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A TECHNIQUE FOR ELUTION OF PROTEINS FROM STARCH GEL

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SUMMARY

A technique for recovery of separated plasma proteins after electrophoresis in starch gel is described. By means of a second electrophoresis applied to sections which have been cut from the main gel almost quantitative recoveries of these proteins, except for γ-globulin were obtained. The trace amounts of protein originating from the gel itself under these conditions were measured. Considerable amounts of starch are eluted at the same time but do not interfere with the estimation of the plasma proteins.

INTRODUCTION

With the increasing use of electrophoresis in gels the need has become apparent for a simple and quantitative method for recovery of the separated materials. A technique involving lifting an agar gel on to a cellophane membrane placed so as to isolate pools of buffer at anode and cathode ends of the slab followed by a period of elution by electrophoresis into these pools has been described[1]. Since the introduction of electrophoresis in starch gel by SMITHIES[2], several methods for elution from such gels have been worked out. That of JARRIGE AND LAFOSCADE[3] depends on cutting a slot in the gel in front of the band of material to be eluted, filling this with buffer and cotton wool and eluting by electrophoresis into the buffer. MORETTI,

BOUSSIER AND JAYLE[4] on the other hand, preferred to cut out the piece of gel containing the material under investigation and elute from it after fragmentation. Since the SMITHIES[2] method for electrophoresis of plasma proteins is now in very wide use, a simpler apparatus than that used by MORETTI, BOUSSIER AND JAYLE has been designed. Using this apparatus conditions for recovery in good yield of all the proteins present in rat serum, with the exception of γ-globulin, have been worked out.

The principle of the new apparatus is that the piece of gel to be eluted has placed on its upper surface a vertical rectangular slab of the same gel containing only dilute buffer solution. This upper slab of gel has the same cross sectional area as the piece

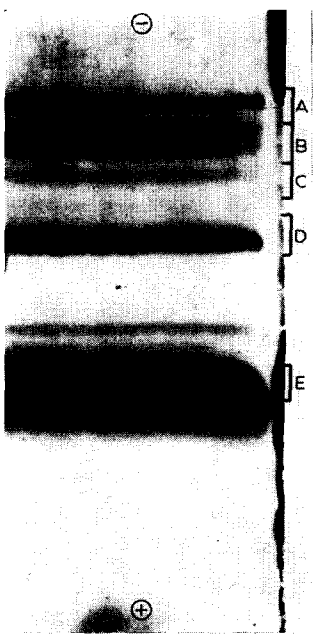

Fig. 1. Pattern of plasma proteins obtained by electrophoresis of 0.15 ml of rat serum for 20 h at 4.0 V/cm.

of gel to be eluted and, because of its excellent mechanical properties, does not require any support. Passage of current through the gel causes migration of protein from the bottom section into a small volume of buffer solution placed immediately below it. This buffer solution is separated from a large volume of the same solution by means of a cellophane membrane and the eluted protein is thus trapped in the small volume. During elution the gel rests in the upper part of a vertical channel cut through three rectangular pieces of perspex (Fig. 2A) which are screwed tightly together to form a single unit. Support for the gel is provided by several narrow polythene bridges stretching across the channel and forming part of a sheet of this material which is held between the two uppermost pieces of perspex. The upper perspex rectangle is of the same thickness as is the gel to be eluted. The thickness of the middle section (6 mm in the present apparatus) determines the volume of buffer into which the protein will migrate. The cellophane membrane is held between the

two lower-most pieces of perspex. As shown in Fig. 2B cross bars are screwed to the top of the perspex rectangles so that the unit can rest on the top of a large filtrate jar. Contact from the top surface of the upper slab of gel is made with a horizontal slab of the same material which in turn is kept in electrical contact with the buffer solution in the cathode vessel by means of wet folded muslin.

In preliminary experiments 0.02 *M* borate buffer was used both in the gel above the section being eluted and for the solution in the electrode vessels. The same buffer

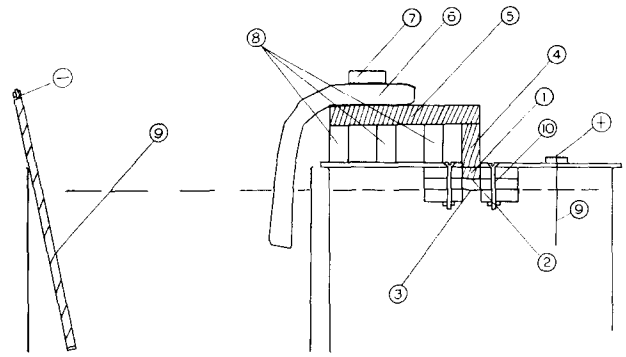

Fig. 2a.

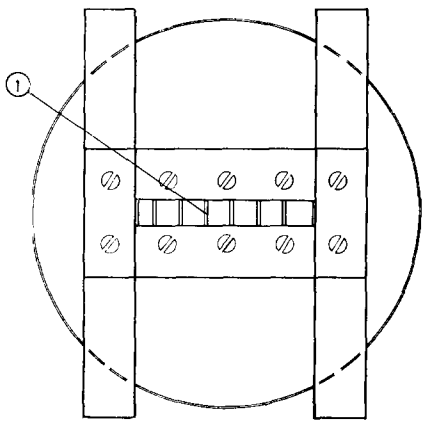

Fig. 2b.

Fig. 2. A. Complete apparatus with elution cell resting on top of a 3-l filtrate jar. Only the upper part of this is shown. 1. Section of gel being eluted. 2. Polythene bridges. 3. Cellophane membrane. 4. Vertical rectangular slab of starch gel (starch pillar). 5. Horizontal slab of starch gel. 6. Wet folded muslin. 7. Glass weight. 8. Perspex supports for gel bridge. 9. Platinum electrodes. 10. Brass screws. B. Plan of elution cell showing polythene bridges (1) which support the piece of gel during elution.

had been used for the original electrophoresis and it was thought likely that the gel would best retain its shape if there was no discontinuity either in the type of buffer ion present or in concentration. In practice, however, a sharp fall in pH occurred at the buffer–gel interface (the lower surface of the piece of gel being eluted). As the region of decreased pH moved upwards the gel became distorted. Furthermore, since the fall of pH was of several units, very poor recoveries of protein were obtained. Fortunately, however, no effect of this kind took place when phosphate ions were used instead of borate. The reason for the decrease in pH of part of the gel which occurs in presence of borate ions is not understood but may be connected with the ability of these ions to complex with starch. Satisfactory recoveries were obtained with 0.02 *M* phosphate buffer at pH 8.3 in the gel bridge and in the electrode vessels. The small amount of borate ions in the section of gel being eluted, compared with the

total amount of phosphate ions in the rest of the gel, is insufficient to prevent an adequate degree of elution. At 300 V the current of 58 mA caused only slight warming of the gel bridge. During electrophoresis the volume of solution into which the protein was being eluted was found to increase slightly. To prevent overflow small amounts were occasionally sucked off from the angle between the upper slab of gel and the surface of the perspex rectangle and kept for estimation with the final eluate. The proportions of protein eluted under these conditions from various sections of the gel are shown in Table I. Fig. 1 shows the pattern obtained by electrophoresis of 0.15 ml of rat serum and the positions from which the sections of gel used for elution were taken. The conditions for the primary electrophoresis were those given by SMITHIES[2] except that the time was longer and the voltage gradient was lower (*cf.* Fig. 1).

In order to assess the degree to which proteins eluted as just described are contaminated with protein and soluble starch originating from the gel, a blank experiment was carried out. This consisted of the electrophoresis of a slab of starch

TABLE I

PROPORTIONS OF ^{131}I-LABELLED PLASMA PROTEINS RECOVERED FROM STARCH GEL

For conditions of elution see text. Eluates were removed from the cell with a Pasteur pipette. Radioactivity of final gel plus eluate is taken as 100 %.

Section of Starch		*% radioactivity in eluate after*	
	cm from origin	*1 h*	*2 h*
A	2.5–3.5	23	70
B	3.5–4.5	31	86
C	4.5–5.5	64	96
D	6.0–7.0	96	98
E	10.0–11.0	95	98

7 cm wide and 0.65 cm deep to which nothing had been added, elution of appropriate sections, and examination of the eluates thus obtained for protein and dry weight. Before analysis the eluates were dialysed for 48 h against water. Subsequent evaporation to dryness revealed dry weight corresponding to just under 30 mg/cm of gel, or approximately 5 % of the starch originally present. Whether some of this quite considerable quantity of starch came from the gel bridge rather than from the actual piece of gel undergoing elution was next investigated. For this purpose an elution was done with a rectangle of cellophane membrane interposed between the section being treated and the starch pillar resting on it. Under these conditions the proportion of soluble starch in the eluate dropped to 2.5 %. The amounts of material reacting as protein by the method of LOWRY *et al.*[5] in eluates made from the blank gel, with and without the interposed cellophane, were also estimated. No effect due to the cellophane could, however, be detected since both eluates were found to contain just under 50 μg protein.

In another experiment 0.15 ml of rat plasma was applied to the gel. Elution of a section corresponding to cut D in Fig. 1 which was at the same distance from the start as had been the blank section above, gave 950 μg protein by the method of LOWRY *et al.*[5] before correction for the blank. Since in this case the blank value to be subtracted was only just over 5 % of the total protein eluted, correction can evidently be made without noticeable loss of accuracy.

Since, however, cut D in Fig. 1 contains one of the stronger bands, larger amounts of plasma would need to be applied to the gel for accurate estimations in this way of the weaker bands. Fortunately, however, certain estimations such as immunological tests in gels[6] or scintillation counting for radioactivity are unaffected both by the considerable quantities of soluble starch or the much lower amounts of protein derived from the gel which are present in the eluates made as described above. If necessary soluble starch can be eliminated from the eluate by chromatography on Dowex-2 by the method of DE PAILLERETS *et al.*[7].

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D-THREOSE 2,4-DIPHOSPHATE INHIBITION OF 3-PHOSPHOGLYCERIC ACID PHOTOREDUCTION BY A SONICALLY RUPTURED SPINACH CHLOROPLAST SYSTEM

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SUMMARY

1. [1-^{14}C]PGA is readily reduced by a sonically ruptured chloroplast system in light. This reduction is inhibited by threose 2,4-diphosphate.

2. PGA accumulates when $^{14}CO_2$ is incubated in light with the sonically ruptured chloroplast system in the presence of threose 2, 4-diphosphate. This indicates that PGA is in the major pathway of photosynthetic CO_2 fixation in the system.

3. Threose diphosphate inhibition of the total $^{14}CO_2$ fixation by the carbon cycle of sonically ruptured spinach chloroplasts is not due to inhibition of carboxydismutase, but due primarily to inhibition of triose phosphate dehydrogenase which in turn limits the formation rate of the photosynthetic carbon cycle CO_2 acceptor, RuDP.

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