A MODIFICATION OF THE METHOD OF IMMUNOELECTROPHORESIS OF THE SERUM PROTEINS

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Grabar [1, 2] and Williams [4] described the so-called immunoelectrophoretic method of investigation of the serum proteins, which is a combination of electrophoretic fractionation in a solid medium and the Ouchterlony immunological precipitation method [3], in which two antigens diffuse from two points in agar, meet antibodies, and are precipitated in the form of lines. During investigation of human serum by the use of this method, Grabar found 19 precipitation bands in the form of arcs, corresponding to 19 antigens in the serum (and this is, therefore, the number of individual proteins).

We have slightly modified the technique of this method in order to secure greater standardization of the experimental conditions.

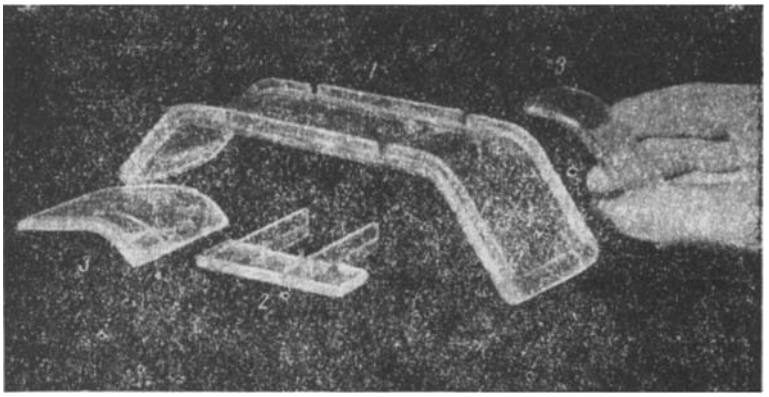

Fig. 1. Bridge-shaped dish for electrophoresis (1) with covers (3) and template (2) for the starting wells and the gutters. (The template is shown inverted).

Analysis of the immunoelectrophoregrams obtained by Grabar's original method shows that, even with slight changes in the conditions of electrophoresis, the picture is quite considerably changed. The arcs are formed in other places — nearer to the gutter with the antiserum or further away from it; their curvature is also altered.

On the basis of our experience we can say that the main conditions to be satisfied especially strictly are: 1) constancy of the distance from the starting well to the gutter filled with antiserum (an error of a fraction of a millimeter in a total distance of 4-6 mm is sufficient to alter the position and curvature of the arcs); 2) the gutter must be parallel to the long axis of the agar plate; 3) the volume of serum undergoing electrophoresis must be constant; 4) the shape of the starting well must be correct. Changes in the thickness of the agar layer also affect the electrical parameters of the experiment.

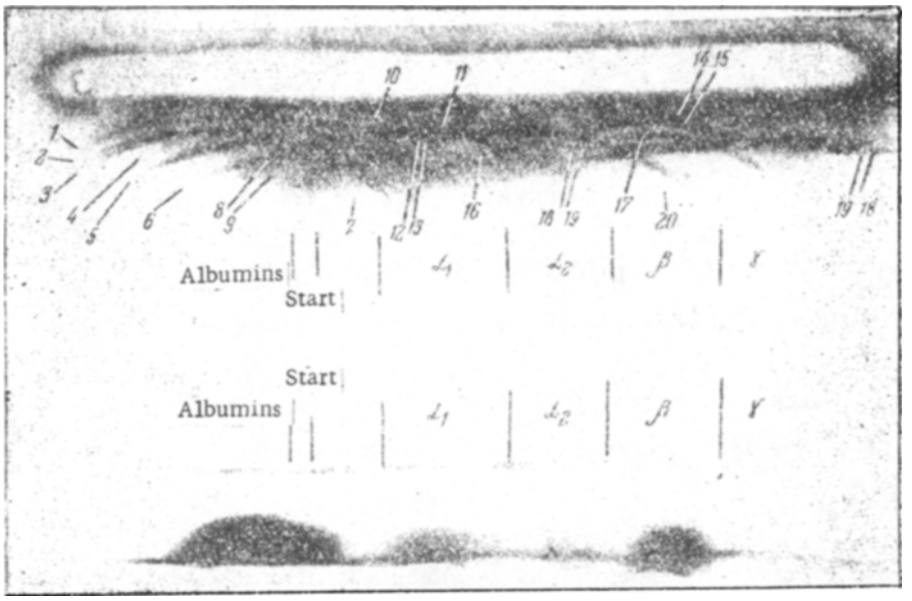

Fig. 2. An immunoelectrophoregram. 1-9) arcs in the albumin region; 10, 11) arcs in the α-globulin region; 12, 13, 16) arcs common to α_1- and α_2-globulins; 14, 15, 17) arcs common to α_2- and β-globulins; 18, 19) arcs of γ-globulins, going beyond the region of α_2- and β-globulins; 20) weak and inconstant line (lower part of the figure is the control half of the electrophoregram, stained without immunological development).

In order to secure standardization of the experimental conditions, we introduced a new detail into practice —the bridge-shaped dish. The glass slide with the agar film was not placed on the edge of the electrode dishes, as was described in the papers by Grabar, but on the bottom of a special dish (Fig. 1) which was shaped like a bridgewith side walls. The width of of the inner arc of the bridge between the walls was twice the width of a glass slide. The side walls were 4 mm high. The total length of the bridge-shaped dish, inclusing the curved legs which were made to stand in electrode dishes, was 24 cm. Two parallel glass slides were placed in the dish, and agar was then poured in. To prevent the agar from flowing down the legs at each side, covers were applied to the latter (see Fig. 1), fitting snugly against the side walls of the legs. The covers were additionally pressed against the side walls of the legs of the bridge by rubber ballons placed over them.

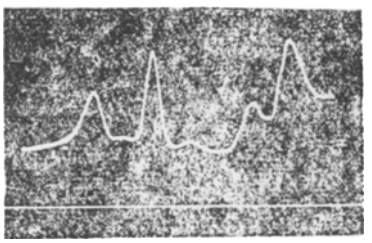

Fig. 3. Cross densitogram of the first 5 arcs in the albumin region, recorded on the MF-4 recording microphotometer.

A special template was placed over the glass side during its immersion in agar, to give the shape of the 2 future gutters and 2 starting wells (see Fig. 1). The starting wells, into which a mixture of serum and agar was later to be poured, could be cylindrical in shape (diameter 2 mm) or square in cross section. The patterns for the gutters for the antiserum were situated on both sides of the starting wells, each at a distance of 6 mm from the pattern for the corresponding well. The patterns of the gutters were strictly parallel to each other; the width of the gutter pattern was 3 mm. All four details were fixed to a connecting piece. The template was so designed that the patterns of the gutters were strictly parallel to the midline of the bridge-shaped dish; this was acrieved by the coincidence of special marking on the body of the dish and on the template.

After the agar had solidified, the template was removed. Correctly shaped starting wells for the serum and gutters for the antiserum remained in the agar. Next into wells was poured a mixture of serum with molten agar at 37° in proportions of 1:3. Excess was poured, in, so that the surface was raised obove the surrounding level. After the agar had solidified, all the excess lying above the general level was cut away with a razor blade

applied along side walls of the bridge. An agar plate of standard thickness, with a perfectly smooth and flat surface, was obtained. The height of the column of mixture of serum and agar was also predetermined. The covers were then removed from the legs of the bridge, and the legs were then placed, together with the overflow of agar attached to them, in electrode dishes for carrying out the electrophoresis.

At the completion of the electrophoresis ($2\frac{1}{2}$ -3 hours at 120 v) the layer of agar on the glass slide was cut away from the general mass around the edge of the slide, and the slide with its attached agar was removed from the bridge. Antiserum was poured into the gutters and the slides were placed in a humid chamber for 2 days. The advantage of this short time of development, which was possible only with agar plates of small size, was that it obviated the necessity of adding antiseptics, for colonies of microorganisms would not grow in 2 days at room temperature. In our experiments to investigate rat serum, 19 arcs of immunological precipitate appeared during the time of development (Fig. 2).

Next, in order to ensure complete removal of the soluble proteins, the slide was washed with physiological saline for not less than 6-7 days. In contrast to Grabar [2], we stained the agar plate with acid blue-black without preliminary drying. The preparations were also photographed undried, for drying deforms the agar slightly and its outlines become less sharp. Ill-defined, pale arcs shown up well by cross densitography on the MF-4 recording microphotometer (Fig. 3); the quantitative distribution of the immunological precipitate in the arc along an axis perpendicular to the axis of electrophoresis can also be ascertained from these densitograms.

We shall now consider a few methods of analysis of the arcs. It is sometimes important to detect very fine differences in the shape and situation of the arcs, since changes in the shape of the arcs indicate changes in the coefficient of diffusion of the corresponding serum protein, or a change in its immune properties which alters the proportions of antigen and antibody necessary for the formation of a precipitate, or, finally, changes in the amount of antigen protein. The most accurate comparison of the shape of the arcs can be obtained by the application method consisting of the juxtaposition of diapositives taken of the two preparations to be compared. This procedure, however, slightly complicates the picture, and it may be difficult to distinguish which arcs belong to which preparation. Comparison is simplified by staining one of the diapositives by the usual methods of toning as used in photography.

In some cases it is desirable to remove the image of certain arcs from the diapositive, which hamper examination. This is easily done by the use of a fine water-color brush, moistened with ammonium thiocyanate and ferrocyanide. In addition, copies made by hand, in ink, may be compared. For this purpose unexposed and developed (transparent) photographic film is applied to a phtograph of the immunoelectrophoregram, and the individual arcs are traced on it with a pen. Each arc is copied on a separate film; this facilitates the subsequent comparison. The second method is far more accurate although, at first glance, it may seem to lack the objectivity of the first method.

In conslusion it may be mentioned that all the improvements described are very simple to carry out.

SUMMARY

A modification of Grabar's immunoelectrophoresis method is described. Electrophoresis is effected on slides placed in a special bridged cuvette which serves both to hold the agar poured over the slides and to carry out electrophoresis.

The amount of agar in the cuvette is somewhat in excess of the required. The overflowing agar is cut off with a razor sliding along specially arranged edges of the cuvette, thus, always ensuring a uniform thickness of the agar layer.

The first compartments for the introduction of the serum and the troughs for the antiserum are also strictly standard in size.

This is achieved with the aid of special patterns (templates) placed on the slides while the agar is poured. Their position is fixed by markers on the cuvette.

Immunoelectrophoregrams obtained by this method are characterized by their constancy.

LITERATURE CITED

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