Improved protein resolution of concentrated tissue extract in zonal column electrophoresis

Zonal electrophoresis in vertical columns of ethanolyzed cellulose[1] has been developed by PORATH[2–5] into a versatile and highly useful preparative and analytical procedure.

Investigations on the soluble proteins of rat liver[6,7] have extended into column electrophoretic studies, begun by one of us at the Institute of Biochemistry, Uppsala University, Sweden. Isolation and analysis of all principal components therein[6,7] require use of extracts of high protein concentrations and viscosities. Introduction of such samples 15 to 25 cm into the column, in allowance for electroosmosis, results in broad and irregular starting zones and consequently inferior protein resolution. PORATH[3] devised a sectional column permitting introduction of viscous samples into the middle of a column. This provided only preliminary fractionation for further efficient electrophoretic resolution. This communication deals with a convenient modification of the PORATH technique. In single-stage runs, thus reducing alterations attending dilution and concentration of labile proteins, it provides improvement in resolution of concentrated extracts and certain technical advantages.

Two pools of soluble proteins of livers of rats fed the hepatocarcinogen, 3'-methyl-4-dimethylaminoazobenzene for 2.5–3 weeks were prepared[7,8]. Each was divided into equal portions, dialyzed against 0.02 I sodium veronal + 0.03 I NaCl, pH 8.6, and made to a concentration close to 10.0 % protein. 7.5 ml were introduced at 6–25 ml/h into a PORATH column of ethanolyzed cellulose, 225 × 3.4 cm, water-cooled peripherally at 1–2° and previously equilibrated with the same buffer (Fig. 1).

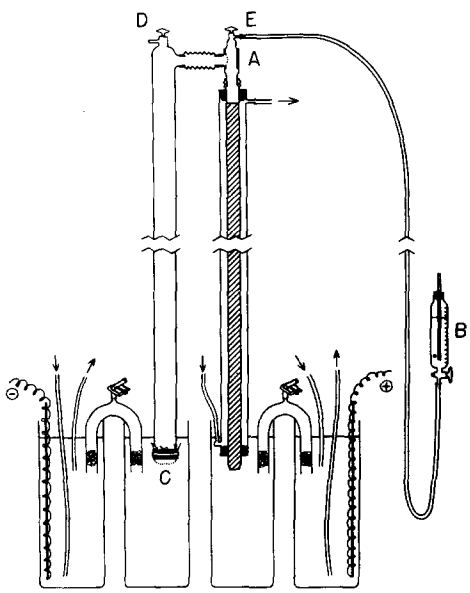

Fig. 1.

Electrophoresis without semi-permeable membrane

To allow for electroosmosis[2,9], the leading boundary of the protein zone was introduced 10.4 cm (minimal) into the column. The visible zone was 3.2–5.0 cm long. Subsequently, electrophoresis was performed with 90 mA passing through the cellulose circuit. Products of electrolysis were in part neutralized by the continuous peristaltic circulation of electrode buffers. To maintain the protein on the column, despite electroosmotic back-movement, the current was stopped at 20, 40 and 60 h, (A) closed off, and protein was compensated down 12 cm, 12 cm, and 4 cm, respectively, as measured by liquid flow at 22–25 ml/h from a graduated 125 ml Mariotte flask (B). After a total of 80 h of electrophoresis, protein was eluted at 30 ml/h and

collected in 10.0-ml fractions at 7°. Protein was measured in the Beckman DU Spectrophotometer at 284 mμ[10] against veronal chloride buffer. For the evaluation of zone sharpness and resolution, distribution of the dye content of azo protein therein[7,11] was analyzed by the formic acid method of WIRTZ, MILLER AND MILLER[11].

Electrophoresis with membrane-closed column

Similarly, the leading protein boundary was introduced into the same column only 2.5 cm. The visible zone was 1.6 cm long. Two layers of Visking dialysis membrane of regenerated cellulose were stretched over the bottom of the vertical liquid arm;

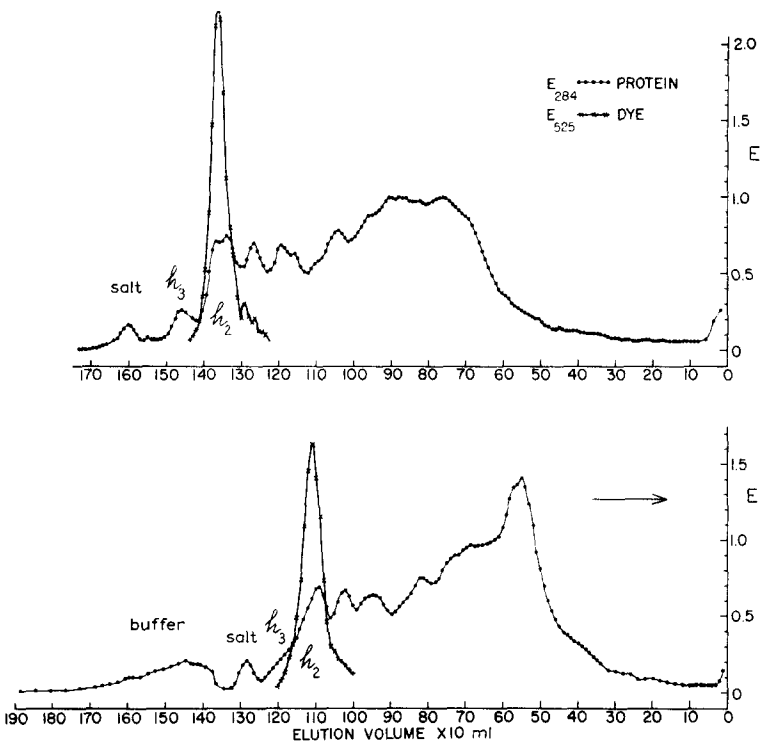

Fig. 2.

overlapping sides were strapped with 0.75 in. rubber splicing tape, over which a stainless-steel worm-drive clamp was firmly fastened. The liquid tube, with the diaphragm at its bottom (C), was filled through the top opening (D). (The membrane must be able to withstand the hydrostatic pressure of the liquid arm without leaking.) A 125-ml Mariotte flask (B), graduated in 1-ml divisions and containing buffer, was attached to the top stopcock (E). The immersed capillary opening was positioned 10 cm above the meniscus of the buffer vessels. With this flask just previously opened to the liquid above the column, the column and vertical liquid arm were joined through glass joint (A). Throughout the ensuing electrophoresis, the flask was positioned for a flow of 2.5 to 3.5 ml/h. Elution and analysis followed as above.

Fig. 2 compares the protein and protein-azo dye separations obtained. Similar

results were realized with both protein pools. Improved resolution in the protein and azoprotein distributions with the membrane-closed column is evident from the sharpness, height, detail and additional peaks obtained (upper pattern). Thus, the h_3 component is considerably better resolved using the present technique than without membrane (lower pattern). The h_2 and other components divide into at least two subcomponents, unlike the latter case. The peak dye content of h_2 azoproteins is considerably taller ($E_{525\ m\mu} = 2.20$) than in the matching run ($E_{525\ m\mu} = 1.63$). Faster minor azoprotein peaks are evident in the former, whereas they merge into an almost smooth leading shoulder in the latter case. With this improved resolution, the peak content of azoproteins has been determined to coincide exactly with the slow subdivision of the h_2 component, the slow h_2 component[7]. Studies[8] utilizing the membrane-closed column are in progress.

The membrane-closed column has additional advantages. (a) Electroosmotic backflow of buffer is greatly reduced. Compared to open-column electrophoresis with discontinuous forward compensations, the sample in the membrane-closed column travels less distance and spreads less therefrom, and is in the column less time. (b) Precise control of compensation may be achieved without use of colored marker substance by the adjustment of the liquid flow rate and amount from the Mariotte flask. (A pump could automatically control flow.) Movement of any component may be thus more accurately controlled or immobilized, in the manner analogous to that of PORATH[2] and GEDIN AND PORATH[9], who compensated during electrophoresis by elevating the buffer level in the vessel here containing (C). Precise control with their technique requires a colored internal marker. (c) Hours required for the movement of protein to the starting position are eliminated. (d) Column and sample are freer from troubles in circulation of buffer. The membrane isolates the liquid in the vertical buffer arm from the flow dynamics in the pumping system.

This work was aided by a grant from the American Cancer Society. The first author expresses his appreciation for the interest, discussions, and kindness extended to him by Professor A. TISELIUS, Dr. J. PORATH, Mr. S. JERSTEDT and colleagues.

Institute for Cancer Research, Philadelphia, Pa. (U.S.A.)

S. SOROF
E. M. YOUNG
M. M. SPENCE
P. L. FETTERMAN

[1] P. FLODIN AND D. KUPKE, *Biochim. Biophys. Acta*, 21 (1956) 368.
[2] J. PORATH, *Biochim. Biophys. Acta*, 22 (1956) 151.
[3] J. PORATH, *Arkiv Kemi*, 10 (1956) No. 38.
[4] J. PORATH, *Arkiv Kemi*, 11 (1957) No. 18.
[5] J. PORATH, Summary of *Doctoral Dissertation*, University of Uppsala, Sweden, 1957.
[6] S. SOROF AND P. P. COHEN, *J. Biol. Chem.*, 190 (1951) 311.
[7] S. SOROF, E. M. YOUNG AND M. G. OTT, *Cancer Research*, 18 (1958) 33.
[8] S. SOROF, M. M. SPENCE, E. M. YOUNG AND P. L. FETTERMAN, *Proc. Am. Assoc. Cancer Research*, 3 (1959) 65 and (1960), in the press.
[9] H. I. GEDIN AND J. PORATH, *Biochim. Biophys. Acta*, 26 (1957) 159.
[10] I. BRATTSTEN, *Arkiv Kemi*, 8 (1955) No. 34.
[11] G. H. WIRTZ, J. A. MILLER AND E. C. MILLER, in H. V. GELBOIN, J. A. MILLER AND E. C. MILLER, *Cancer Research*, 18 (1958) 608.

Received August 18th, 1959