A METHOD OF AGAR MICROELECTROPHORESIS FOR STUDYING PROTEINS IN THE AQUEOUS HUMOR OF THE ANTERIOR CHAMBER OF THE EYE AND PERILYMPH OF THE INTERNAL EAR

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The method described in this work is one of the varieties of agar electrophoresis [4, 5]. The advantage of this method is that the electrophoretic process itself approaches the moving-boundary electrophoresis of Tiselius to a greater degree than in other methods of investigation, owing to the homogeneity of the gel and the presence of an aqueous medium containing almost 99% of the carrier. Agar in 1-1.5% solution forms true, very homogeneous gels where adsorption of proteins is reduced to a minimum, the proteins being in a native state and not being subjected to any physical or chemical effects capable of causing their denaturization [1, 4, 5]. Moreover, the transparency of the agar film makes it possible to use direct photometry for a quantitative evaluation of results [8] and to document the films by means of a contact print [1], which also is an advantage of the agar method in comparison with other types of electrophoresis.

The task of the study was to analyze the fractional composition of the proteins of the aqueous and of the perilymph. For this purpose a method of agar microelectrophoresis on slides was developed. The basis for the proposed method was the determination of microquantities of protein in solutions by means of agar electrophoresis which was described in the literature [9].

METHOD

The technique of the method we used is relatively simple and can be quickly adjusted when using apparatuses for paper electrophoresis with certain special adaptations.

To prepare the agar gel we used high-quality agar obtained in the bacteriological laboratories. After washing the agar in distilled water, for several days it was covered with a veronal-acetate buffer with pH 8.6 and ionic strength of 0.05 so that we obtained a concentration from 1 to 1.5%. The flask with the agar was held on a water bath until it was completely melted, and then the hot solution was filtered through a cotton gauze filter into small flasks with thymol crystals. The solidified agar was stored in a refrigerator and used as needed.

Before starting the experiment the agar was melted in the flask on a water bath and the hot solution was poured onto slides (2.5 · 7.5 cm), which were used in the microscopic examination. Before the slides were covered they were carefully degreased with a mixture of alcohol and ether.

At first we used the apparatus of our own design (Fig. 1) with a system of flowing buffer. A regulated rectifier producing a direct current up to 250 mA and a voltage of 250 V served as the power source. Later we used the Soviet instrument ÉFA-1 for paper electrophoresis with dual buffer vessels, since the pH of the buffer did not substantially change if electrophoresis lasted 1-2 h.

The ÉFA-1 apparatus consists of an alternating-current rectifier, 2 baths with dual buffer vessels, and a densitograph. Into each bath of this instrument we placed 3 specially prepared plexiglas molds (Fig. 2) on the bottom of

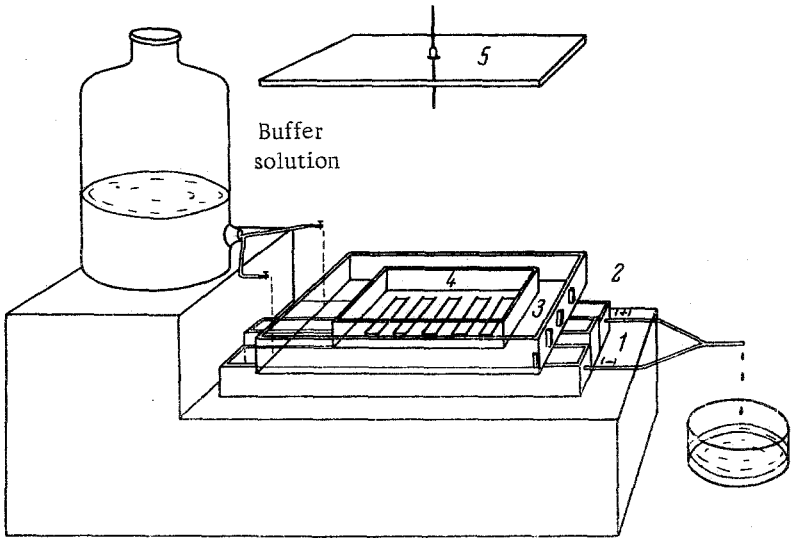

Fig. 1. Apparatus for microelectrophoresis. 1) Two cuvettes of plexiglas with drainage tubes for the buffer (the electrodes which are situated in these cuvettes are not shown in the figure); 2) vessel with ice; 3) compartment for ice in chamber; 4) chamber for placing the molds with the agar-coated slides. The paper bridges connecting the mold with the buffer solution and passing through special holes in the bottom of the chamber are not shown; 5) cover and thermometer.

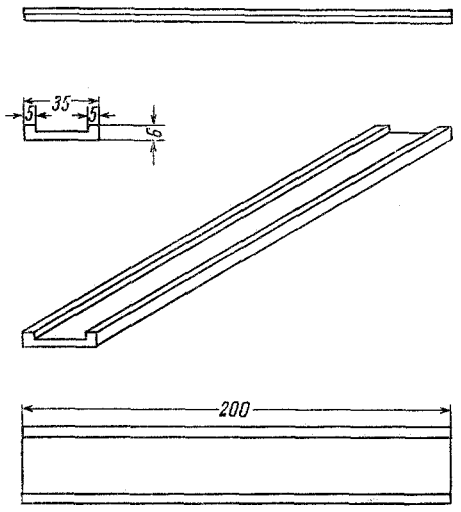

Fig. 2. Plexiglas mold for 2 slides (75 × 25 mm) when carrying out electrophoresis in the ÉFA-1 apparatus.

which were 2 clean slides. The height, i.e., distance from the surface of the slide to the edges of the mold rims, was 2 mm; thus, the layer of agar did not exceed this value. By means of filter-paper bridges the molds were connected with the dual vessels filled with the same veronal-acetate buffer, but with an ionic strength of 0.033. Pieces of ice were laid on the bottom of the baths to cool the agar during electrophoresis.

The investigated material was inserted into the agar by 2 methods.

1. A razor blade, 8-10 mm wide attached to a wooden handle, on which a drop of 5-10 μl was applied, was submerged into the layer of agar so that it touched the surface of the slide. Upon removing the blade the drop of the investigated liquid remained in the slitlike channel that was formed.

2. A hole was made in the gel by means of a special punch (1 × 10 mm) and a small "bricklet" of agar was removed, and the small pit thus formed was filled with the investigated solution, using a fine pasteur pipette.

The process of electrophoresis usually lasted $1\frac{1}{2}$-2 h at a potential gradient of 10-12 V/cm and current strength of 10 mA on each slide. At the end of electrophoresis the slides were placed in a 5% solution of acetic acid for fixing the proteins. The experiment demonstrated that fixation is required for at least 12 h. This prevents crystallization of the agar during drying. Drying was done at 36° under filter paper so that cracks did not appear in the agar film and the agar uniformly adhered to the slide.

The film was stained after drying and removal of the filter paper. The proteins were rather well stained with 0.5% solution of Amido Schwarz 10 B in a 5% solution of acetic acid, which required 15-30 min depending on the

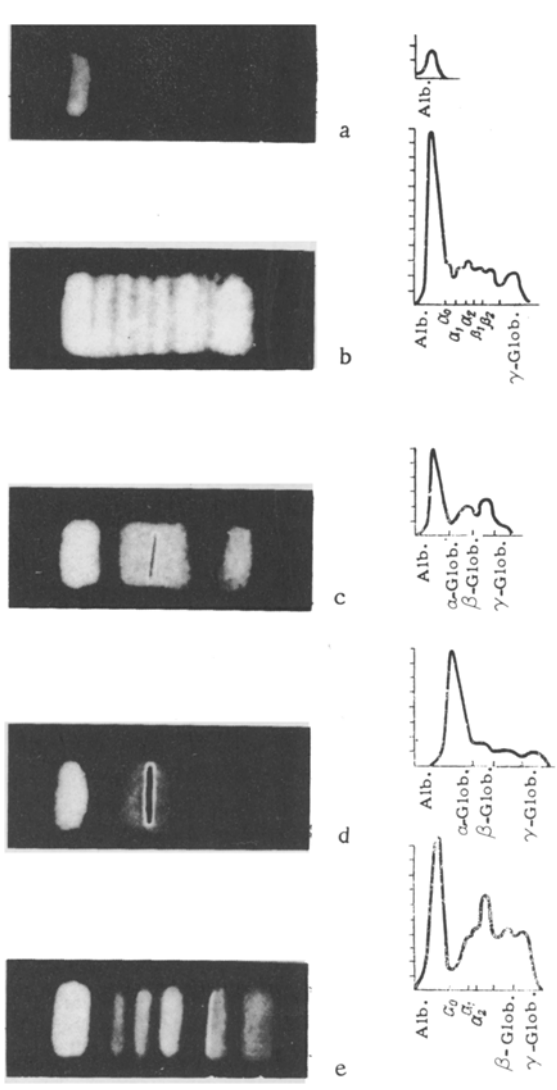

Fig. 3. Electrophoretic patterns and proteinograms of the proteins of normal and pathological aqueous and perilymph. a) Normal cat aqueous; b) secondary cat aqueous; c) normal cat perilymph; d) human perilymph obtained 30 min after death; e) cat perilymph in chemically induced labyrinthitis.

protein concentration. The background was washed with a 5% solution of acetic acid until it was completely decolorized. The again dried films were subjected to a quantitative analysis on any densitometer suitable for this purpose. We used the ÉFA-1 and MGF (East Germany) densitographs. The numerical values relative to the content of the fractions were obtained by weighing individual peaks cut from the densitograms on an analytical balance.

The main virtue of the method being proposed here is the simplicity of the technique and the possibility of investigating very small volumes of liquid with a relatively low content of protein.

RESULTS

Figure 3 shows the electrophoregrams and the curves of normal and pathological aqueous and perilymph corresponding to them.

In electrophoresis of the cat secondary aqueous humor, 5-10 μl of this fluid was sufficient to obtain a distinct electrophoretic pattern, which contained up to 7 fractions: albumin, α_0-, α_1-, α_2-, β_1-, β_2-, and γ -globulins. However, electrophoresis of 10-20 μl of normal cat aqueous yielded only 1 albumin peak, which is explained by its very low content of protein, 20 mg % [7]. Therefore, in this case preliminary concentration of the fluid by some method is necessary.

As regards the investigation of normal cat perilymph where, according to the data of certain authors [2, 6], the protein concentration is 142-268 mg%, electrophoresis of 10-20 μl of this fluid elicited albumin, α- and β - fractions, and sometimes it was possible to record γ -globulin. The same can be said for human perilymph obtained 30 min after death.

Analysis of the perilymph in induced serous labyrinthitis in cats elicited an entire spectrum of proteins; 5-10 μl of perilymph is completely sufficient for electrophoresis.

An examination of Soviet and foreign literature did not reveal information concerning the protein composition of perilymph in induced labyrinthitis. Such data, just as the discovery of 7 protein fractions in the secondary aqueous humor of the cat, were obtained for the first time, which was possible to do with the use of the method of electrophoresis described.

SUMMARY

A modified technique of agar electrophoresis is suggested; this method is applicable to the study of microquantities of solutions with low protein content. The technique allows the analysis to be made of the fraction protein composition of such biological fluids as aqueous humor of the eye and internal ear perilymph. The findings relating to the fluids under normal and pathological conditions are presented.

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