in bone grafting are fully understandable.

The blood serum of rabbits was studied after immunization with an emulsion from a bone homograft. Altogether 16 sera taken at different times after immunization were investigated. The sera were first titrated in the ring-precipitation test. The formation of a clearly visible precipitate was observed during the reaction between antigen diluted 1:10 (10% saline extract) and serum diluted 1:2. The formation of a precipitation ring was visible after 55-60 min depending on the properties of the test serum.

On phase-contrast microscopy with the use of a point source of heat the first signs of the reaction were observed 15 min after mixing the reagents. The external appearance of the complexes formed resembled in many ways the precipitate of albumin with antibodies against it, but the impression was given that these structures were lighter and more azure in color, almost transparent, and hardly detectable in phase contrast. Aggregates of the complexes settled on the floor and walls of the cell, gradually reducing the translucency of the test medium.

Investigations with bacterial antigens showed that under certain conditions (temperature, character of the frontal surface of the cell, its degree of electrification), active adhesion of the antigen particles to the frontal walls of the cell with the test fluid can be observed. A characteristic feature of the phenomenon is the formation of curious patterns resembling fibrous crystals. A detailed investigation of the agglutinated masses under magnification of 1000× of the microscope also revealed a regular distribution of the separate particles in the aggregate of the complexes.

The ability of antigens to be adsorbed on Pyrex glass has recently been described [2]. A small quantity of egg albumin can be made to adhere to glass of this type and it still retains its ability of react with antibodies [3].

Considering that the individual components of complexes formed by precipitation and agglutination are clearly visible in phase contrast and have certain specific features, microfilming could be profitably used as a research method.

LITERATURE CITED

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