Journal of Chromatography, 172 (1979) 49–56

CHROM. 11,618

VERSATILE INSTRUMENTATION FOR ONE- AND TWO-DIMENSIONAL MICROSCALE POLYACRYLAMIDE GEL ELECTROPHORESIS*

VOLKER ANSELSTETTER**

Institut für Physiologische Chemie der Universität Erlangen-Nürnberg, Wasserturmstrasse 5, D-8520 Erlangen (G.F.R.)

(First received September 26th, 1978; revised manuscript received November 23rd, 1978)

SUMMARY

An all-glass electrophoresis cell for slab gels has been developed. It has excellent separation qualities in polyacrylamide gel electrophoresis. An instrumentation is presented for the use of this cell with various techniques of one- and two-dimensional electrophoresis. Special attention is paid to gradient gel electrophoresis. The features of the instrumentation are discussed in detail and a few examples are given to demonstrate the quality of the electrophoretic separation.

INTRODUCTION

Polyacrylamide gel electrophoresis has proved to be a valuable tool in the investigation of many problems of biochemistry and cell biology and a considerable number of electrophoretic techniques are now available. During our investigations of haematological disorders we found an aberrant pattern of erythrocyte membrane proteins in congenital dyserythropoietic anaemia, type II with our recently developed two-dimensional technique[1,2]. For a deeper insight into the molecular pathology of this disease, however, it is necessary to study erythropoietic cells in culture. As a consequence, an electrophoretic instrumentation has been developed for the rapid analysis of very small amounts of macro-ions.

Since sensitivity can be greatly increased by radioactive labelling, attention has mainly been paid to the design and the dimensions of the electrophoresis cell, to the principle of gel-casting and to the design of the electrophoresis apparatus. The instrumentation described in this paper is routinely used for the investigation of samples with volumes of 1–25 μl. Separations are completed in a maximum of 4 h and slab gels are dried in 1 h for radio-autographic studies.

* A preliminary report was presented at the Joint Autumn Meeting 1977, organized by the Biochemical Societies of the G.F.R., Switzerland and France, held in Freiburg, G.F.R., October 2–5, 1977, and an abstract has appeared in *Hoppe-Seyler's Z. Physiol. Chem.*, 358 (1977) 1174.

** Present address: Zentrum für Klinische Grundlagenforschung der Universität Ulm, Parkstrasse 11, D-7900 Ulm, G.F.R.

MATERIALS

Glass plates and strips for the electrophoresis cell are cut from plates 1.9 and 1.2 mm thick, respectively. Middle spacers are glued to glass plates with an adhesive, based on epoxy resins (UHU plus, endfest 300; Lingner & Fischer, Bühl, G.F.R.). Glass plates and strips are cleaned in a warm (*ca.* 40°) solution of sodium dodecylsulphate (*ca.* 0.5%) by sonication. Appropriate stands are used to ensure that glass parts are not touched by hand during the cleaning procedure. The electrophoresis cells are sealed with the special adhesive tape, tesafilm 108, which is 25 mm wide (Beiersdorf, Hamburg, G.F.R.). All the apparatus is made from Plexiglas (Roehm and Haas, Darmstadt, G.F.R.) plates of various thicknesses (3, 4, 8 and 10 mm) or machined from Plexiglas blocks or tubes. Parts are glued together with Acrifix 92 (Roehm, G.F.R.). Platinum wire is used for the electrodes. Gel chambers are filled by a multichannel pump (Desaga PLG multipurpose peristaltic pump 13 21 00; Desaga, Heidelberg, G.F.R.). Gel slabs are dried with the Model SE 540 slab gel dryer (Hoefer Scientific Instruments, San Francisco, Calif. U.S.A.).

METHODS

Assembly of the electrophoresis cell

The glass plates (150 × 1.9 × 70 mm) and strips (10 × 1.2 × 70 mm) for the assembly of the electrophoresis cell are shown in Fig. 1A. One strip is glued to the plate on the left as middle spacer. To produce the side spacers, the other two strips are placed lengthwise in the middle of the special adhesive tape, their narrow edges facing the sticky surface (Fig. 2A). The tape is then cut flush with the glass strips. The assembly of the electrophoresis cell is pictured in Fig. 2B. The two glass plates are made flush with the glass strip and are placed upright on to the tape of the side spacer at both sides of the glass strip (arrows 1 and 2 in Fig. 2B). The tape is then raised and smoothed against the plates by rotating the assembly on that part of the stand which is covered by foam rubber, as indicated by the curved arrow in Fig. 2B. The sealing of the lower aperture of the cell is shown in the right half of Fig. 2B:

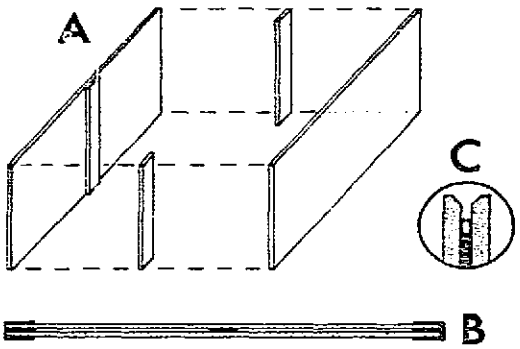

Fig. 1. (A) Exploded diagram of the electrophoresis cell. (B) Top view of the assembled cell (for assembly see Fig. 2). (C) Sagittal section of the electrophoresis cell, upper end (3×). Diagrammatic representation of gel rod and slab gel being in position for two-dimensional electrophoresis. Measurements are given in the text.

the tape comes *ca.* 1.5 cm up both sides. All glass surfaces that later come into contact with gel solution or adhesive tape must be clean and free of grease and therefore must not be touched with fingers during manipulation of the cell assembly.

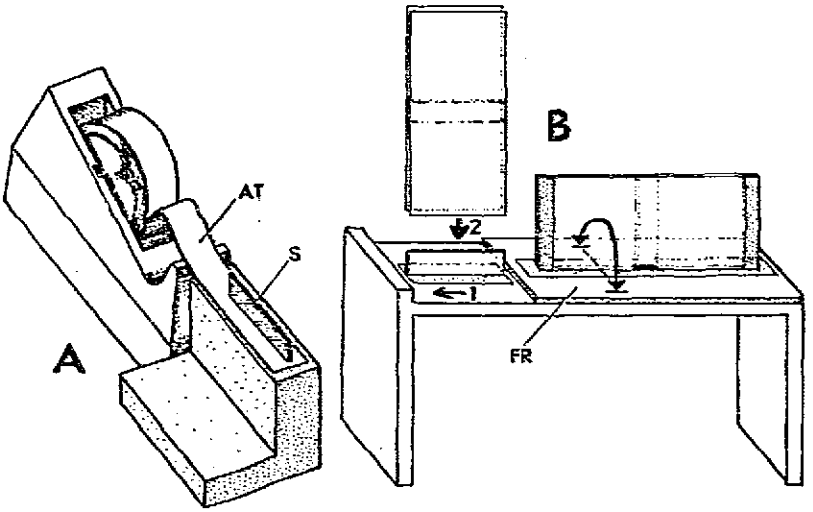

Fig. 2. Assembly of the electrophoresis cell. (A) Preparation of side spacer. (B) Assembly of cell. AT, adhesive tape; S, spacer; FR, foam rubber.

Casting of slab gradient gels

The arrangement for casting linear or exponential gradient gels is outlined in Fig. 3. The electrophoresis cells are put upright into an appropriate stand and are held in place by a wedge. To cast linear gradients the vessels B and C are connected by a piece of silicone tubing which is then closed with a clip. The outlets of the mixing chamber (B) are connected by silicone tubings of equal length to the electrophoresis cells via a multichannel pump (P). The tubings are connected to the cells with small pieces of plastic tubing squeezed into the upper apertures of the cells. A calculated volume of the low concentration gel solution is poured into vessel C and the clip is opened briefly to allow the connecting tube to fill (the volume of the low concentration gel solution exceeds that of the high concentration gel solution by the volume of the connecting tube). The multichannel pump is turned on to pump counterclockwise at minimum speed. Then the respective volume of high concentration solution is poured into vessel B, mixing is started and the clip is removed. Mixing in vessel B is achieved by a magnetic stirrer or by an overhead corkscrew stirrer. The drive direction of the pump is turned to clockwise, the pump still working at minimum speed to achieve synchronous filling of all outlets. Then the speed is raised to complete filling of the cells within 5–10 min. When the gradient solution is just entering the cells the speed should be decreased briefly to avoid the trapping of air bubbles in the corners of the cells. Finally, gradients are overlaid with water at minimum pumping speed. The arrangement of inlet and outlets in vessel B is shown in Fig. 3D. The bottoms of vessels B and C are machined in such a way that round corners and a slight descent towards the central openings are produced.

Vessel C is not needed for casting exponential gradient gels; the central inlet

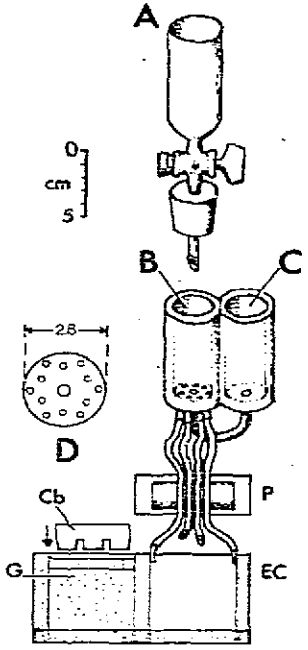

Fig. 3. Arrangement for casting gradient slab gels. A–C, vessels for taking up gel solutions; D, enlarged drawing of the bottom of the mixing vessel B (inserted measurement in cm) with central inlet (I.D. 3 mm) and peripheral outlets (I.D. 1.5 mm); P, multichannel pump; EC, electrophoresis cell, right chamber: filling with gradient solution, left chamber: preparation of sample wells by insertion (↓) of a comb (Cb); G, polymerized gradient gel.

into vessel B is closed by a stopper. A calculated volume of high concentration gel solution is poured into vessel B and mixed by a magnetic stirrer. An airtight connection is made between the burette (A) and the opening of vessel B, the stopcock of the burette being open. The stopcock is closed and the calculated volume of low concentration gel solution is poured into the burette. The stopcock is opened again and the pump is started to fill the electrophoresis cells as described above.

If sample wells are desired on gradient gels, a gel solution is poured on to the polymerized gradients and combs are inserted into this solution until they touch the surface of the gradient gels (see the left gel chamber of the electrophoresis cell in Fig. 3). Cast gels can be sealed in plastic bags and stored in a humid atmosphere (gel buffer) for a few days without loss of quality. Conditions for producing gradients with this equipment are described in the legends to Figs. 5–7. For general calculations of gradients see refs. 3 and 4.

Slab gel electrophoresis

The apparatus for slab gel electrophoresis is shown in Fig. 4 with the electrophoresis cell in place. An appropriate volume of electrophoresis buffer is poured into the lower buffer reservoir so that the bottom of the electrophoresis cell dips into the buffer when the cell is put in place. The upper buffer reservoir is inserted and tightened against the electrophoresis cell with a cord of foam rubber, as outlined in Fig. 4.

Electrophoresis buffer is poured into the upper reservoir until a mark *ca.* 1 cm

above the electrophoresis cell is reached. The electrophoresis buffers in both reservoirs are adjusted to the same level. Samples are loaded on to the gel and the electrophoresis is started. At the end of the run the electrophoresis cell is dismantled and the gels recovered. The electrophoresis buffers are usually discarded. In two-dimensional electrophoresis the first-dimension gel rods are loaded on to the slabs before the upper reservoir is filled with electrophoresis buffer (*cf.* Fig. 1C).

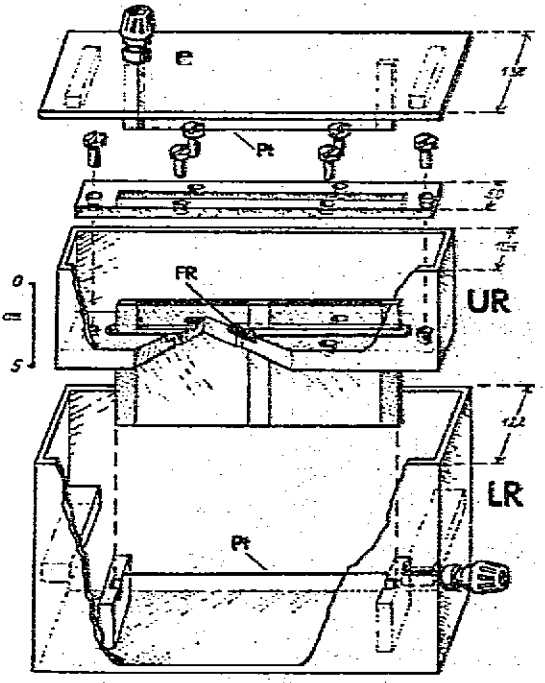

Fig. 4. Perspective view of the apparatus for slab gel electrophoresis. The electrophoresis cell is in place for electrophoresis. FR, foam rubber; Pt, platinum wire; UR, upper buffer reservoir; LR, lower buffer reservoir. Screws are of plastic. Dimensions are in centimeters

Gel rod electrophoresis

Gel rods are cast in disposable glass capillaries (Blaubrand intramark, 200 μl, I.D. 1.5 mm; Brand, Wertheim, G.F.R.) which are usually cut into pieces 40 mm long. For casting, the lower apertures of the capillaries are sealed with Parafilm, and the capillaries are filled with gel solution by means of a syringe equipped with a blunt-ended needle. At the beginning of the filling process the Parafilm is touched by the end of the needle to prevent air bubbles becoming trapped. The gel solution is finally overlaid mostly with water using a fine glass pipette. If appropriate stoppers are used as adapters, electrophoresis or isoelectric focusing can be performed with almost any apparatus available for gel rod electrophoresis. Gels are pushed out of the capillaries by injecting water between the gel and the glass surface using a syringe equipped with a fine needle. For conditions of electrophoresis with this type of capillaries see refs. 5 and 6. An example of isoelectric focusing is described in the legend to Fig. 7.

DISCUSSION

The design and the dimensions of the electrophoresis cell, the principle of gel-casting and the design of the electrophoresis apparatus bring about superior separation qualities and facilitate the performance of a series of electrophoretic techniques.

The electric current is passing absolutely uniformly through the gel. Consequently, individual bands are separated strictly parallel to each other, even if the sample has been spread all over the top of the gel. This indicates that conditions are optimal both for the recovery of separated proteins from gels, *e.g.* by subsequent electrophoretic elution, and for the comparative investigation of different samples by one- or two-dimensional electrophoresis. As an example, results obtained by polyacrylamide gradient gel electrophoresis in the presence of sodium dodecylsulphate are shown in Fig. 5.

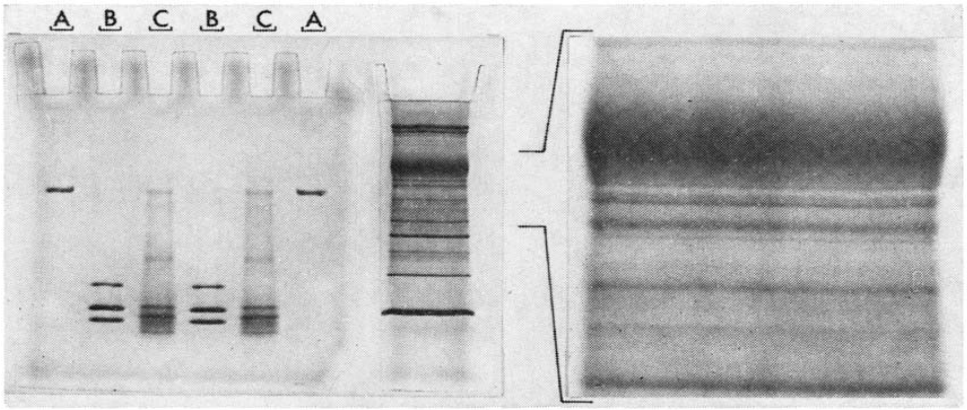

Fig. 5. Gradient gel electrophoresis in the presence of sodium dodecylsulphate; Coomassie blue staining patterns. Left: Determination of molecular weight. Samples are (A) 0.9 μg of bovine serum albumin (68,000); (B) 0.5 μg of each of the low-molecular-weight standards (Combithek, Boehringer, Mannheim, G.F.R.) trypsin inhibitor (21,500), cytochrome *c* (12,500), aprotinin (6500); (C) *ca.* 1.5 μg of (glyco-)proteins from bronchial mucus. Middle and right: Separation of 30 μg of erythrocyte membrane proteins. Protein was measured by the method of Horstmann[7]. All sample solutions are: 75 m*M* tris(hydroxymethyl)aminomethane (Tris)–HCl, pH 8.9; 70 m*M* sodium dodecylsulphate (SDS); 128 m*M* 2-mercaptoethanol; 2 m*M* EDTA; 20% glycerol (v/v). Linear gradient gel: total concentration of monomers (T) = 5.75–21%, cross-linking concentration (C)[8] = 1.75–3%; 1.75 m*M* ammonium persulphate (apers), 3–1.25 m*M* N,N,N′,N′-tetramethylethylenediamine (TEMED); 400 m*M* Tris–HCl, pH 8.9; 5.25 m*M* SDS. Gel for preparation of sample wells: T = 6%, C = 2%; 3 m*M* apers, 3 m*M* TEMED; 400 m*M* Tris–HCl, pH 8.9; 5.25 m*M* SDS. Electrophoresis buffer: 25 m*M* Tris, 192 m*M* glycine, 3.5 m*M* SDS, pH 8.3 Electrophoresis (constant power): 10 min at 0.25 W, 10 min at 0.5 W. 10 min at 1 W, 90 min at 2 W.

A circulation or change of electrophoresis buffer is not necessary because the volume ratio of electrophoresis buffer to gel is high and also because the electrophoresis time usually does not exceed 2–3 h per separation step.

Heat evolved during electrophoresis is adequately dissipated by diffusion. Therefore, cooling coils which are connected to an external low-temperature bath are not serially installed. If the electrophoresis is to be performed at low temperature, the use of pre-cooled buffers normally suffices. The enzyme glutathione reductase, for instance, has been separated in this way without any loss of activity[9].

The use of polyacrylamide gradients greatly enhances resolution in gel electrophoresis. Best results will be obtained if gradients can be prepared to any specification dictated by the sample to be separated. Different types of gradient gel can be prepared by use of the system outlined in Fig. 3. The shape and quality of the gradients obtained are best revealed by a so-called transverse gradient electrophoresis, *i.e.* the sample is separated perpendicular to the direction of the gradient (Fig. 6). Alternatively, gradients can be prepared according to the method of Margolis and Kenrick[10], but the quality of separation was less satisfactory with these gradients.

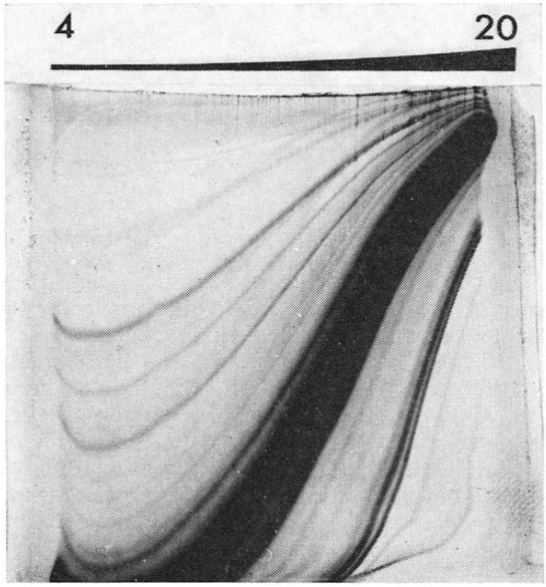

Fig. 6. Electrophoresis across a 4–20% exponential gradient (transverse gradient electrophoresis); Coomassie blue staining pattern. *Ca.* 250 μg of reduced and SDS-treated serum proteins were separated. Exponential gradient gel: T = 4–20%, C = 1–2.5%. All other conditions as described in the legend to Fig. 5. Electrophoresis (constant power): 10 min at 0.25 W, 20 min at 0.5 W, 30 min at 1 W, 60 min at 2 W. The embedding of the gel for transverse gradient electrophoresis caused a slightly impaired migration of proteins in the side regions of the gel.

Gel rods are used as first-dimension gels in two-dimensional techniques. The rods are squeezed into the electrophoresis cell and are pressed firmly on to the top of the slab gel by means of a strip of plastic. To facilitate this process and to prevent damage to the gel rods the upper edges of the glass plates that face the gel are cut away (see Fig. 1C). An example of a two-dimensional separation is shown in Fig. 7.

For large-scale experiments, bigger electrophoresis set-ups which take three or six cells have been built along the lines of the one-cell apparatus shown in Fig. 4. Furthermore, the use of a set of glass strips and plates of different size and thickness makes it possible to cast gels of different dimensions. This allows one to choose the best ratio of sample load to gel surface area. The chemicals used in the preparation of gels should be of the highest purity available; this system is reasonably economic because consumption of reagents is low.

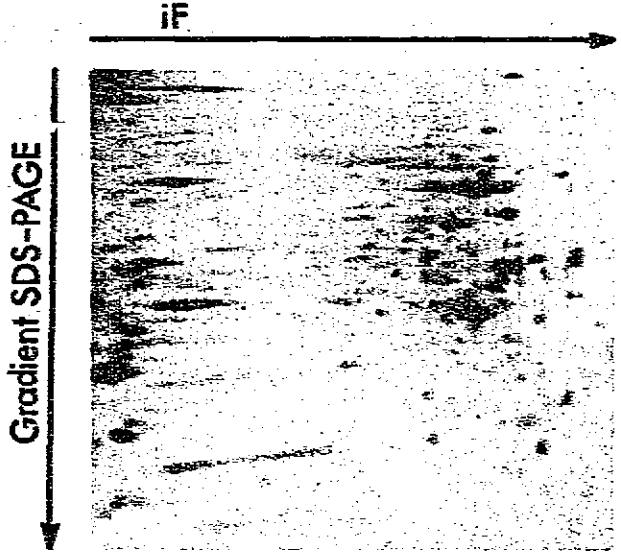

Fig. 7. Two-dimensional electrophoresis of a crude fraction of ^{14}C-Phe labelled cytoplasmic proteins from Ehrlich ascites tumour cells; autoradiogram (28 d exposure). Proteins were separated according to O'Farrell[11] with the following modifications. *Isoelectric focusing (first dimension)*: Sample: 7.6 μg of protein (2380 dpm); 5 m*M* Tris–HCl, pH 8.9; 10 m*M* Triton X-100; 75 m*M* 2-mercaptoethanol; 2 m*M* EDTA; 9 *M* urea. Gel: T = 5%, C = 1%: 2.2 m*M* apers., 2.6 m*M* TEMED; 32 m*M* Triton X-100; 2.5% Biolyte 3/10 (v/v), (Bio-Rad Laboratories, Richmond, Calif., U.S.A.), 9 *M* urea. Protein was measured by the method of Horstmann[7]. Catholyte (upper reservoir): 50 m*M* NaOH; anolyte: 25 m*M* H_2SO_4. Focusing was at constant power with 0.1 W per gel for 40 min, the voltage increasing from 150 to 600 V. *Equilibration:* Focused gels were equilibrated for 5 min in 100 m*M* Tris–HCl, pH 8.9; 70 m*M* SDS; 70 m*M* 2-mercaptoethanol; 1 m*M* EDTA. Being in position for two-dimensional electrophoresis (*cf.* Fig. 1C), the gel rods were slightly covered with that solution (made 20% in glycerol). *Gradient gel electrophoresis in the presence of SDS (second dimension):* Linear gradient gel: T = 6–21%, C = 1.5–3%. All other conditions as described in the legend to Fig. 5.

ACKNOWLEDGEMENTS

The author thanks Professor Hans-Joachim Horstmann for much helpful discussion and for drawing Figs 1–4; Mr Matthias Wirth for expert technical work and for his contribution to the design of the apparatus; and Dr Stefan Frank for a gift of ^{14}C-Phe labelled cytoplasmic proteins of Ehrlich ascites tumour cells. This work was supported by grants from the Deutsche Forschungsgemeinschaft.

REFERENCES

1 V. Anselstetter and H. J. Horstmann, *Eur. J. Biochem.*, 56 (1975) 259.
2 V. Anselstetter, H. J. Horstmann and H. Heimpel, *Brit. J. Haematol.*, 35 (1977) 209.
3 H. Noll, *Nature (London)*, 215 (1967) 360.
4 Anonymous, *Separation News*, Pharmacia Fine Chemicals, Uppsala, Sweden, January 1974.
5 N. Tulchin, L. Ornstein and B. J. Davis, *Anal. Biochem.*, 72 (1976) 485.
6 J. S. Condeelis, *Anal. Biochem.*, 77 (1977) 195.
7 H. J. Horstmann, *Anal. Biochem.*, (1979) in press.
8 S. Hjerten, *Arch. Biochem. Biophys., Suppl.*, 1 (1962) 147.
9 V. Anselstetter and G. Weidemann, in preparation.
10 J. Margolis and K. G. Kenrick, *Anal. Biochem.*, 25 (1968) 347.
11 P. H. O'Farrell, *J. Biol. Chem.*, 250 (1975) 4007.