REVIEW

STARCH ELECTROPHORESIS
III. STARCH GEL ELECTROPHORESIS

H. BLOEMENDAL

Department of Biochemistry, Antoni van Leeuwenhoek-Huis: The Netherlands Cancer Institute, Amsterdam (The Netherlands)

(Received January 12th, 1960)

A promising electrophoretic method was introduced by SMITHIES[5,6], who applied starch gel as supporting medium*. The idea of using gel electrophoresis was not new in itself; successful experiments with agar gels had been carried out previously by GORDON *et al.*[90,91]. The same supporting medium has already been applied as long ago as 1923 for the separation of inorganic ions[92].

Starch, as compared with other gels, has certain advantages owing to its uncommonly high resolving power. However, the reason why, for instance, serum splits up into more bands than observed in other stabilizing materials is not yet wholly clear. There is a possibility that some of the extra zones are found as a result of interaction between the borate buffer used and certain proteins linked to carbohydrate. Furthermore, a molecular sieving effect may occur. Small molecules will penetrate the swollen gel grains more easily than molecules of larger size. The same principle forms the basis of a new technique called "gel filtration" which may be successfully applied for desalting of protein solutions and separation of these solutions from peptides and amino acids[93]. In this way BOSCH AND BLOEMENDAL[94] were able to free ribonucleic acid components from sodium chloride after fractionation with salt gradients on Ecteola columns.

Whereas a growing stream of papers is being published on starch gel electrophoresis as an analytical tool, no convenient technique for large scale preparative work has so far been described. The paper by BERNFELD AND NISSELBAUM[95] deals with the separation of larger quantities of protein, but these workers used a gel that was not prepared from hydrolysed starch only.

METHODS

1. *Apparatus*

Starch gel electrophoresis may be carried out in similar rectangular plastic boxes as described in the first part of this review[63]. A suitable tray originally used by SMITHIES[6] had the following dimensions: depth, 6.5 mm; width, 20 mm; length, 250 mm. In

* *Note added in proof.* When this review was in print a detailed bibliography of the literature with special reference to studies of serum proteins was published by SMITHIES[161].

starch block electrophoresis a vertical position of the box appeared to be preferable[8,63]; according to a recent paper by SMITHIES[96] a vertical arrangement of the tray also gives better results in the separation of serum components in starch gel electrophoresis. Fig. 12 shows this improved apparatus which is also used in our laboratory.

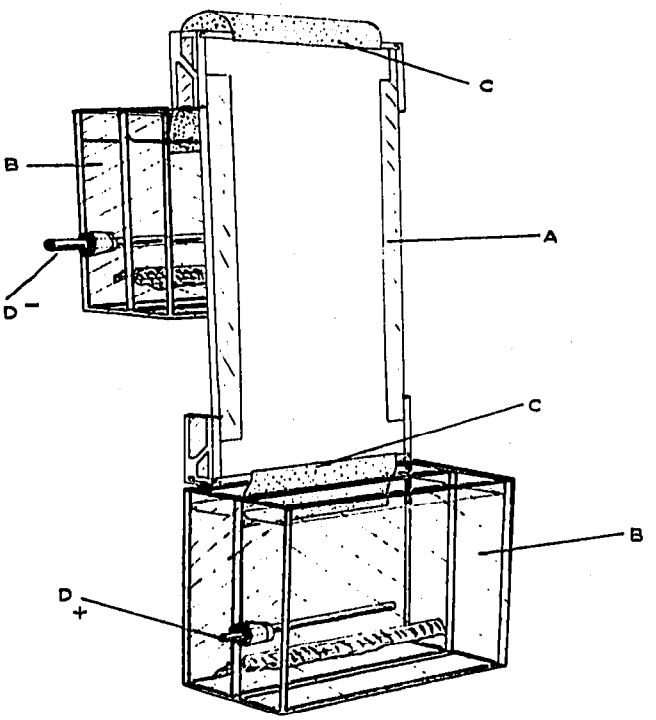

Fig. 12. Vertical starch gel electrophoresis. A. Tray containing the gel. B. Buffer vessels. C. Filter paper wicks. D. Carbon electrodes.

2. *Electrodes*

The construction of simple plastic electrode vessels is described in Part I [63]. Platinum, carbon or reversible silver–silver chloride electrodes may be used. The reversible electrodes are easily prepared by electrolysis of silver wire in 0.5 *M* HCl. The electrode is immersed in a tray containing 10 % sodium chloride solution. Filter paper or agar bridges are used to connect the electrode compartment with a vessel containing buffer solution (in serum fractionation 0.06 *M* NaOH + 0.3 *M* H_3BO_3 is mostly used).

3. *Preparation of the gel*

The essential point in the procedure of starch gel electrophoresis is the preparation of a good gel, made from soluble starch. Starch from different batches does not always possess the same gelling properties. Hydrolysed starch ready for use is now commercially available (*e.g.*, Connaught Medical Research Laboratories, University of Toronto, Canada).

Good gels are obtained in the following way:

(a) Pure potato starch is washed with distilled water and borate buffer (or any other buffer solution to be used in the electrophoretic experiment), followed by rinsing with distilled water and dehydration with acetone.

(b) 250 g of the dry starch is suspended in 495 ml acetone containing 5 ml

concentrated HCl. This mixture has to stand for 1 h at a temperature of 37°.

(c) A solution of sodium acetate or carbonate is added until the pH is 7. The starch is then resuspended in distilled water and washed thoroughly on a Buchner funnel in order to remove sodium chloride and acetate ions.

(d) The starch is dried by dehydration at 40°.

(e) The hydrolysed starch is mixed with buffer (10–15:100 w/v) with constant swirling. When a temperature of approximately 90° has been reached, the flask is de-aerated under slightly reduced pressure during a few seconds.

(f) The hot gel is poured into the tray and immediately covered with a flexible sheet of plastic, the surface of which is coated with a thin layer of paraffin oil.

(g) The excess of gel is forced out by carefully pressing on the sheet so that air bubbles are avoided. After 2–3 hours cooling in a refrigerator or in a cold room the plastic sheet is removed and the gel is covered with a perspex plate or with a layer of paraffin wax. In the latter case the melted paraffin is poured on the gel just short of congealing (temperature about 45°).

The starch may also be mixed with Hyflo-Super Cel and amylose[95], in this way a stiff gel is obtained in which the electroosmotic flow is extraordinarily low. This gel is easily cut after the electrophoretic run and preparative work becomes possible.

The gel is connected with the outer compartments of the electrode vessels by means of filter paper strips enclosed in parafilm or thin plastic sheets.

Gels may be stored overnight in a cold room or in a refrigerator.

4. *Insertion of the sample*

There are three different methods of introducing the sample.

(a) A slit is cut transversely in the gel and filled with the solution to be separated. SMITHIES' improved apparatus has a device[96] which allows the formation of a slit when the gel is poured.

(b) The sample is soaked into a small strip of filter paper. An opening is cut in the gel and the wet paper is put in by means of two forceps. When only small amounts of the sample are available, this method is preferable.

A similar insertion technique is applied when starch gel electrophoresis is carried out in combination with paper electrophoresis[97]. After the sample has been separated on filter paper, the strip is inserted into a slit in the starch gel and the second run is carried out at right angles to the first.

(c) The sample is mixed with starch powder so that a homogeneous paste is obtained. This paste is carefully put into a slit cut in the gel. When the electrophoresis is carried out vertically, melted paraffin wax is poured on the solution so that the slit filled with the sample is sealed. Then the whole gel surface is covered with paraffin wax, after which the tray is brought into a vertical position.

5. *Electrophoresis*

In serum analysis the starch gel electrophoresis is mostly carried out as described originally by SMITHIES[5,6,96] (see also Fig. 13).

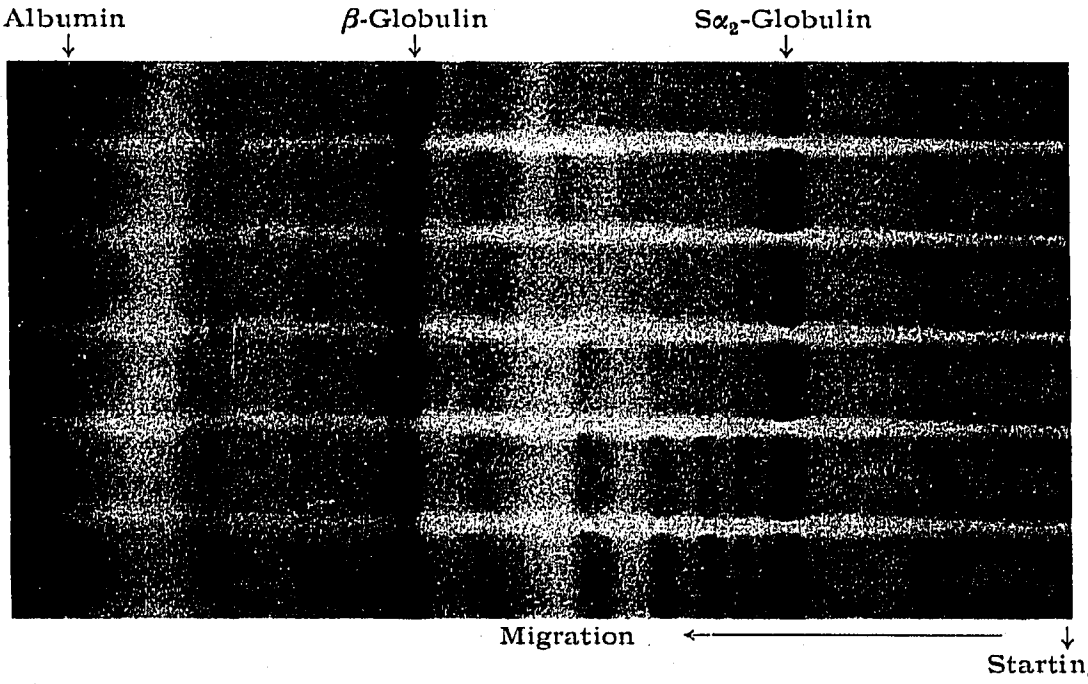

Fig. 13. Serum separation obtained with vertical starch gel electrophoresis (reprinted with the kind permission of Dr. O. SMITHIES and the Editor of *The Biochemical Journal*[96]).

POULIK[98] pointed out that a discontinuous buffer system may have considerable advantages for the separation of certain proteins.

In Table V the working conditions for the fractionation of several substances are given.

TABLE V

Material	*Buffer*	*Ionic strength, molarity*	*pH*	*V/cm*	*mA*	*h*	*Reference*
Histone	Unbuffered NaCl solution (adjusted with HCl)	0.02 μ	4.1–4.9	3.2	3.5	6	101
Haemocyanin	Borate	0.02 *M* + 0.008 *M* NaOH	8.03	9	—	12	104
Haemoglobin	Borate	0.03 *M*	8.5	6	—	5	112
Human plasma	Borate	0.025 *M*	—	450 V	—	18	124
Parotid protein	Borate	0.03 *M*	8.4	8.7	4.5	5	138
Azotobacter protein	Veronal	0.1 μ	8.6	540 V	50	20	139
Gastricsin	Acetate	0.1 μ	5	4.55	—	22	144
Thyrotropin	Acetate	0.012 μ	5	260 V	11	3–4	145
Bromocresol green	Borate	0.05 *M* + 0.02 *M* NaOH	8.5	5	1.4	5.5	146

6. *Influence of temperature*

Not much is known exactly about the effect of the rise of temperature on starch gel electrophoresis. Like in other stabilizing media and in free electrophoresis the mobilities are temperature-dependent. Heat development is stronger in the strips than on the surface so that distorted zones may be the result of irregularity of the temperature

distribution. Using low ionic strength buffers and small strips the temperature effect is reduced, provided the voltage gradient is not higher than 5–6 V/cm.According to GOLDBERG[99] the temperature at which the separation of serum haemoglobin is performed is not critical over a range of 10–25°. At 30°, however, the wicks forming the connection between gel and electrode vessel tend to dry out.

7. Location of substance

Different procedures are recommended for staining the gels. After the electrophoretic run the gel is carefully taken from the tray. Trays with removable side walls are very convenient. The gel is cut parallel to the bottom surface of the tray so that two equal strips are obtained. A razor blade or microtome may be used to split the gel in

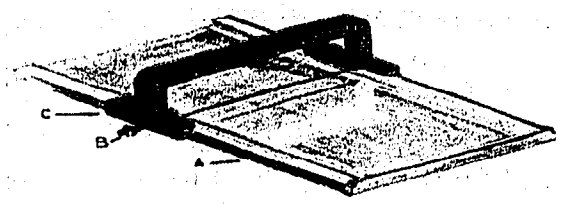

Fig. 14. Slicing device for starch gel electrophoresis. A. Bottom of electrophoresis tray. B. Screw for stretching the thread. C. Metal slide.

a single movement. In our laboratory a very simple apparatus has been constructed to divide the gels (Fig. 14). A thin stainless steel thread is stretched over a slide which fits the bottom of the electrophoretic tray.

Staining is carried out by pouring dye on the cut surface. Many workers employ protein staining with a saturated solution of amido black 10B in methanol–water–glacial acetic acid (50:50:10) during 10–30 min. Excess of dye is removed by 3–4 washings with the solvent. After the last washing the gel is kept in the methanol–water–acetic acid mixture for 1–2 h. Proteins which do not give stable complexes with amido black may be stained with bromophenol blue, light green or other dyes well-known in paper electrophoresis. Staining may also be carried out with a saturated solution of Buffalo black in a mixture of methanol–water–acetic acid (50:50:1) for 2 min. The solvent is used as washing fluid. After removal of the excess of stain the strips are left overnight in the solvent[100].

Another technique includes fixation of the gel in 5 % acetic acid before staining. In this case the colouring mixture consists of 1 g amido black 10B, 500 ml 1 *M* acetic acid and 500 ml 0.01 *M* sodium acetate. The strips are washed in 5 % acetic acid[6].

According to NEELIN AND CONNELL[101] nigrosine stains more dilute components more heavily than does amido black. However, it obscures fine zones in a densely stained region.

Lipoprotein may be stained in a solution containing 1.2 g Sudan black, 40 ml water, 600 ml ethanol and 2 ml NaOH for 16 h. Complete decoloration takes 6–8 days in 60 % alcohol[102]. A saturated solution of oil red O in a mixture of methanol–

acetic acid–water (60:10:30) may also be used in order to make lipoprotein visible[103].

Copper-containing proteins, such as haemocyanin, are detected by placing the horizontally sectioned gel for 3–4 h in a solution containing 50 ml 10% sodium acetate and 3 ml 0.1 % solution of dithio-oximide in alcohol. A positive test results in the development of a greenish black colour[104].

Haemoglobins are detected with the benzidine reagent according to FRANKLIN AND QUASTEL[105] or as described for agar electrophoresis[106] but without zinc acetate solution. Haem proteins may also be stained by *o*-tolidine[107].

The a_2 region may be made visible by adding haemoglobin to serum protein. The result is a faintly coloured band which makes the detection of the a_2 region possible without staining[108].

OWEN *et al.*[109] use another colour reaction suitable for the detection of small amounts of haptoglobins in human serum. The complex of these proteins with haemoglobin shows peroxidase-like activity. *o*-Dianisidine appeared to be the best reagent, giving a stable brownish colour, whereas it was not absorded on peroxidase-free gel.

8. *Quantitative evaluation of the strip*

FINE AND WASZCZENKO[110] prepared gels suitable for direct photometric estimation. Since several protein dyes in alcoholic solution cause permanent opacity of the gels these authors used the aqueous staining method of URIEL AND GRABAR[111].

The strips are fixed overnight in 5 % acetic acid and then stained for 10 min. Decoloration is achieved by successive washings in 5 % acetic acid.

The stained gel is placed on a glass plate covered with 1.5 % melted agar and dried in an oven. The glass plate is withdrawn after the agar has solidified. A sheet of Whatman No. 1 filter paper is applied to the surface. The gel enclosed in agar is left in an oven at 37° for 40 h. In this way a transparent film is obtained.

A simpler method is described by VESSELINOVITCH[100]. Following staining, the strips are immersed in a 10 % solution of acetic acid and boiled for 30–60 sec. After the solution has cooled the strips may be removed and estimated photometrically.

GOLDBERG[99] applied densitometric scanning of photographs of the strips in order to obtain quantitative evaluation of the starch pattern.

The coloured bands producedby some dyes fade on storage of the gels. JACOBS[150] describes a photographic routine method which allows permanent recording of the strips.

9. *Elution*

After the electrophoretic run and staining of one of the strips cut horizontally, the uncoloured half may be cut vertically into segments of 0.5 or 1 cm width. Comparison with the stained strip makes location of the separated protein fractions possible.

The protein may be removed from the gel by freezing and thawing of the segments followed by extraction with distilled water or buffer. This procedure does not allow quantitative elution of the substance, as does the starch block technique.

Another more complicated method is digestion of the starch with amylase before protein elution.

APPLICATIONS

(a) *Serum proteins*

Starch gel electrophoresis allows the identification of more haemoglobin fractions than do other electrophoretic techniques. ROSA *et al.*[112] observed a difference in haemoglobin pattern in six inbred strains of mice.

DE GROUCHY[113,114], who originally used the borate buffer system of SMITHIES, studied the behaviour of normal and abnormal human haemoglobin in a discontinuous buffer system (Tris–citrate–borate). Better solution was obtained and minor components, not detected by other types of electrophoresis, were observed.

GOLDBERG[99] also used a discontinuous buffer system in analyzing human serum haemoglobin.

BANGHAM AND BLUMBERG[115] studied the distribution of electrophoretically different haemoglobins between cattle breeds from Europe and Africa. There was a certain correlation between the absence of bovine B haemoglobin and a relatively high tolerance to trypanosomiasis.

Haptoglobins (= α_2-globulins) combine specifically with oxyhaemoglobin. The complex appears to be relatively stable, while uncombined oxyhaemoglobin is rapidly broken down with the formation of methaemalbumin. From these observations ALLISON AND REES[116] concluded that haptoglobins determine the renal threshold for haemoglobin. These authors ascribe haemoglobinuria to low levels or absence of haptoglobins in plasma.

Genetically controlled variations in haptoglobins of human sera were demonstrated by SMITHIES and co-workers[6,117,118].

Using borate buffer ASHTON[119] failed to detect similar differences in other mammalian sera. However, using phosphate buffer, it could be demonstrated that differences in cattle serum protein patterns were under genetic control[120]. In a preliminary communication[121] ASHTON described five serum β-globulin phenotypes of cattle. A sixth phenotype was recognized later[122] so that it was possible to propose a genetic mechanism for the system. The same author observed 3 β-globulin types, 5 α-globulin types, and at least 3 albumins in horse sera[123].

FINE *et al.*[124,125] found similar patterns to those of SMITHIES but the γ-globulin was distinctly divided into two components. The distribution of zones in the electrophoretic pattern was as follows: 2 γ-globulins on the cathodic side of the origin. Staining with Sudan black revealed the presence of α- and β-lipoprotein on the anodic side. Furthermore, an S-α_2 band, 9 zones between S-α_2 and the β-c-globulin, 2 F-α_2-globulins and 5 zones between the fast α_2 and the albumin were detected by the usual staining method.

HARRIS *et al.*[126] demonstrated in a series of 153 sera from Africans new β-globulins. According to SMITHIES[6,117,118] differences in these globulin patterns are controlled genetically. The author referred to the new globulins as β-CD_1 and β-CD_2. β-Globulin polymorphism in humans distinguishes the white and the black races[127]. So sera from 425 normal adult blood donors were examined by the two-dimensional method in order to demonstrate the absence of β-globulin D in whites. One-dimensional starch gel electrophoresis was not always reliable for detecting this protein[128].

ASHTON[120] obtained 8 β-globulins in sheep sera. Further separation of the zones detected resulted in 14 β-globulin phenotypes. According to that author, sheep sera show the most complex β-globulin polymorphism ever found in mammals[103].

SMITHIES AND POULIK[97] obtained originally more than fifteen resolved components from serum proteins by two-dimensional electrophoresis. In a later communication[131] the occurrence of more than twenty components was reported.

WOODS *et al.*[132] demonstrated different haemocyanines in sera of certain crustaceous species.

MOULLEC AND FINE[133] who examined the sera of 406 blood donors claim that the frequencies of the genes Hp^1 and Hp^2 do not differ very much in 40–60 % of the cases examined. This conclusion holds for most of the populations living in Europe and the white population of the U.S.A.

LATNER *et al.*[134] demonstrated that the major binding of vitamin B_{12} by serum proteins occurs by the α- and β-globulins.

FINE AND CREYSSEL[135] studied the electrophoretic behaviour of abnormal proteins in myeloma and macroglobulinaemia. A typical β-myeloma showed three abnormal components as compared with normal sera. However, macroglobulins gave no characteristic pattern.

FINE AND BURSTEIN[102] fractionated human serum lipoproteins. The most important component migrated between the origin and α-globulin. According to the authors the small mobility is caused by a high molecular weight of the lipoprotein fraction.

DE GROUCHY[136] described a method for identification of caeruloplasmin in human serum. The zone was detected by incubation with a solution of *p*-phenylenediamine. The caeruloplasmin coincided with F α_2-globulin and was absent in sera of Wilson's disease.

^{131}I-Thyroxine added to human serum was run on starch gel. The thyroxine-binding protein migrated ahead of the albumin in a sharp band in the first pre-albumin position[137].

(*b*) *Other proteins, enzymes, and hormones*

HOERMAN[138] separated parotid saliva proteins. Reproducible migration patterns were obtained in which 9–12 components were resolved. An advantage was that only small volumes of native parotid secretion were required.

PERT AND KUTT[103] observed in normal cerebrospinal fluid 10–12 protein components corresponding to the serum protein in the same individual but with different relative concentrations. In neurologic diseases considerable alterations of the protein pattern were noted.

Starch gel electrophoresis of *Azobacter* proteins[139] revealed the presence of 12 components, differing in amino acid composition.

NEELIN AND CONNELL[101] applied starch gel electrophoresis in combination with cation exchange chromatography to chicken erythrocyte histone. Both mobility and resolution were dependent upon ionic strength, the nature of anions, pH, and on the protein concentration.

POULIK demonstrated the complex nature of diphtheria toxins[140]. In paper electrophoresis an apparent homogeneity was observed. Furthermore the lethal factor from these toxins could be isolated [141].

DIXON AND SMITHIES[142] separated enzymes from cabbage juice. Initially the enzymes showed peptidase activity towards leucyl and glycyl peptides and glycyl transfer activity. It was more difficult to fractionate these activities by other methods.

MARKERT AND HUNTER[143] separated the esterases in B2 mouse tissues. These esterases were compared, using α-naphthyl butyrate as a substrate.

TANG *et al.*[144] studied the properties of crystalline gastricsin. When electrophoresis of the enzyme was carried out on paper and starch under similar conditions, it only migrated on starch gel. Furthermore no activity could be recovered from paper strips, probably on account of strong adsorption or denaturation. From the gel the proteolytic activity could be extracted.

PIERCE and coworkers[145] purified thyrotropin. The hormone was separated into diverse fractions after chromatography on DEAE cellulose. One of these fractions was submitted to starch gel electrophoresis and appeared to split up into six fractions These findings were in contrast to results obtained with paper and moving-boundary electrophoresis.

(*c*) *Non-protein substances*

Except in one instance, starch gel electrophoresis has hitherto not been utilized in the separation of organic substances other than proteins.

FRANGLEN AND GOSSELIN[146] obtained a very remarkable result. These workers succeeded in separating metastable polymers of the dye bromocresol green. From this investigation it appeared clearly that the presence of two distinct bands after the electrophoretic run in starch gel is not always an indication of heterogeneity.

CONCLUSION

Numerous investigations have demonstrated without doubt that—from an analytical standpoint—the starch gel technique has certain advantages over the starch column method, and even over the starch block method. For preparative purposes, however, the gel technique has up to now not been found convenient. There must be certainty that the separated fractions are homogeneously distributed through a cross section of the medium. In starch gel strips the separation is never completely homogeneous throughout the gel when the strip is sliced after the electrophoretic run. For analytical purposes this is of no importance, as only the stained surface of the strip cut horizontally is considered. In preparative work, however, the variations in different layers of the gel cause overlapping of the separated zones and the stained surface does not correspond with the pattern inside the strip.

In several cases starch block electrophoresis appears to combine the remarkable resolving power of the gel technique with the possibility of isolating the separated fraction after the electrophoretic run. So BEARN AND FRANKLIN[147] made a comparison

of starch gel and starch block patterns of the haptoglobin–haemoglobin complexes from the three main genetic groups. Starch block electrophoresis also enabled a distinct differentiation of the three groups to be made. This was important for the isolation of sufficient material for ultracentrifugal studies.

WOODIN[148] submitted partially purified leucocidine from *Staphylococcus aureus* to electrophoresis in starch gel. Complete recovery of the protein was not obtained. On the other hand, nearly quantitative recovery was observed with the starch block technique.

In a recent paper MILLER AND BERNFELD[149] claim that a combination of starch granules and starch gel has the advantages of both media. This important finding awaits further verification.

Summarizing, it may be stated that starch gel electrophoresis is a powerful analytical tool, chiefly for the study of proteins. The utmost attention must always be paid even to the smallest detail of an obtained pattern. As a result, the success of the method depends to a great extent on the experience of the worker.

REFERENCES

1-62 At the end of Part I, *J. Chromatog.*, 2 (1959) 121–135.
63-88 At the end of Part II, *J. Chromatog.*, 3 (1960) 1–10.
89 H. BLOEMENDAL, *J. Chromatog.*, 3 (1960) 1.
90 A. H. GORDON, B. KEIT, K. ŠEBESTA, O. KNESSL AND F. ŠORM, *Collection Czechoslov. Chem. Communs.*, 15 (1950) 1.
91 A. H. GORDON AND P. REICHARD, *Biochem. J.*, 48 (1951) 569.
92 J. KENDALL AND E. D. CRITTENDEN, *Proc. Natl. Acad. Sci. U.S.*, 9 (1923) 25.
93 J. PORATH AND P. FLODIN, *Nature*, 183 (1959) 1657.
94 L. BOSCH AND H. BLOEMENDAL, unpublished work.
95 P. BERNFELD AND J. S. NISSELBAUM, *J. Biol. Chem.*, 220 (1956) 851.
96 O. SMITHIES, *Biochem. J.*, 71 (1959) 585.
97 O. SMITHIES AND M. D. POULIK, *Nature*, 177 (1956) 1033.
98 M. D. POULIK, *Nature*, 180 (1957) 1477.
99 C. A. J. GOLDBERG, *Clin. Chem.*, 4 (1958) 484.
100 S. D. VESSELINOVITCH, *Nature*, 182 (1958) 665.
101 J. M. NEELIN AND G. E. CONNELL, *Biochem. Biophys. Acta*, 31 (1959) 539.
102 J. M. FINE AND M. BURSTEIN, *Experientia*, 14 (1958) 411.
103 J. H. PERT AND H. KUTT, *Proc. Soc. Exptl. Biol. Med.*, 99 (1958) 181.
104 J. R. WHITTACKER, *Nature*, 184 (1959) 193.
105 A. E. FRANKLIN AND J. H. QUASTEL, *Science*, 110 (1949) 447.
106 J. M. FINE, J. URIEL AND J. FAURE, *Bull. soc. chim. biol.*, 38 (1956) 349.
107 T. O'KELLY AND J. KOHN, *J. Clin. Pathol.*, 8 (1955) 249.
108 S. BAAR, *Nature*, 182 (1959) 259.
109 J. A. OWEN, H. J. SILBERMAN AND C. GOT, *Nature*, 182 (1958) 1373.
110 J. M. FINE AND E. WASZCZENKO, *Nature*, 181 (1958) 269.
111 J. URIEL AND P. GRABAR, *Ann. inst. Pasteur*, 90 (1956) 427.
112 J. ROSA, G. SCHAPIRA, J. C. DREIFUSS, J. DE GROUCHY, G. MATTÉ AND J. BERNARD, *Nature*, 182 (1958) 947.
113 J. DE GROUCHY, G. SCHAPIRA AND J. C. DREIFUSS, *Rev. franç. d'études clin. biol.*, 3 (1958) 377.
114 J. DE GROUCHY, *Rev. franç. d'études clin. biol.*, 3 (1958) 877.
115 A. D. BANGHAM AND B. S. BLUMBERG, *Nature*, 181 (1958) 1551.
116 A. C. ALLISON AND W. REES, *Brit. Med. J.*, (1957 II) 1137.
117 O. SMITHIES AND W. F. WALKER, *Nature*, 176 (1955) 1256.
118 O. SMITHIES AND W. F. WALKER, *Nature*, 178 (1956) 694.
119 G. C. ASHTON, *Nature*, 179 (1957) 824.
120 G. C. ASHTON, *Biochem. J.*, 67 (1957) 32 P.
121 G. C. ASHTON, *Nature*, 180 (1957) 917.
122 G. C. ASHTON, *Nature*, 182 (1958) 370.

123 G. C. ASHTON, *Nature*, 182 (1958) 1029.
124 J. M. FINE, J. LOEB AND E. WASZCZENKO, *Nature*, 182 (1958) 452.
125 J. M. FINE, E. WASZCZENKO, J. LOEB AND J. MOULLEC, *Rev. hématol.*, 12 (1957) 698.
126 H. HARRIS, E. B. ROBSON AND M. SIREISALCO, *Nature*, 182 (1958) 452.
127 O. SMITHIES, *Nature*, 180 (1957) 1482.
128 O. SMITHIES, *Nature*, 181 (1958) 1203.
129 G. C. ASHTON, *Nature*, 181 (1958) 849.
130 G. C. ASHTON, *Nature*, 182 (1958) 1101.
131 M. D. POULIK AND O. SMITHIES, *Biochem. J.*, 68 (1958) 636.
132 K. R. WOODS, E. C. PAULSEN, R. L. ENGLE, JR. AND J. H. PERT, *Science*, 127 (1958) 519.
133 J. MOULLEC AND J. M. FINE, *Nature*, 184 (1959) 196.
134 A. L. LATNER AND ANGEL HABIB ZAKI, *Biochem. J.*, 66 (1957) 54 P.
135 J. M. FINE AND R. CREYSSEL, *Nature*, 183 (1959) 392.
136 J. DE GROUCHY, *Rev. franç. d'études clin. biol.*, 3 (1958) 621.
137 C. RICH AND A. G. BEARN, *Endocrinology*, 62 (1958) 687.
138 K. C. HOERMAN, *J. Lab. Clin. Med.*, 53 (1959) 64.
139 G. N. ZAITSEWA AND A. N. BELOZERSKII, *Biokhimiya*, 62 (1958) 687.
140 M. D. POULIK, *Nature*, 177 (1956) 982.
141 M. D. POULIK AND E. POULIK, *Nature*, 181 (1958) 354.
142 G. H. DIXON AND O. SMITHIES, *Biochim. Biophys. Acta*, 23 (1957) 198.
143 C. L. MARKERT AND R. L. HUNTER, *J. Histochem. and. Cytochem.*, 7 (1959) 42.
144 J. TANG, S. WOLF, R. CAPUTTO AND R. E. TRUCCO, *J. Biol. Chem.*, 234 (1959) 1176.
145 J. G. PIERCE, L. K. WYNSTON AND M. E. CARSTEN, *Biochim. Biophys. Acta*, 28 (1958) 434.
146 G. FRANGLEN AND C. GOSSELIN, *Nature*, 181 (1957) 1182.
147 A. G. BEARN AND E. C. FRANKLIN, *Science*, 128 (1958) 596.
148 A. M. WOODIN, *Biochem. J.*, 73 (1959) 225.
149 E. E. MILLER AND P. BERNFELD, *J. Chromatog.*, 2 (1959) 519.
150 R. JACOBS, *Biochem. J.*, 74 (1960) 45P.
151 O. SMITHIES, *Advances in Protein Chem.*, 14 (1959) 65.