Relationship Between Electroendosmosis and Plaque Production in Different Agars[1] (36357)

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Agar is used extensively in virology and bacteriology and it has been shown (1) to consist of two fractions, agarose and agaropectin. Several authors (2–4) have found that substances included in the agaropectin fraction have an inhibitory effect on the formation of plaques by some viruses. Many different methods for treating agar to remove these virus inhibitors have been used but the results vary, often from batch to batch. Comparing the effect of agars on plaque formation is time consuming but the present paper shows that the degree of electroendosmosis, measured by electrophoresis of serum, serves as a useful and rapid test for the suitability of an agar for virological work.

The agars used were selected from commercially available products, and some samples were treated in various ways to improve the quality of the agar. "Cold washed" agar was washed in cold running tap water for 48 hr. "Hot washed" was washed in two changes of water at 80°. The "PEG precipitated" material was agarose precipitated from a 4% agar solution by the addition of an equal volume of a 40% polyethylene glycol solution at 80° (5); the agarose was precipitated three times before being tested. The material that was not precipitated by this method was collected from the supernatant fluid and called "agaropectin."

The agars were compared physically by measuring the degree of electroendosmosis produced when rabbit serum was subjected to electrophoresis through the agar gel, using the method of Russell, Mead and Polson (5). Solutions of the agars (1% w/v) were prepared in veronal buffer at pH 8.2 (6) and 2 ml poured onto a 3-in. microscope slide and allowed to gel. A drop of rabbit serum was added to a small hole made in the gel and a potential of 100 V applied across the agar strip. After passing current for 25 min the proteins were stained with naphthalene black (7), and the agar washed in a solution of 5 parts methanol, 5 parts water, 1 part acetic acid (v/v/v) and allowed to dry. The quality of the agar determines the degree of electroendosmosis. The poorer gels with more particles that are negatively charged show more movement of the gamma globulin towards the negative (cathode) end of the slide (Fig. 1). In order to quantitate this movement the distance from the origin to the end of the globulin (x) was measured by a comparator and expressed as a percentage of the movement from the front of the albumin to the back of the gamma globulin (y).

The influence of the different agars on virus plaque formation was estimated by inoculating chick embryo fibroblast cells with the same dilutions of a cytoplasmic bovine orphan (CBO) virus (Van den Ende strain). The cells were suspended in the agar solution made up according to the method of Cooper (8), with 5% fowl serum added. After incubation at 37° for 3 days the plaques were counted. The number of plaque forming units per ml of the inoculating medium was calculated and it was thus possible to gauge the "plaquing efficiency" of the agars, that is the number of plaques formed in the presence of the agar when a standardized virus suspension was added.

The results of the experiments are shown in Fig. 2, and a line of best fit has been

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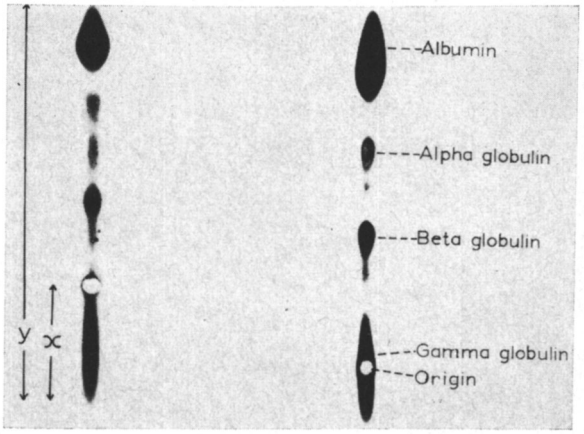

FIG. 1. Results from electrophoresis of rabbit serum in agar gels. Both samples are formed from Ionagar No. 2 (Oxo Ltd., London). Left—untreated; Right—improved by washing in hot water.

drawn. There appears to be a correlation between the amount of electroendosmosis shown by each agar and the ability of the virus to form plaques in this particular agar. The inhibitory substance is negatively charged since it caused an endosmotic flow of water towards the cathode. This agrees with the conclusion reached by others (3, 4, 9) that it is sulfated polysaccharide.

The line of best fit levels off in this particular virus/cell/medium combination above the 50% electroendosmosis point. Even though the agars further to the left of this point contain progressively fewer charged molecules their "plaquing efficiency" does not improve at the same rate as before. It is probable that this is because the fowl serum present in the medium neutralizes some of the sulfated polysaccharides, for Vigier (9) has demonstrated that calf serum reduces the effect of agar extract on the formation of foci of Rous cells in chick embryo cultures. For this reason media which contain no added serum will be most sensitive to the presence of sulfated polysaccharides in the agar.

For this particular system, if the agar falls to the left of the 50% electroendosmosis point further steps to purify it are unnecessary as they would have no significant effect on the "plaquing efficiency" of the agar. However if the agar shows more than 50% electroendosmosis the "plaquing efficiency" for this virus is low and steps should be taken to improve the quality of the agar before results of plaque counts can be considered to be comparable with other agars.

Summary. A comparison of the electroendosmosis exhibited by an agar and its ability to allow a CBO virus to form plaques in chick embryo fibroblasts shows that these parameters are correlated, indicating that the inhibitor is a negatively charged material, agreeing with the conclusion that it is sulfated polysaccharide. The degree of electroendosmosis may be used as a measure of the suitability of an agar for use in plaque production. Some of the inhibitor appears to be neutralized by fowl serum in the particular virus/cell/agar system used.

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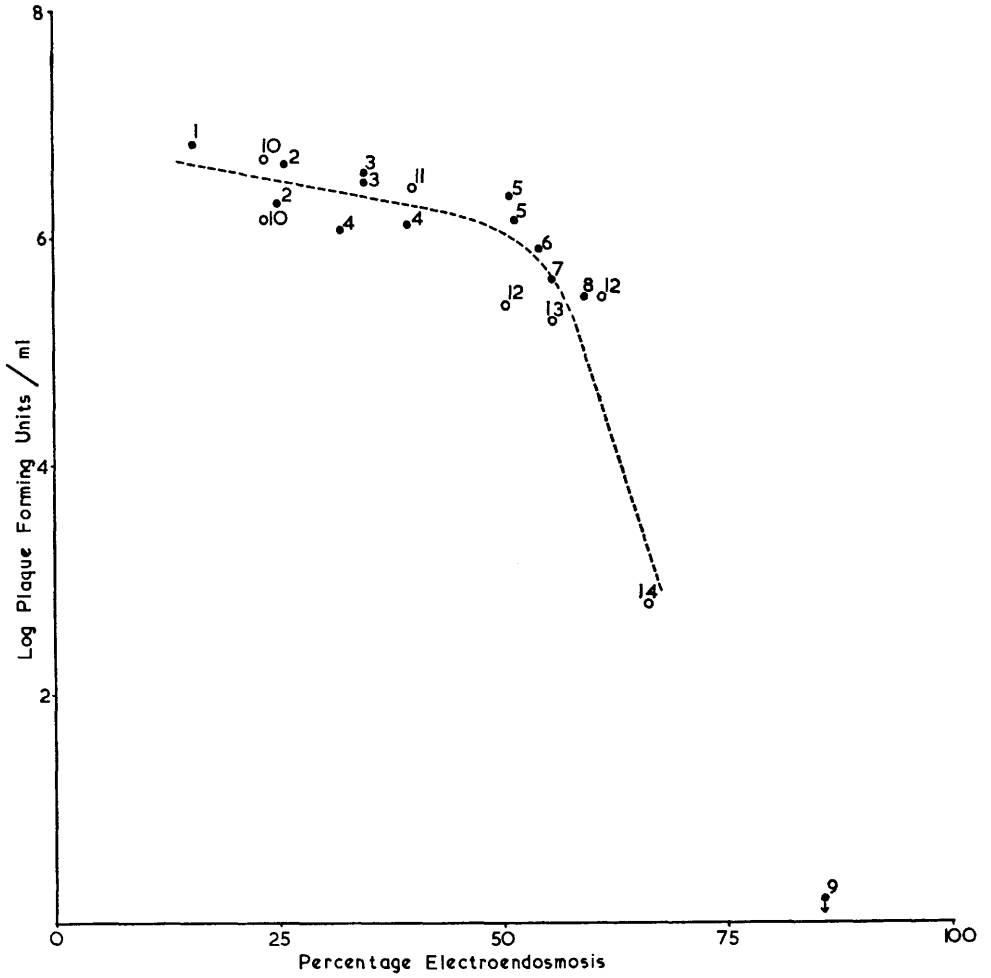

FIG. 2. Comparison of percentage electroendosmosis of each agar and the number of plaque forming units shown by it when a standard virus suspension is added to the cell/agar medium. In some cases an agar was tested more than once.

● 1—Ionagar No. 2 (Oxo Ltd., London) precipitated 3 times with PEG. 2—Ionagar No. 2 (Batch 51) untreated. 3—Ionagar No. 2 (Batch 69) untreated. 4—Ocean Gold (Vitamin Oils Ltd., Cape Town). 5—Special Noble Agar (Difco Labs., Detroit). 6—Case Agar (Case Labs., Chicago). 7—Bacto-Agar (Difco Labs., Detroit). 8—Japanese Agar (British Drug Houses, Poole, England). 9—Bacteriological Agar (Fischer Scientific, New Jersey). The chick embryo fibroblasts all died. Oxoid Agar No. 3 (Oxo Ltd., London).

○ 10—PEG precipitated. 11—Hot washed. 12—Cold washed. 13—Untreated. 14—Agaropectin derived from the supernatant after PEG precipitation.