IMMUNODIFFUSION ANALYSIS OF PROTEIN MIXTURES IN OBJECTS 150 μ IN DIAMETER

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Methods of analysis based on the principle of immunologic counterdiffusion are now being used in many branches of biology. For surveys of these methods, see [1, 9].

Interesting attempts are being made to apply the immunodiffusion method to the investigation of microscopic objects, especially to the analysis of unique objects, i.e., those which cannot be accumulated (for example, the oocyte, or a group of cells in tissue culture). In every case when a particular biochemical method has to be adapted to the study of these unique objects, new methods of work must be used. An example of this is the Linderstrom-Lang method using a Cartesian diver instead of a Warburg's apparatus to measure the respiration of the oocyte [2, 7, 8, 10].

Application of the method of nucleotide electrophoresis to the study of microscopically small quantities has also called for new techniques [3-6].

In the present investigation, in order to adapt the immunodiffusion technique to the study of microscopic objects a method based on Ouchterlony's principles [9], but with new operations, was proposed. First, difficulties of introducing the material into agar had to be overcome. Second, even when a micromanipulator is used, it is impossible to make a gutter of microscopic dimensions for antiserum in the gel at a required distance from the object; further, this distance must not exceed the diameter of the specimen by more than 3 times. This gutter must be shallow, otherwise the precipitates will be dome-shaped instead of cylindrical, and consequently, they will not be clearly visible on transillumination.

Introduction of the Test Object into Agar (Fig. 1). The model of the object was a block measuring 150 × 150 μ cut from a film prepared from a mixture of 2% agar with human serum. The object was first embedded in a microdrop of agar, which was cooled until it solidified and was then stuck with liquid agar to a tapered strip of gel made from agar with the addition of ink. This last operation is necessary in order that the location of the object may be determined later, when it cannot be seen clearly. A small drop of agar was then poured on top, and above it was placed a disk of 1% agar gel 0.1 mm in thickness, the disk was melted in the drop, and excess of gel was sucked from it. After solidifying the agar formed a continuous plate. The preparation was then stripped from the slide and overturned. All these operations were carried out under mineral oil. When electrophoresis was also performed this was done before turning the preparation over.

Application of Antiserum. All the requirements mentioned earlier were met by what is, in principle, a new technique: instead of a gutter with liquid antiserum a wedge-shaped block prepared from a mixture of 3% agar with antiserum, in the ratio 1:2, was used. The wedge with antiserum (Fig. 2d) was placed on the surface of the main agar plate (c) on one side of the specimen, and under continuous observation under the microscope it was moved toward the specimen by means of a micromanipulator to the required distance. This distance was checked with great accuracy with an ocular micrometer. The optimal distance was 200 μ. This was followed by exposure for 15 h in a humid chamber, during which time counterdiffusion took place and the precipitates formed. The material was then washed and stained with amido black. Several precipitates were found under the microscope, shaped like characteristic arcs (Fig. 3).

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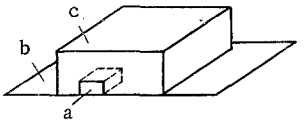

Fig. 1. Introduction of the specimen into agar. a) Specimen to be examine; b) surface of slide; c) agar plate.

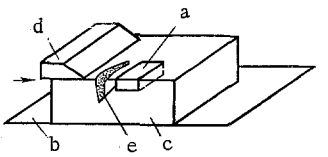

Fig. 2. Mutual arrangement of details at the time of immunodiffusion development. d) Wedge with antiserum; e) precipitate. Remaining legend as in Fig. 1. The arrow indicates the direction of movement of the wedge.

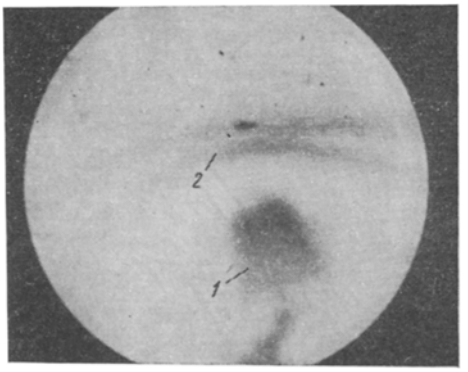

Fig. 3. Photomicrograph of immunoprecipitates. 1) Specimen tested; 2) precipitate in the shape of an arc. Development with rabbit antiserum against α- and β-globulin fraction of human serum. 60 ×.

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