REVIEW

STARCH ELECTROPHORESIS

I. STARCH BLOCK ELECTROPHORESIS

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INTRODUCTION

Starch is used as supporting medium in three zone electrophoretical techniques:

I. Starch block electrophoresis, introduced by KUNKEL AND SLATER[1,2].

II. Starch column electrophoresis, developed independently by CARLSON[3] and FLODIN AND PORATH[4].

III. Starch gel electrophoresis, first described by SMITHIES[5,6].

The name zone electrophoresis has been introduced by TISELIUS[7] in order to indicate a difference with moving boundary electrophoresis. While in the moving boundary or free electrophoresis one works in dilute solutions with the inevitable result of only partial separation of different fractions, in zone electrophoresis the application of a solid material as supporting medium allows the separation of a mixture into discrete zones.

Various materials have been used as stabilizing medium. Especially for the separation of proteins, peptides, and probably also ribonucleic acids, starch has some advantages over paper, gelatin, agar, silica, asbestos fiber, or glass powder.

(i) Adsorption of most proteins on starch is only small.

(ii) Elution of starch segments after the electrophoretic run is easier than in the case of gelatin or agar gel which is an important factor for preparative purposes.

(iii) Starch forms more easily a homogeneous paste with buffers than does cellulose or glass powder.

(iv) Electrosmosis is lower than in most other supporting media[2] although this phenomenon may cause poor separation also in the starch block technique, mainly when electrophoresis is carried out with horizontal arrangement of the starch block[8]. Starch electrophoresis is generally considered to be a rather gentle purification method for proteins, that is to say that there is no more chance of denaturation than there is in the method of salting-out or precipitation with acids, alcohol or acetone. SORKIN *et al.*[9] on the contrary claim that during the electrophoretic run irreversible denaturation processes lead to considerable loss of protein. The question arises whether this holds true for their special case or that heat development, caused by the 17–20 mA current in their experiments with 0.1 μ buffer solutions, was responsible for the observed denaturation. Buffers of lower ionic strength than 0.1 μ usually give better results.

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HARRIS AND MEHL[10] found a decrease of 40–50 per cent in enzymatic activity during electrophoresis of crude intestinal alkaline phosphatase. These authors could not give an explanation for this effect but were able to show that incomplete recovery from starch would cause a loss of 12–13 per cent only.

Hereafter we shall discuss the most important applications, and our own experience with starch block electrophoresis, which has been introduced as routine technique in the Laboratory of Anatomy and Embryology ((University of Amsterdam) and in the Department of Biochemistry of the Netherlands Cancer Institute.

METHODS

1. Apparatus

Starch block electrophoresis is carried out either in semicylindrical glass troughs or in rectangular plastic boxes which can easily be constructed in every laboratory. Glass troughs can be obtained by cutting Pyrex tubing in half longitudinally[11]. In our laboratory perspex boxes 40 × 2 × 4 or 80 × 2 × 8 cm appeared to be convenient for analytical and large scale preparative work. The use of a starch block simply packed in wax paper as originally described by KUNKEL AND SLATER is not advisable. When very high voltages are required a water-jacketed box may be used[12].

2. Electrode vessels

Reversible Ag–AgCl electrodes as well as agar or filter paper bridges between the electrode vessels are sometimes used[12–14, 56]. Very simple electrode vessels are made of

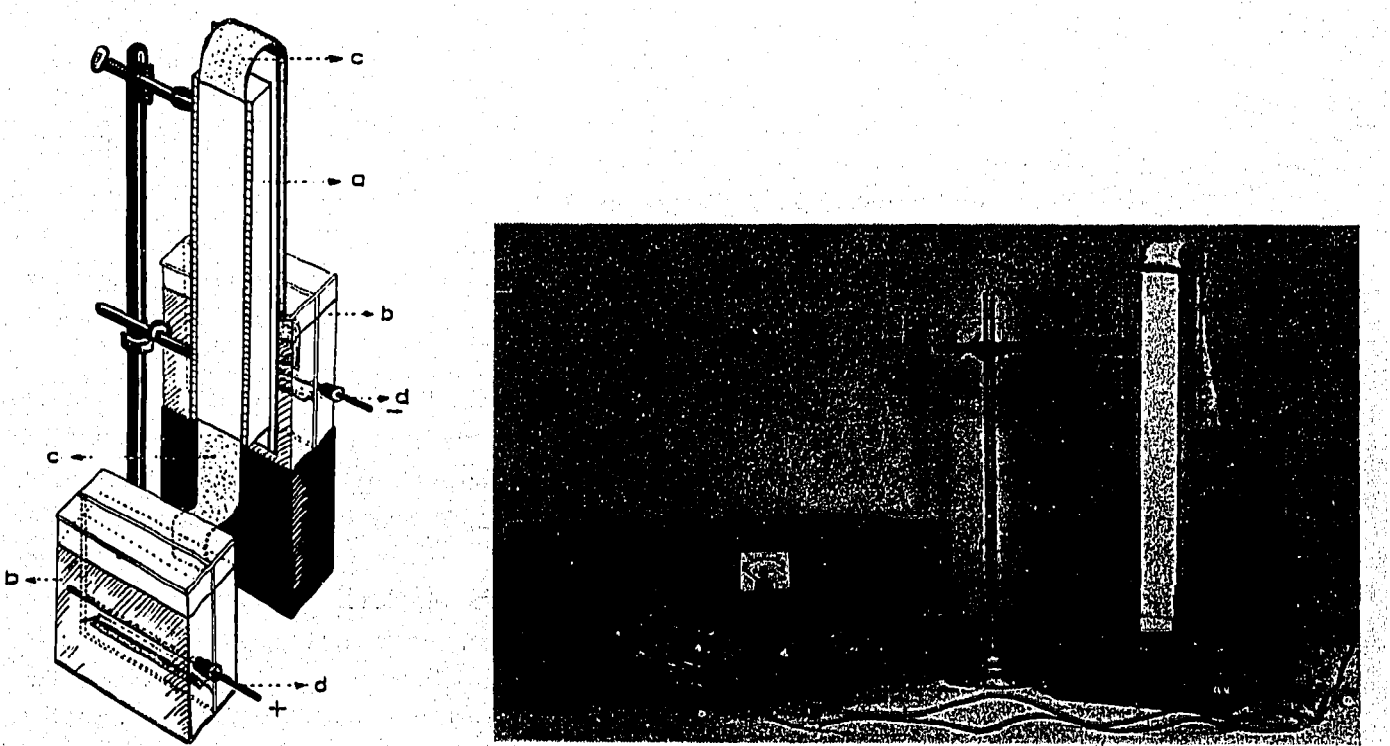

Fig. 1. Starch block electrophoresis apparatus [vertical arrangement of the block (a), perspex electrode vessels (b) with carbon electrodes (d)].

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perspex 18 × 7 × 14 cm. Each vessel is divided into two compartments by means of a perspex plate (Fig. 1, b). A cotton wick pressed into a slit in this plate serves as fluid bridge. Separated compartments are necessary to maintain the pH constant in that part of the vessel which is connected with the starch block. While there is a considerable change of pH in the outer electrode compartments, there is but a very slight decrease or increase, respectively, of pH in the inner compartments of the anode and cathode vessel. When experiments are carried out for longer than 24 hours it becomes necessary to renew the buffer solution in the electrode vessels. Inexpensive carbon rods (Fig. 1, d) appear to be satisfactory in various buffers, although at higher voltages carbon anodes show a tendency to disintegrate. In these cases they may be replaced by coiled platinum wire. Balance in the electrode vessels is obtained by connecting cathode and anode with a rubber tube. This tube has to be closed during the electrophoretic run. Balancing of the levels is unnecessary when the box is arranged vertically and the cathode vessel is placed higher than the anode vessel (Fig. 1). When a series of troughs at different pH's or ionic strengths are run at the same time, the anode chamber will have to be divided in subcompartments into which the troughs dip, as described by PAIGEN[12]. This arrangement protects the troughs from any change induced by electrosmosis. For the cathode vessel this division is not required.

During the electrophoretic run the vessels are closed with perspex plates or a sheet of parafilm.

3. *Preparation of starch*

Potato starch is the material most commonly used in starch electrophoresis. Depending on the purpose of a given investigation the starch has to be purified more or less carefully before use. If very exact analytical results are required, the starch is washed several times with distilled water; these washings are best carried out in the following manner. The starch is mixed with water in a ratio 1:3, stirred vigorously and the particles are allowed to settle. After 30 minutes the supernatant is decanted. Insoluble impurities and the smaller starch particles are removed. After repeated washings the starch is partially dried by sucking on a large Buchner funnel. The starch then is washed two or more times with the buffer in which the electrophoretic run will be carried out. KUNKEL recommends the use of warm buffer solution[14]. After these washings and drying of the starch, it is mixed once more with buffer and stirred until a homogeneous paste is obtained which is poured into the trough. Excess fluid is removed by absorption on thick filter paper. GRANICK AND MAUZERALL[15], who isolated an enzyme which converted *d*-aminolevulinic acid to phosphobilinogen, claim that the recovery could be increased when the starch is pre-washed with 0.25% bovine albumin. In order to prevent evaporation the whole starch block may be packed in water-repellent paper or covered with a sheet of parafilm. When open troughs or boxes are used the surface of the starch may be covered with a layer of molten paraffin wax[16].

The starch block (Fig. 1, a) is connected with the outer compartments of the electrode vessels by means of filter paper strips (Fig. 1, c) folded five or more times and inserted between the outer end of the block and the perspex box. The strips are

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enclosed in parafilm or thin plastic sheets. The connection may also be accomplished by means of plastic sponges, moist cloth, or agar bridges.

Recently RAACKE[60] found that marked changes in the conductivity and pH take place in the starch medium. These changes, caused by the ion-exchange properties of starch can largely be avoided by washing the starch with buffer before electrophoresis.

4. *Insertion of the protein mixture*

The box, connected with the electrode vessels, is left for at least 3–4 hours in the cold room for equilibration. Experiments may also be carried out in a refrigerator.

In case the proteins migrate towards the anode, a starch segment 0.5 or 1 cm wide is cut out of the block at 5–7 cm from the cathode side of the box. When the starch is covered with paraffin a window is cut in the wax layer after equilibration in the cold room.

In order to ensure a correct insertion of the mixture under investigation the following procedure is recommended[8]. The protein solution is mixed with starch so that a paste is obtained of the same consistency as the starch block in the box. Then

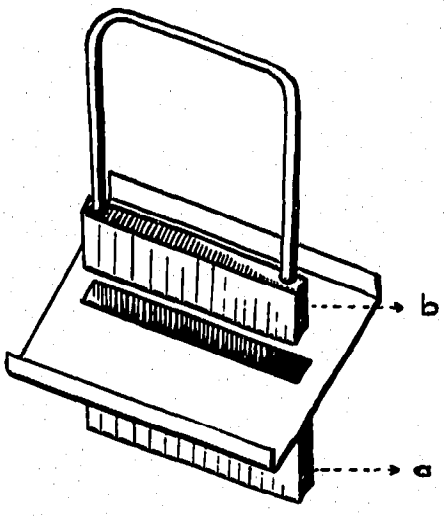

Fig. 2. Stainless steel frame for the insertion of protein-starch mixture (a). Plastic pestle with brass handle (b).

a steel frame with sharp edges (Fig. 2, a) and the exact proportions of a transverse segment is pressed through the window in the wax layer into the block, the starch within the frame is withdrawn and the inserted segment is pressed by means of a perspex pestle (Fig. 2, b) that fits precisely in the frame. Direct application of the protein by means of a pipette which penetrates the starch block is not advisable on account of irregular flow of the solution which often results in poor separation after the electrophoretic run. Narrow zones may be obtained by careful injection of the sample into the block by means of a hypodermic syringe fitted with a blunt needle[12]. When very small quantities of material have to be separated the solution may be sucked into a piece of Whatman 3MM paper, which has the same cross-section as the starch block. An opening is cut into the starch with a spatula and the wet paper is

carefully put in by means of two forceps. Direct contact of the paper with the starch is obtained by slight pressure on the starch surface. In this manner tritium-labeled glycyl-alanine was separated from trace amounts of glycine and alanine[17].

5. *Electrophoresis*

The circumstances under which electrophoresis in starch medium is carried out differ from case to case. In Table I a survey is given of a number of substances studied, buffers, pH's, voltages, current, and duration of the electrophoretic run.

TABLE I

Material	*Buffer*	*Ionic strenght (μ) Molarity (M)*	*pH*	*V*	*mA*	*h*	*Ref.*
Normal and pathological serum lipoproteins	Barbital	0.1 μ	8.6	100–500	25–60	24	1
	Phosphate	0.05 μ	6.5				1
Serum lipoproteins	Barbital	0.1 μ	8.6	400	—	24	2
Bovine lens proteins	Barbital	0.025 μ	7.8	420	6–8	25	8
Tuberculo-proteins	Barbital	0.1 μ	8.6	360	17–20	30	9
Intestinal alkaline phosphatase	Barbital	0.02 μ	8.6	200	7–9	12	10
Growth hormone (Somatotropin)	Acetate	0.017 M	4	175	30	24	11
	Carbonate	0.1 M	11.2	175	30	72	11
Tobacco mosaic virus	Phosphate	0.01 M	7.1	380	—	3¼	12
Rabbit reticulocytes	Tris	0.05 μ	7.65	270	90	27	15
Chicken erythrocytes	Phosphate	0.13 μ	6.9	300	80	19	15
Glycyl-alanine (Tritium-labeled)	Acetate	0.02 M	3.9	180	4	45	17
β-Galactosidase	Pyrophosphate	0.025 M	8.5	370	11	16¼	18
Bacterial RNA	Tris	0.015 μ	7.6	5.5V/cm	—	15	19
Staphylococcal cell extracts	Tris	0.05 M	7.6	600	—	4½	26
Chymotrypsinogen	Cacodylate	0.1 μ	6.6	3.2V/cm	—	65	27
α-Corticotropin	Acetate	0.1 μ	4.0; 5.2	200	—	24	28
	Cacodylate	0.1 μ	6.6; 7		—		28
	Barbital	0.1 μ	8.3		—		28
Inorganic anions	Acetate	—	7	300	5–15	1	20
Inorganic cations	Lactate	—	3.5; 6.5	800	10–30	½	20
	Phthalate	—	4.5				20
Thyroid stimulating hormone	Phosphate	0.1 μ	7.4	300–600	30–50	16–22	31
^{14}C-labeled plasma protein	Barbital	—	—	400–500	24–32	24–36	37
Diphteria antitoxin	Barbital	0.1 μ	8.6	500	50–60	18–20	38, 3
Follicle stimulating hormone	Acetate	0.1 μ	4	440	10	20	40
Albumin, α-globulin	Barbital	—	8.6	112	27	36	43
Prolactine S. acetamide	Carbonate	0.1 M	11.2	3.5V/cm	—	48	46
Mammalian tyrosinase	Barbital	0.1 μ	8.5	300	11	19	48
Trypsin	Acetate	0.1 M	4	300	—	10	49
Ribonucleoproteins	Phosphate	0.1 μ	9	350–435	—	5¾	50
	Phosphate	0.08 μ	8.2	150	—	12	50
Proteins from microsomes	Phosphate	1.5 M	7	225	11–13	12	53
Proteins from mitochondria	Phosphate	0.05 M	7.4	6V/cm	20	16–18	55

6. *Influence of temperature*

When heat-labile substances are under investigation effective cooling often becomes a serious problem. Control experiments carried out in our laboratory showed a heat effect as indicated in Fig. 3. Working in a cold room without further precautions is not sufficient in itself. The rise of temperature, illustrated in Fig. 3, may be restricted

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to 3° if the temperature of the cold room is kept at —4° during electrophoresis. According to PAIGEN[12] 10 watt over a 24 hours period may be applied if the block is cooled with circulating ice water. The use of a water-jacketed box is then necessary. ROTMAN AND SPIEGELMAN[18] obtained efficient cooling by having the trough in contact both from above and below with lead bricks such as are commonly used for radioactive shielding.

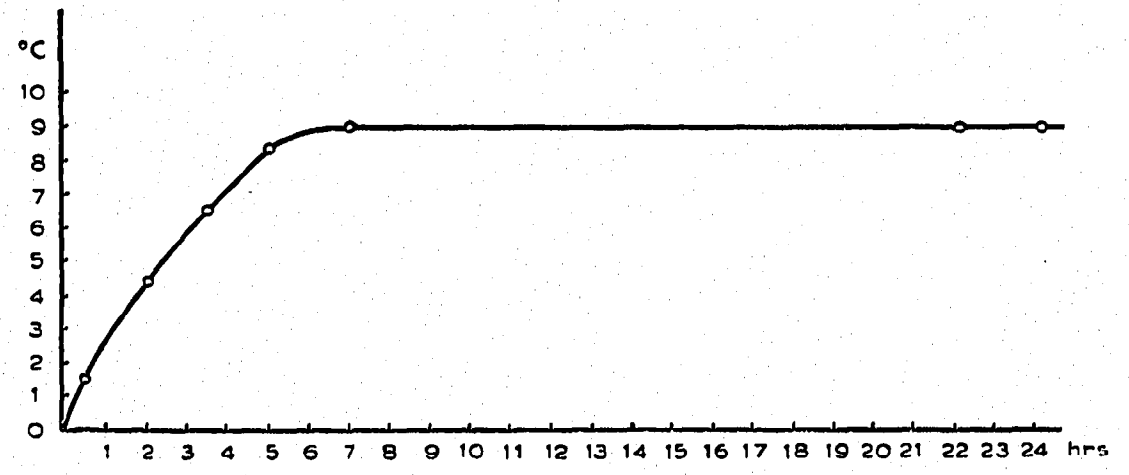

Fig. 3. Rise of temperature in a 40 × 2 × 4 cm starch block during electrophoresis at 200 V, 6–8 mA; in phosphate buffer 0.025 μ. Temperature of the cold room: 2° C.

7. Location of substance

After the electrophoretic run, the paraffin layer, the parafilm or perspex cover is carefully removed and a strip of Whatman paper is pressed against the starch surface. It is dried for 5 minutes at 80–100°. In case starch particles adhere to the strip they are removed with a soft brush. The strip then is coloured with Amido Black or any other protein dye. In this manner one is able to find the approximate position of protein components which is important mainly for preparative work when sectioning of the whole block is not necessary. The position of nucleic acids is determined by observation of a "print" on Whatman 3MM filter paper under ultraviolet light at 260 mμ[19]; protein, however, will interfere. Radioactive substances may be detected by examination of the paper under a strip counter. Examination of the starch block under ultraviolet light may also be useful when the material shows fluorescence. The most exact location of components is realized by cutting the block, transferring the starch segments to centrifuge tubes, followed by elution and protein estimation in the supernatant after short centrifugation. Settling of the starch without centrifugation is not advisable on account of possible interference of very small particles with protein or RNA determination. The run of sera can be followed by mixing them with a quantity of stain which is bound to the albumin fraction[16].

8. Cutting

CARLSON[3] already noted that it is difficult to cut the starch with precision. According to our experience errors of 10–20% may arise when segments of 0.5 cm are cut in the way originally described by KUNKEL AND SLATER[2]. The method of PAIGEN[12] too gives

results of insufficient exactness when proteins, the mobilities of which differ only little, are under investigation.

In order to circumvent these troubles a calibrated box permitting more exact cutting was originally used by us[8]. A guillotine-like frame (Fig. 4, a) is moved over this box. The frame holds a stainless steel knife with the proportions of the cross-section

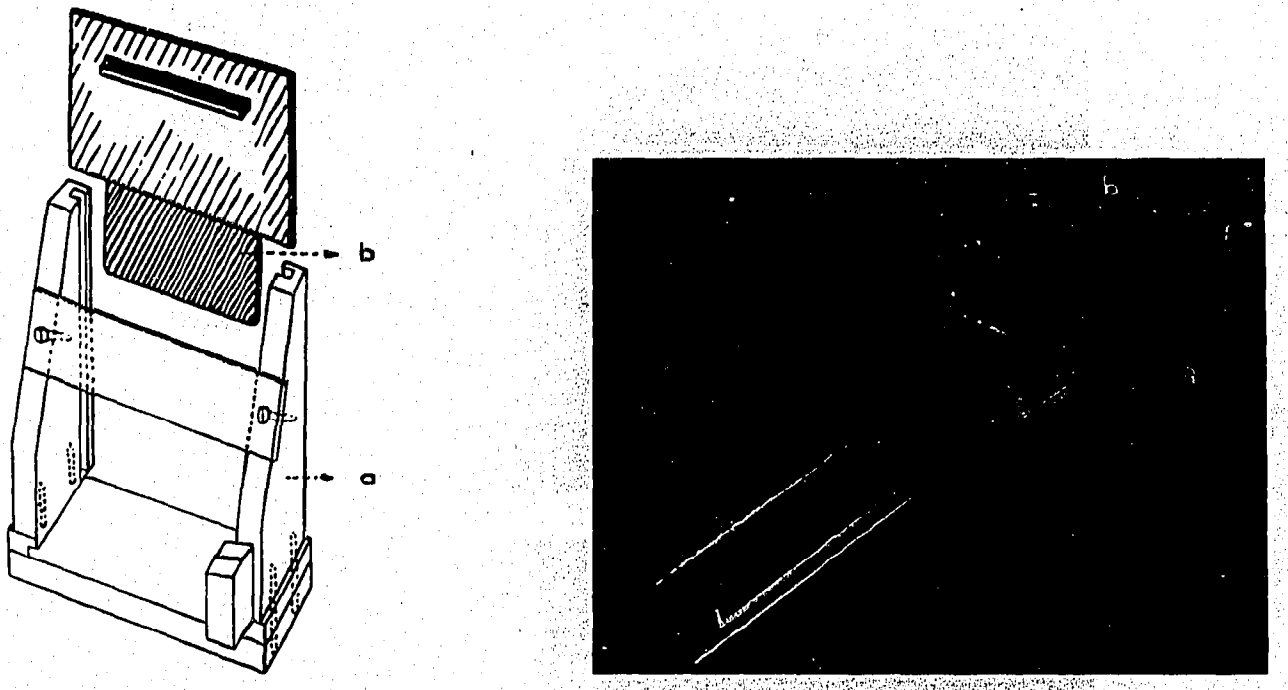

Fig. 4. Cutting device for starch block electrophoresis. (a) Perspex frame. (b) Stainless steel knife.

of the starch block (Fig. 4, b). Although this method improves the slicing technique considerably, small distortions of the starch block may occur which result in poorly reproducible patterns after protein estimation. Furthermore, this procedure is time-consuming, particularly when long boxes are used. Substantial improvement is obtained with the apparatus shown in Figs. 5A and 5B.

A perspex box with removable side walls (Fig. 5, a) fits in a cutting device illustrated in Fig. 5, b. The latter consists of a steel frame joined to a metal plate by means of hinges. Thin stainless steel threads are spanned on the frame at 1 cm intervals[20].

After an electrophoretic experiment is finished the two side walls of the box are removed and the remaining bottom of the box with the starch block is placed in a fixed position on the metal plate of the cutting apparatus. The frame is turned down with the aid of handles (Fig. 5, c). The bottom of the box is supplied with notches at 1 cm intervals into which the threads fit. Cutting of the block is performed in one single manipulation and exactly equal starch segments are obtained.

9. *Elution, protein and nucleic acid determination*

The separate starch segments are eluted with ice cold water or buffer solution by stirring and centrifugation during 10 minutes. To obtain optimal recovery the follow-

ing method is recommended. Each starch segment is put on a narrow-stemmed glass filter. Buffer solution added above the packed starch displaces the fluid in the segments by slight suction[14,37]. It has, however, to be considered beforehand whether the protein will not be denatured on the filter surface. The eluates can be concentrated by

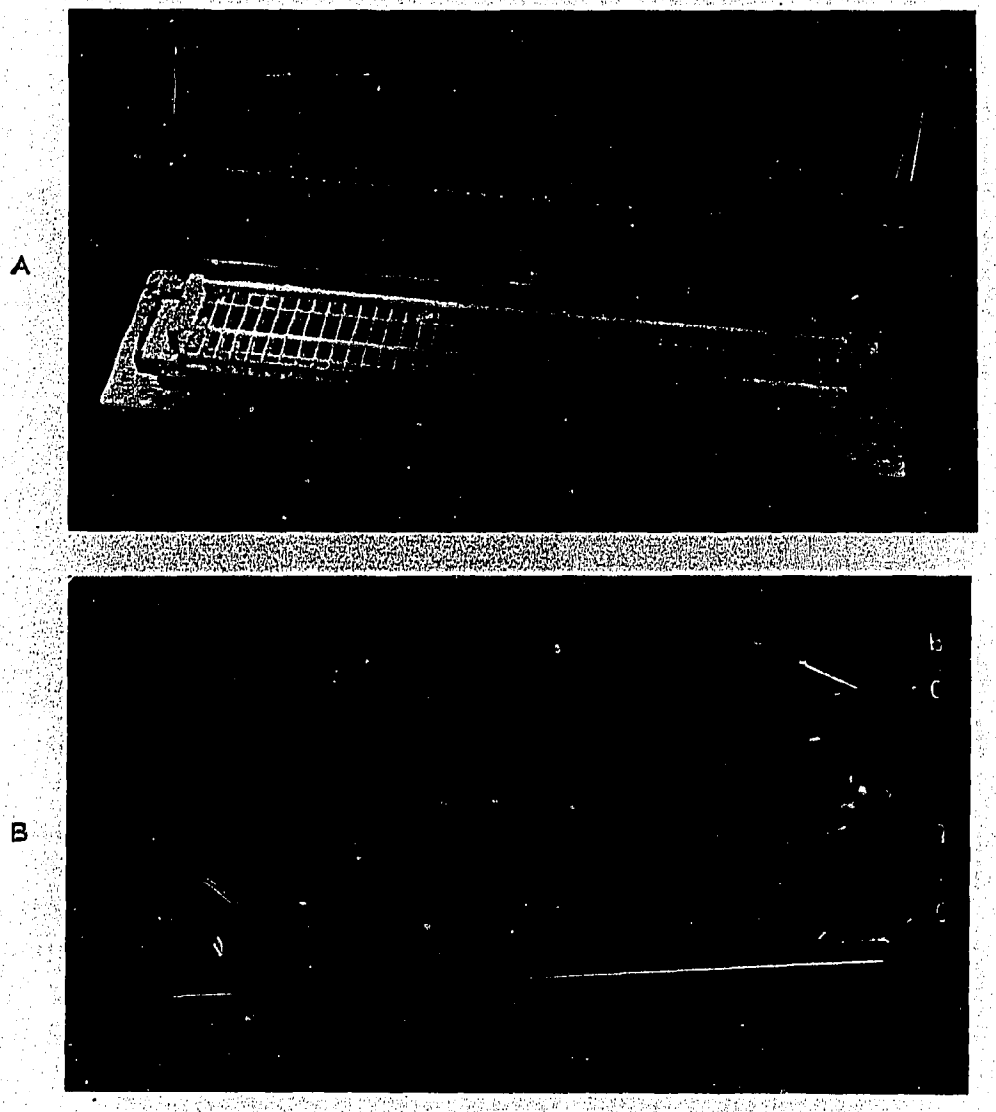

Fig. 5. [A]. Improved cutting apparatus for starch block electrophoresis. [B]. (a) Calibrated box with removable side walls. (b) Cutting device. (c) Handles.

dialyzing against dextran solution or polyvinyl pyrrolidone[21] or by freeze drying. An aliquot of the supernatant fluid is taken from each tube and the protein content is determined according to the method of FOLIN as modified by LOWRY *et al.*[22]. Also direct measurement of the protein extinction at 280 mμ is possible if the solution is completely clear. Direct measurement of nucleic acid contents may be carried out at 260 mμ, but protein or starch traces interfere. Ribonucleic acid and desoxyribonucleic acid are conveniently estimated according to the orcinol[23] and indole[24] methods, respectively. In the case of radioactive material samples of the eluate may

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be counted in a Geiger-Müller or Flow counter[25,26]. (For hormone and enzymatic activity measurements compare the references discussed in the paragraph dealing with applications, p. 131.)

10. Influence of electrosmosis

In some experiments poor separations are obtained due to a horizontal arrangement of the starch block, or when the buffer solution levels in the electrode vessels are of equal height. Under these circumstances the electrosmotic flow towards the cathode counteracts the electrophoretic migration towards the anode. The starch acquires a negative charge in relation to the buffer, since it is the stationary phase and the buffer moves towards the negative electrode. Dextran, a well known substance

TABLE II

Medium	$\mu_{el} \times 10^5$	d_{el}/d_{alb}
Filter paper (Whatman 3 MM)	1.5	0.30
Potato starch	2.5	0.62
Washed sea sand	4.3	1.95
Ground glass (35– 60 mesh)	5.1	3.64
Ground glass (150–200 mesh)	5.9	9.87
Soft glass bead (200 mesh)	5.6	6.20
1% Agar	4.7	2.60

for estimating the degree of electrosmosis in paper[13] is not conveniently detected in starch[27]. Table II shows the electrosmotic flow for various media expressed in terms of mobility and in relation to the distance of migration of albumin (in barbital buffer 0.1 μ; pH 8.6) as observed by KUNKEL AND SLATER[2]. It appears that electrophoresis on potato starch (with the exception of filter paper) undergoes the smallest influence of the electrosmosis phenomenon.

For the determination of electrophoretic mobilities, μ_{el}, in starch it is necessary to know the displacement of the protein due to electrosmosis, d_{el}[28]. One method to study this is running two known proteins in free as well as in starch electrophoresis; d_{el} then may be calculated from equation (1)

$$\frac{\mu_1}{\mu_2} = \frac{d_1 - d_{el}}{d_2 - d_{el}} \qquad (1)$$

wherein

μ_1 = the mobility of protein 1 in free electrophoresis at the same pH applied in starch electrophoresis,

μ_2 = the mobility of protein 2 in free electrophoresis at the same pH applied in starch electrophoresis,

d_1 = the distance over which protein 1 migrates in the starch block,

d_2 = the distance over which protein 2 migrates in the starch block (under identical conditions of time and field strengths).

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μ_{el} may be computed from the following equations (2) and (3):

$$F = \frac{V}{l} \tag{2}$$

$$\mu_{el} = \frac{d_{el} \cdot l}{Vt} = \frac{d_{el}}{Ft} \tag{3}$$

wherein

l = length of trough (cm), F = field strength, V = average voltage across the ends of the box, t = time (sec).

When the isoelectric point of a protein has to be determined, another calculation may be carried out. The unknown protein is subjected to an electrophoretic run, side by side with a protein of known isoelectric point. In this case equation (4) is used:

$$P_x = P_x\,(\text{obs}) - (P_i\,(\text{obs}) - P_i) \tag{4}$$

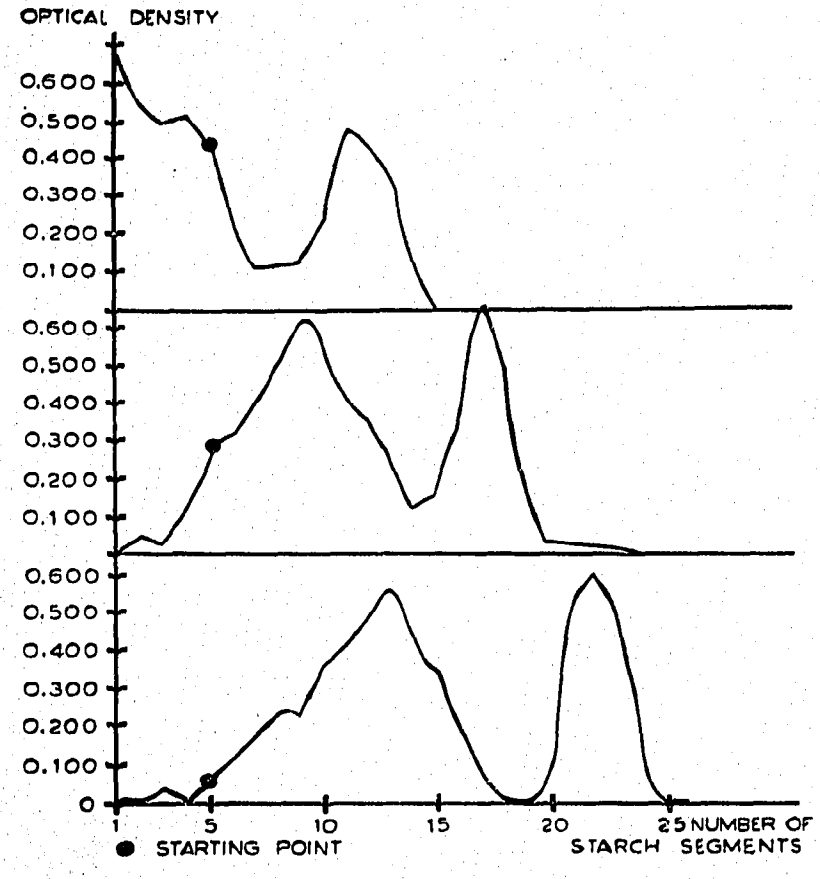
wherein

P_x = the unknown isoelectric point,

P_i = the isoelectric point of the known protein,

P_x(obs) and P_i(obs) = isoelectric points of unknown and known protein, respectively, obtained by plotting the distance of migration against pH.

Fig. 6 demonstrates the effect of electrosmosis on the separation of the water-soluble proteins of the bovine eye lens[8].



(a) Horizontal arrangement of the starch block; buffer vessels at equal height.

(b) Horizontal arrangement of the starch block; difference in levels of buffer = 20 cm.

(c) Vertical arrangement of the starch block; difference in levels of buffer = 20 cm.

Fig. 6. The influence of electrosmosis on the separation of water-soluble lens proteins.

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APPLICATIONS

As appears from Table I starch block electrophoresis is applied for the separation of various proteins, hormones, enzymes and nucleic acids. Furthermore, attempts have been made to submit inorganic ions to this separation technique[29]. It seems, however, that until now paper or column chromatography give better results for inorganic ions. In addition to the separation and isolation of certain compounds from mixtures the method is repeatedly used as a homogeneity criterion.

(*a*) *Proteins and protein hormones.* The α and β-lipoproteins were readily separated and could be followed by phospholipid and cholesterol analysis on the starch segments. No success was achieved in an attempt at direct staining. Further subdivision of these lipoproteins was obtained with low ionic strength buffers[1,2].

BLOEMENDAL[8] separated α-crystalline from the other water-soluble proteins. This protein migrated as a single boundary when subjected to free electrophoresis at different pH's above and below its isoelectric point[30].

FØNSS-BECH AND LI[11] studied the behavior of somatotropin isolated from the anterior lobe of ox pituitaries. These authors showed that their preparation migrated as a single zone at different pH's. Furthermore, they were able to estimate the isoelectric point.

RAACKE AND LI[28] described the determination of corrected electrophoretic mobilities of different proteins. In their procedure the isoelectric point of corticotropin was found to be 6.6 in monovalent buffers.

POSTEL[31] fractionated serum and pituitary preparations. The uptake of ^{131}I by the chick thyroid gland was used as indicator of TSH activity. The thyroid-stimulating activity of a commercial "pure" TSH preparation appeared to be associated with the protein peak, and was transported primarily with the γ-globulins of human serum when added *in vitro*.

KUNKEL AND WALLENIUS[32] succeeded in showing a new hemoglobin in normal adult blood. This component did not appear clearly in free electrophoresis.

GERALD, COOK AND DIAMOND[33] identified normal oxyhemoglobin and hemoglobin M in a hemolysate obtained from a patient affected with methemoglobinemia.

KUNKEL, TAYLOR AND DU VIGNEAUD[34] were able to carry out a biological assay of an oxytocin fraction isolated after starch block electrophoresis.

MÜLLER-EBERHARD AND KUNKEL[35,36] described a subfractionation of human γ-globulin. The experiments were partially performed in polyvinylchloride as supporting medium. This material is more suitable for carbohydrate analysis, as starch is less satisfactory on account of contamination of carbohydrate derived from the medium.

MILLER AND BALE[37] applied the starch block technique in order to separate ^{14}C-labeled plasma proteins produced by the normal rat and by the isolated perfused liver. The latter incorporated radioactive lysine into the plasma albumin, α-globulin, β-globulin and fibrinogen.

KUHNS[38,39] separated precipitating and non-precipitating skin sensitizing diphteria antitoxin.

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Concentration of FSH activity was achieved by means of starch electrophoresis[40]. Two fractions were obtained representing 42 and 23%, respectively, of the total protein applied. According to the author the low recovery was due to losses of dialyzable material in one of the fractions.

The utility of the described method as purification technique in combination with other isolation procedures was shown by LI and coworkers[41]. They succeeded in obtaining a peptide with high ACTH activity from sheep pituitary. The whole isolation and purification scheme involved: dioxan fractionation, starch electrophoresis, column chromatography and counter current distribution. The same laboratory reported the purification of interstitial cell-stimulating hormone[42]. This hormone was subjected to saline extraction, ethanol precipitation, ammonium sulphate fractionation, column chromatography and zone electrophoresis on starch.

LARSON *et al.*[25] identified thyroxin radiochromatographically in a globulin fraction from serum which was separated by means of zone electrophoresis on starch.

GOLDSWORTHY AND VOLWILER[43] studied the mechanism of protein turnover with labeled plasma proteins of the dog. The starch block technique was applied in combination with the COHN fractionation method[44,45] in order to obtain γ-globulin, fibrinogen, α-globulin and albumin.

Preparations of reduced lactogenic hormone when submitted to zone electrophoresis on starch[46] showed only one peak. The mobility of the reduced hormone appeared to be higher than that of the native prolactin.

SORKIN *et al.*[9] applied a zone transfer technique from starch into glass powder. These investigators are among the few to obtain poor yields with starch block electrophoresis.

(*b*) *Enzymes.* WETTER[47] fractionated proteases from *Mortierella renispora* Dixon-Stewart. Nearly quantitative recovery was obtained for both the activity and nitrogen content. When the separation was performed by means of Whatman No. 3 paper, the yields were extremely poor.

HARRIS AND MEHL[10] achieved approximately 200-fold purification of a crude preparation of alkaline phosphatase from bovine intestinal mucosa. However, the amounts of purified product were only small. The results of these authors indicate that the enzyme may have been present initially in a complexed form and that this complex was disrupted during sequential electrophoretic runs in several starch blocks.

Efforts to remove protein impurities present in chromatographically prepared mammalian tyrosinase did not result in further purification. Approximately 99% of the activity was recovered[48].

LIENER AND VISWANATA[49] separated autolysed trypsine into an active fast moving component and a less active fraction which corresponded to dialyzable split products.

ROTMAN AND SPIEGELMAN[18] investigated extracts of *Escherichia coli* for β-galactosidase activity. One single run could effect a 30-fold purification whereas 80% of the available enzyme could be recovered.

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(*c*) *Ribonucleoproteins and nucleic acids.* ELSON[50] subjected ribonucleoproteins to zone electrophoresis on starch before and after treatment with urea. According to his findings there are indications that hydrogen bonds play an important role in the linkage of RNA with the protein.

PAIGEN[12] applied the method to tobacco mosaic virus strains. Separation into three components was possible.

PARDEE, PAIGEN AND PRESTIDGE[16] studied the electrophoretic behavior of RNA, DNA and protein from *Escherichia coli* and *Bacillus megatheria*. They were able to show that bacterial RNA migrates only in two distincts zone. The electrophoretic patterns obtained depended on the buffers employed, a phenomenon which is also well known in paper electrophoresis[51,52]. Approximately 90% of the nucleic acid and 70% of the protein were recovered.

(*d*) *Proteins from cell fractions.* Lately, starch block electrophoresis is being used also for the study of subcellular fractions. GRANICK AND MAUZERALL[15] described the fractionation of a soluble enzyme fraction from red cells. They observed one main component migrating towards the cathode, which appeared to be hemoglobin, whereas three colorless enzyme fractions moved towards the positive electrode. A total of 30 ml fluid could be fractionated.

ARCOS AND ARCOS[53] made an attempt at detecting structural alterations in liver microsomes during chemical carcinogenesis. Swelling had been used to detect these alterations[54]. The swelling of microsomes from liver tissue was paralelled by the release of a group of soluble proteins not sedimenting at 105,000 *g*. These proteins were resolved into two fractions on starch. Microsomes from hepatoma on the contrary showed low swelling with relatively high protein release. These investigators did not include the proteins of hepatoma in their electrophoretic studies.

WIRTZ AND ARCOS[61] made a comparative study of the electrophoretic pattern of rat liver and hepatoma supernatant fluids. The so-called protein "h" was isolated and its molecular weight, amino acid composition, and bound azo-dye were determined.

BERESOVSKAYA[55] isolated an enzyme from mitochondria which catalyzed the amino acid synthesis from pyruvic acid and ammonia. The active protein was present in a fast migrating fraction which had a higher mobility than had serum albumin. The electrophoretic separation resulted in a 300–350 fold purification of the enzyme as compared with the original liver homogenate.

GALE and coworkers[26] isolated a fast moving fraction from extracts of disrupted staphylococcal cells which were previously incubated with ^{14}C-L-glutamic acid as the only labeled amino acid precursor. The authors provide evidence that this fraction represents an intermediate stage in protein synthesis. If this is so the L-glutamic acid containing intermediate should be very stable as the heat development in a starch block under the conditions of their experiment (20 V per cm, pH 7.6) may be considerable.

BLOEMENDAL AND BOSCH[62] fractionated the ^{14}C-leucine labeled pH5 enzyme from rat liver cytoplasma. An important working condition was air-cooling of the starch block to —5°.

References p. 134/135.

(*e*) *Homogeneity studies.* RAACKE[27,28,56] used starch electrophoresis for homogeneity studies of ribonuclease, lysozyme, bovine serum albumin, chymotrypsinogen and chymotrypsin. All preparations investigated yielded more than one peak after variation of buffers and the application of a pH-range from 4.7 to 9.1. These results emphasized once more the importance of pH variation. On the other hand one has to realize whether the peaks observed are a proof of heterogeneity or that the substance under investigation was no longer stable at any "extreme" pH in the alkaline or acid range. Such stability boundaries were observed *e.g.* in the case of the lens protein α-crystalline below pH 4 and above pH 8.9[57]. According to RAACKE starch electrophoretical homogeneity experiments may be considered to be as sensitive as the reversible boundary spreading test[58] and superior to the moving boundary method.

WADA *et al.*[59] concluded to the homogeneity of soy bean hemagglutinin after finding one peak at one pH. In this connection it must be mentioned that a number of workers are as yet satisfied with one single pH value. The conclusion reached then can only be that the protein is *electrophoretically* homogeneous at *this* pH.

CONCLUSION

Starch block electrophoresis is of growing importance as a useful tool in protein chemistry for analytical studies as well as for isolation on preparative scale. However, reproducible results will be obtained only if the working methods are exactly standardized. In a few cases polyvinylchloride as a supporting medium may be more satisfactory than starch; working conditions, however, remain identical.

Poor separation may sometimes be the result of horizontal arrangement of the troughs. Gravity then may cause accumulation of protein in the lower starch layers, leading to uneven mobilities at different levels of the block. Furthermore, the electrosmotic flow may counteract the electrophoretic migration. Vertical position of the starch block is therefore preferable.

In summary, the starch block technique opens up the possibility for separation of various protein mixtures; it is often successfully applied in combination with other isolation methods and, in some cases, gives good results where other fractionation procedures fail.

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