MICRO STARCH GEL ELECTROPHORESIS

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Because of its high resolving power, starch gel electrophoresis according to SMITHIES[1,2] is a widely used method for the separation of proteins. The fact, however, that it takes several hours to run and requires several microliters of sample may be a disadvantage. In the course of a study of newborn-mouse serum, we therefore tried to combine the resolving power of the starch gel method with the speed and the small sample volume of micro agar electrophoresis[3]. The resulting micro starch gel electrophoresis has some characteristics of its own as compared with the original methods.

EXPERIMENTAL

Materials

All reagents were analytical grade and solutions were made in distilled water.

Hydrolysed starch was purchased from the Connaught Medical Laboratories, Toronto, Canada.

Serum was obtained from healthy mice, strain C_{57} Black.

Methods

1. Preparation of the gel

To 50 ml 0.024 *M* boric acid and 0.0096 *M* NaOH, pH 8.4 at 20°, 1.25 times the amount of starch as prescribed for horizontal starch gel electrophoresis is added, and the mixture is heated in a conical flask just short of boiling with constant swirling over a naked flame. Removal of air bubbles under reduced pressure must be very rapid; to get reproducible results with this small volume, standard conditions are even more important than in the original starch gel method.

The gel is now poured on 12 cover slips (24 × 50 × 0.1 mm) as are used for histological slides. These have previously been glued on a glass plate of 25 × 30 × 0.3 cm, using very little petroleum jelly. On the four corners of this plate square pieces of glass, 1 mm thick, are laid. Another glass plate, of the same size as the first, is used as a cover. Over the latter glass plate a sheet of wet, thin cellophane is smoothly stretched. The cover plate, its cellophane covered side facing downwards, is obliquely lowered

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on the gel, as indicated in Fig. 1. Firm pressure is applied to remove superfluous gel, and a weight of about 2 kg is placed upon the cover plate.

After 15 min the cover plate is carefully removed, leaving the cellophane which is subsequently drawn off the gel. The histological cover slips with the overlying gel

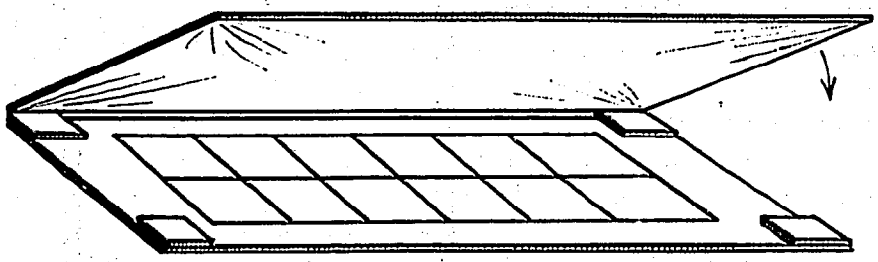

Fig. 1. Diagram of glass plates and cover slips used for the preparation of the gels, as described in the text.

are cut out and removed from the supporting glass plate. These cover slips, each now covered by a 1 mm thick gel layer, may be used immediately or stored in a humid atmosphere for up to 24 h.

2. Introduction of the sample

Two or more rectangular fragments of a razor blade, mounted in a metal block, are used to imprint slits into the gel. The cutting edges and surfaces of these fragments should be absolutely clean and smooth, to prevent irregularities of the slits and of the protein zones.

Undiluted serum is drawn into a thin glass capillary, diameter 0.2 mm. Inserting this capillary into, and subsequently moving it along the slit, will result in the application of the right amount of sample, about 0.2 μl for a 7 mm slit. This procedure requires some experience, it is not feasible to correct excess sample application by blotting since this will adversely affect the electrophoresis results.

3. Electrophoresis

The apparatus used was designed for micro agar electrophoresis by Dr. J. OORT from the Pathological Laboratory of the University of Leyden, to whom we are grateful for permission to copy and to publish this design. For illustrations of the apparatus see Fig. 2. The midcompartment is filled, to a level of 1 cm above the platform, with 1.5 % agar, dissolved by heating in 0.3 *M* boric acid and 0.2 *M* NaOH pH 8.4 at 20°. After cooling, a slot, 3.5 cm wide and with vertical walls, is cut in the agar. The gel bearing cover slips will be placed over this slot. The electrode compartments are filled with the same buffer used for the preparation of the agar. If cathode and anode are interchanged after every run, the electrode buffer must be changed after 10 and the agar after 50 runs.

Pentane is poured into the midcompartment up to 0.5 cm above the agar. A continuous air current, produced by a vacuum pump, gives a nearly constant temperature of 10° when 2 strips are placed in the apparatus. It is recommended to cool the system some minutes before the first run of a series, when the pentane is still warm. 300 V from a stabilized source is applied to the electrodes; the potential in the starch gel is 85 V/cm. The current is 0.25 mA per strip when the apparatus is in thermal equilibrium.

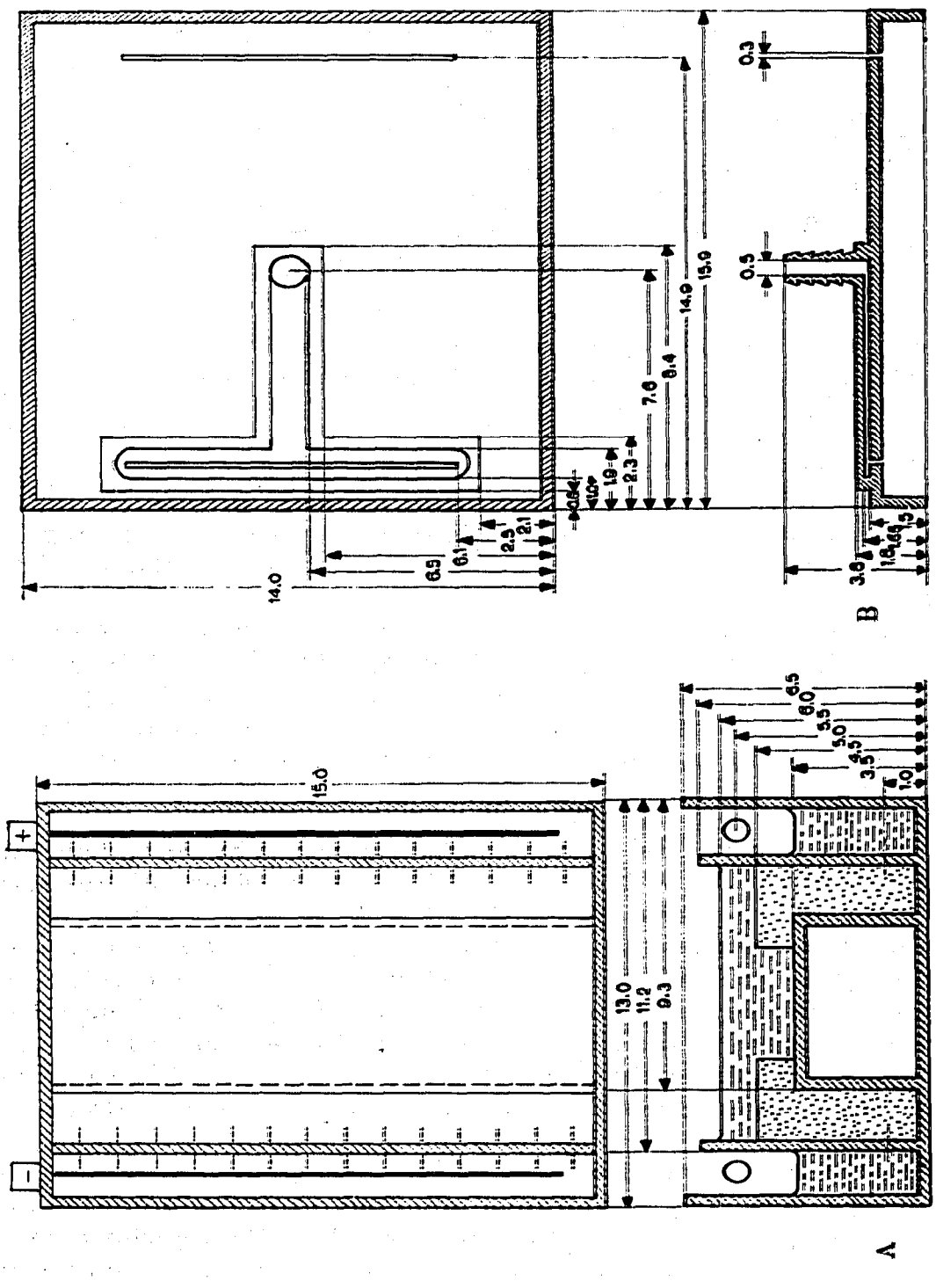

Fig. 2. (A) Electrophoresis apparatus without cover shown from above and in transverse section. Measurements in centimeters. Material is Perspex, 3 mm thick. Mid- and side-compartments are shown filled with agar overlaid with pentane, and electrode buffer respectively. Holes between compartments have a diameter of 1 mm. (B) Cover of the electrophoresis apparatus; *cf.* Fig. 2 (A). Views shown are from above, in longitudinal and in transverse section.

In 15 min a good separation of serum proteins will be achieved. Longer duration of electrophoresis results in less sharply delineated but more widely separated zones, which may be of advantage for scanning of electropherograms.

4. Staining and preserving of the strips

After electrophoresis, the gel strips are removed from the cover glasses and transferred to a saturated solution of Amido Black 10B in methanol–water–acetic acid (50:50:10, v/v).

Staining and washing can be done very conveniently in 5 histological staining trays, the first being filled with the dye solution and the others with the solvent. After staining for 30 sec the gels remain in each of the other trays for 1 min, so that the whole process is completed within 5 min.

The small size of the strips renders it possible to keep a great number of electropherograms in a container filled with solvent. It is, however, also feasible to dry them between cellophane and filter paper under slight pressure. Drying without these precautions leads to curling and opacity of the gels.

RESULTS AND DISCUSSION

The resolution, using this micro starch gel electrophoresis, is equal to that reached by the original vertical macro method. From normal human serum it is possible to obtain 18 protein fractions. The electropherograms also show the same pattern, but the relative distances of the protein zones are slightly different in both methods, as is shown in Fig. 3. This may be due to the different distances travelled by the proteins through the gel, the supposed sieving effect being smaller in the case of micro starch gel electrophoresis.

When applied to sera (*e.g.* rat serum) which contain a large amount of amylase, the micromethod has the advantage that there is practically no breakdown of the starch gel during the short time of electrophoresis and the protein zones are as sharp as with other sera.

When the thickness of the strips is kept constant, the gel concentration required for optimal results seems to be proportional to the potential. 300 Volts, as applied in the described method, requires 1.25 times the concentration used by SMITHIES[1]. A lower concentration gives very diffuse bands and a higher concentration undesirably reduces the mobility of the proteins. It is useless to increase the potential, since the increased heat production will cause an irregular distribution of the protein bands in the different gel layers, as is evident when comparing the two sides of the strips. Unless the strips are to be made translucent by drying, the latter phenomenon may not be very disturbing. In this case one may use histological object slides, which are easier to handle, instead of cover slips. Their greater thickness causes insufficient cooling of the under side of the gel. Consequently, the mobility there will be greater than in upper layers.

The discontinuous buffer system of POULIK[4], as well as the combination of this system with 7 *M* urea[5], can also be used with this micro method. The urea gel takes an hour to harden, and electrophoresis also requires double the time mentioned above. Melting of the agar at the heated contact points with the starch gel can be prevented by using starch gel instead. With this method essentially the same results were ob-

tained with the lens protein, α-crystalline, as were published by BLOEMENDAL *et al.*[6], using the macro method.

In preliminary experiments, the electropherograms obtained by the method described here were found to be suitable for the application of histochemical staining techniques. It has also been found that the strips may be scanned automatically with a Chromoscan from Joyce Instruments, Newcastle-upon-Tyne, England. This apparatus, recording reflected light from the strips, is specially adapted for starch gel electrophoresis. A very narrow split is of course required here.

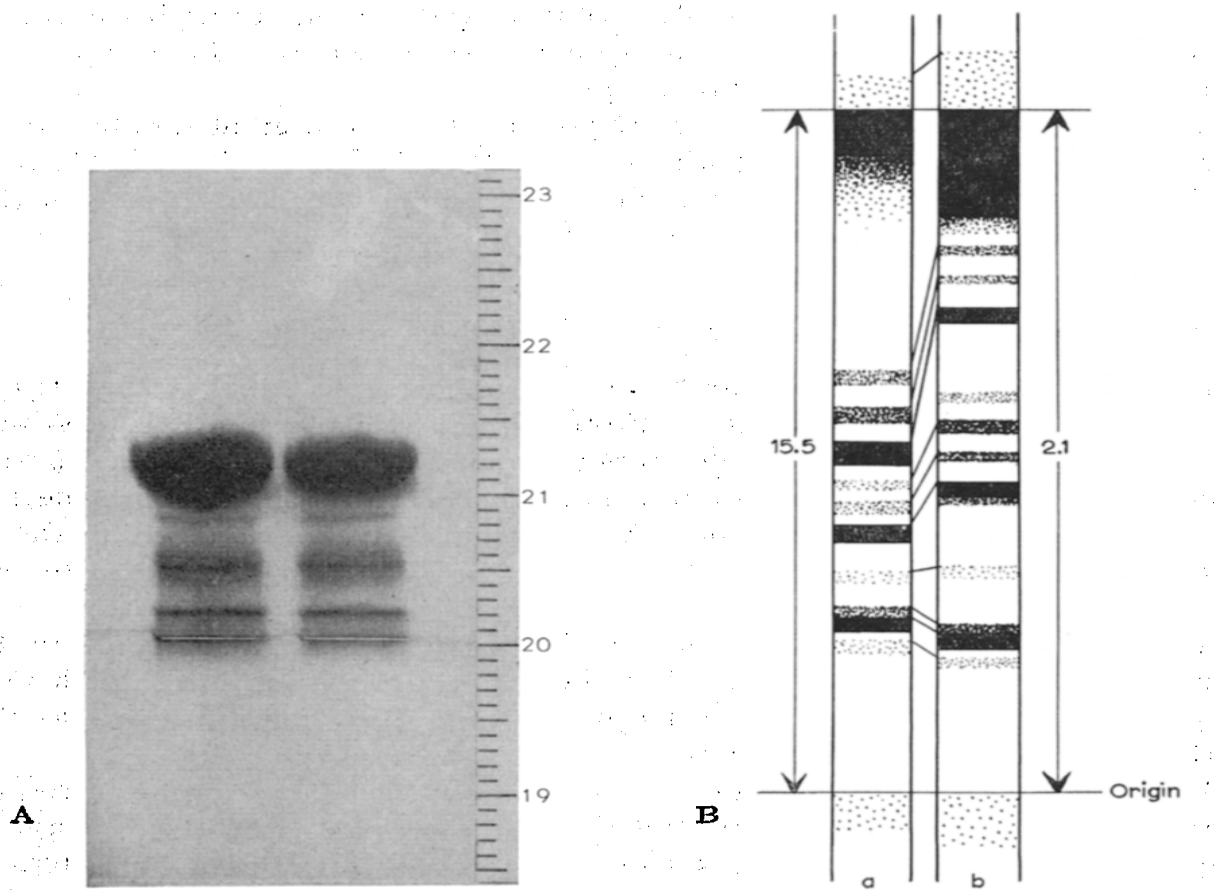

Fig. 3. (A) Electropherogram of mouse serum after micro starch gel electrophoresis. Both slits contained ± 0.2 μl of C_{57}BL male serum. Duration of electrophoresis is 15 min. Staining: Amido Black 10B. Ruling in centimeters. (B) Comparison of electropherograms obtained with (a) macro and (b) micro starch gel electrophoresis. For both electropherograms identical mouse serum was used.

SUMMARY

A micro method for starch gel electrophoresis is described, which has the same resolving power as the original method, but has moreover the following advantages:

(1) It is more rapid, since preparing and hardening of the gel takes 20 min; for serum the electrophoresis takes 15 min, and staining plus washing takes 5 min only.

(2) It requires a very small sample only (0.2 μl for serum).

(3) The gels are small and easy to handle and to store.

ADDENDUM

After the completion of this manuscript, our attention was drawn to the micro electrophoresis method described by MOURAY *et al.* (H. MOURAY, J. MORETTI AND J.-M. FINE, *Bull. Soc. Chim. Biol.*, 43 (1961) 993). This method is intermediary between SMITHIES' macro method and the micro method described above. MOURAY *et al.*, using object glasses, require a migration time of 1 1/2–2 hours and a sample volume of 5–10 μl.

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