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Electrophoretic Resolution and Comparison of Brain Proteins

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Abbreviations: SDS, sodium dodecyl sulfate; K, kilodaltons.

Running title: Electrophoretic Analysis of Brain Proteins

The technique of polyacrylamide gel electrophoresis has been successfully applied to a variety of problems over the past several years. Recently, a method has been described (AMES, 1973) which employs a discontinuous SDS buffer system (LAEMMLI, 1970) in a thin slab polyacrylamide gel electrophoresis apparatus (REID and BIELESKI, 1968; STUDIER, 1973) for the resolution of bacterial membrane, periplasmic and soluble proteins. We report here the advantages of this method for resolution and comparative analysis of proteins from whole brain homogenates.

C57Bl/6J normal and quaking mutant mice (Jackson Laboratories, Bar Harbor, Maine), 11 weeks old, were anaesthetized with ether and decapitated. The whole brain (including olfactory bulbs and medulla) was removed and rinsed in 0.9% NaCl solution and blotted dry. Each brain (approximately 0.4 g) was homogenized in 4 ml of sample buffer (0.064 M Tris, pH 6.8; 5% glycerol; 5% β-mercaptoethanol; 2% SDS) with a Potter teflon homogenizer. A 1 ml aliquot of each homogenate was then diluted with 3 ml of sample buffer and heated in boiling water for 2 min (during which time the solution becomes totally transparent). This solution was then centrifuged (Sorvall centrifuge; 5 min at 1000 x g), the pellet discarded, and the supernatant retained for use. Protein determination (GEIGER and BESSMAN, 1972) revealed that 10 μl of each supernatant contains approximately μg of protein. Two very similar types of electrophoretic systems were used.

The first system (Hoefer Scientific Instruments, San Francisco) sandwiches a 0.75 mm thick slab gel between two glass plates (30 cm long, 18 cm wide), which is then vertically mounted on a lucite support equipped with a continuous-flow cooling system and both upper and lower electrode buffer reservoirs.

The technique, using an upper 5% stacking gel and a lower 9% separating gel, is essentially that of STUDIER (1973), as modified by AMES (1973). In addition, we made a single modification. Voltage (300V) was applied to the separating gel for 8 h (pre-run) before pouring the stacking gel. A lucite or teflon "comb" of the same thickness as the gel, with teeth 0.8 cm wide was inserted into the stacking gel after pouring. Following gel polymerization, removal of these teeth provided wells for loading the samples. Brain sample volumes were 10 μl per well. A series of molecular weight standards were individually added (5 μg in 10 μl each) to two of the wells; the molecular weight assignments are according to WEBER and OSBORN (1969): β-galactosidase (Worthington; 130K), phosphorylase a (Worthington; 94K), bovine serum albumin (Sigma; 68K), glutamic dehydrogenase (Sigma; 53K), and cytochrome c (Sigma; 12.4K). With the exception of β-galactosidase, these gave a linear semi-logarithmic plot of molecular weight vs. relative mobility in the region 12.4K-94K. A separate well contained a tracking dye (0.1% brom phenol blue, Allied Chemical Corp.) in sample buffer; no tracking dye was used in any of the brain samples. Voltages were applied as follows: 10 min at 50V, 10 min at 300V, and 4 h at 650V. Gel heating was no problem at these voltages since pre-running had lowered the current to under 40 mA through both gels. After the run, gels were removed from between the glass plates, stained with Coomassie brilliant blue, and destained sequentially (FAIRBANKS, STECK and WALLACH, 1971). A gel which was run using this first system is shown in Fig. 1. Over 80 bands were observed. Two normal (N1, N2) and two quaking (Q1, Q2) mouse brain protein patterns are compared side by side.

The gel length and pre-running conditions of this system enable the lower molecular weight region (< 20K) of the protein patterns to be well-resolved, and several distinct differences in band intensities have been noted (arrows, Fig. 1). The markedly decreased intensities of bands in the quaking mutant patterns relative to the corresponding bands in the normal patterns (seen more clearly in the original gel than in the photographic reproduction) are interpreted by us to be the result of the genetic defect associated with the mutant, which has been shown to exhibit extensive hypomyelination of the central nervous system (SIGMAN, DICKIE and APPEL, 1964; SAMORAJSKI, FRIEDE and REIMER, 1970). This interpretation is further supported by a previous electrophoretic study (GREGSON and OXBERRY, 1972), which found decreases in 3 protein bands of isolated myelin fractions from quaking mutants.

During the addition of the molecular weight standards to the gel, a small amount (0.5 μg each) of the β-galactosidase, phosphorylase a and bovine serum albumin spilled over into sample wells N1 and Q2, providing internal markers in those wells (asterisks, Fig. 1). These internal markers showed approximately the same mobility whether run in the standard, the normal, or the quaking samples. We can infer, then, that other SDS-soluble cell constituents (lipids, DNA, RNA, etc.) do not influence the relative protein mobilities. A further argument that lipid levels do not influence protein migration follows from the fact that quaking mice have reduced levels of brain lipid, and yet, band mobilities of internal markers and other proteins appear to be the same in normal and mutant samples. An unexpected finding is that one of the low molecular weight bands (arrow #4), which is almost

negligible in the normal pattern, shows increased intensity in the mutant. Further analysis of this difference may lead to a more definitive biochemical characterization of the hypomyelinating disorder.

Though the myelin basic proteins of bovine brain (EYLAR and THOMPSON, 1969; OSHIRO and EYLAR, 1970; LONDON, 1971) and rat brain (AGRAWAL et al., 1972) are known to be of comparable molecular weight, the assignment of the low molecular weight proteins (arrows) seen in this gel as myelin proteins would require further analysis (isolation and electrophoresis of a purified myelin fraction, and comparative amino acid analysis). In this study, we assume that (a) proteins of corresponding molecular weights bind the stain equivalently from sample to sample, and (b) the observed intensities of the stained bands are proportional to the amount of protein present in each band.

The second system uses non-commercial apparatus which has short, thin (10 cm long, 12 cm wide, 0.8 mm thick) polyacrylamide slab gels (AMES, 1973), with a discontinuous SDS buffer system (LAEMMLI, 1970) as in the first system. No cooling or pre-running was used. In addition to the previously used molecular weight standards, catalase (Sigma; 60K), ovalbumin (Sigma; 43K), d-amino acid oxidase (Worthington; 37K), and histidine-binding protein J (courtesy of G. F. Ames; 25.5K) were also used. A mixture containing approximatley 50 μg of each protein in 1 ml of sample buffer (LAEMMLI, 1970) was heated in boiling water for 2 min and 15 μl of that mixture was then applied to a single well. Brain samples N1 and Q1 (10 μl each) were applied to the gel; in addition, 10 μl of 1:1 dilutions of both N1' and Q1' with sample buffer LSB-A (0.064 M Tris, pH 6.8; 9% glycerol; 5% β-mercaptoethanol;

2% SDS; 0.001% bromphenol blue) were applied to the gel. The gel was run for 1.5 h at 30 mA constant current. Staining and destaining were performed as before. A gel which was run using this sytem is shown in Fig. 2. In both patterns of the normal samples (N1, N1'), a thin band at about 45K (arrow) is clearly visible which is not apparent in the mutant protein patterns (Q1, Q1').

Again, we attribute this decrease of normal protein to the aberrant genetic expression of the mutant. A semilogarithmic plot of molecular weight vs. relative mobility was linear in the region 25K-94K.

The first system, which produced the gel shown in Fig. 1, had greater resolution and lower background between bands. Differences between normal and mutant patterns in the low molecular weight region, not apparent in Fig. 2, are easily distinguishable in Fig. 1. Greater resolution (separation) does not always mean that visual differences will be enhanced. A single band difference between normal and mutant at 45K, shown in Fig. 2, is not apparent in Fig. 1, where this band is resolved into 4 components. In such cases of low band intensities, densitometer scans would probably give more accurate comparisons than visual inspection.

We conclude that the application of this electrophoretic method to the analysis of brain proteins has several advantages:

(1) Reproducibility. Brain protein patterns from different animals of the same strain were identical when run on the same slab gel;

(2) Resolution. Over 80 bands were observed per sample on the long gels;

(3) Sensitivity. Even with a system as complex as whole brain,

protein patterns from normal and mutant mice showed reproducible differences. Comparisons are possible with as little as 10 μg of protein per well. Subcellular fractionation or brain dissection into specific anatomical aprts could further enhance sensitivity;

(4) Molecular weight determination. Within a wide molecular weight range, standards have the same realtive, linearly-related mobilities (both in the same slab and from slab to slab). Protein mobilities appear to be molecular weight dependent and independent of cell constituents soluble in SDS. Brain protein bands can therefore be assigned molecular weights;

(5) Mutant studies. By comparing protein patterns of various regions of the brain from normal and mutant mice, one can identify which region and which major protein bands are affected by the mutation in question;

(6) Variable resolution of specific molecular weight regions. By altering the system dimensions and electrophoresis conditions, one can "spread out" regions of interest and "compress" others. A multitude of parameters (pH, ionic strength, temperature, gel pore size, applied current and voltage, stacking gel-separating gel interface, reagent purity, pre-running, etc.) have been found to be significant in the longer gels (S. J. Fliesler, unpublished);

(7) Facile comparative analysis. In contrast to gels run in cylindrical tubes, slab gels are polymerized, run, stained and destained as a single unit. Protein patterns, as a result, exhibit more uniform mobility from well to well and fewer staining/destaining artifacts. Slab gels can also be dried onto a sheet of filter paper (Maizel, 1971).

In this form, gel storage, photography and autoradiography are greatly simplified.

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REFERENCES

AMES G. F. (1973) J. Biol. Chem., in press.

AGRAWAL H. C., BURTON R. M., FISHMAN M. A., MITCHELL R. F. and PRENSKY A. L. (1972) J. Neurochem. 19, 2083-2089.

EYLAR E. H. and THOMPSON M. (1969) Arch. Biochem. Biophys. 129, 468-479.

FAIRBANKS G., STECK T. L. and WALLACH D.F.H. (1971) Biochemistry 10, 2606-2617.

GEIGER P. J. and BESSMAN S. P. (1972) Anal. Biochem. 49, 467-473.

GREGSON N. A. and OXBERRY J. M. (1972) J. Neurochem. 19, 1065-1071.

LAEMMLI U. K. (1970) Nature 227, 680-685.

LONDON Y. (1971) Biochim. Biophys. Acta 249, 188-196.

MAIZEL J. V., JR. (1971) In Methods in Virology (Edited by MARAMOROSCH K. and KOPROWSKI H.) Vol. 5, Chapter 5, pp. 179-246, Academic Press, New York.

OSHIRO Y. and EYLAR E. H. (1970) Arch. Biochem. Biophys. 138, 606-613.

REID M. S. and BIELESKI R. L. (1968) Anal. Biochem. 22, 374-381.

SAMORAJSKI T., FRIEDE R. L. and REIMER P. R. (1970) J. Pathol. Exp. Neurol. 29, 507-523.

SIDMAN R. L., DICKIE M. M. and APPEL S. H. (1964) Science 144, 309-311.

STUDIER F. W. (1973) J. Mol. Biol. 79, 237-248.

WEBER K. and OSBORN M. (1969) J. Biol. Chem. 244, 4406-4412.

Fig. 1. Photograph of an SDS-acrylamide slab gel (30 cm long) after electrophoresis of whole brain homogenates. Arrows (→) indicate band differences between normal and quaking mutant mice. Asterisks (*) indicate presence of molecular weight standards as internal markers. Symbols: O, origin; N1 and N2, brain homogenates from two different normal C57Bl/6J mice; Q1 and Q2, brain homogenates from two different quaking mutant C57Bl/6J mice; MW, molecular weight standards. Molecular weight standards: 130K, β-galactosidase; 94K, phosphorylase a; 68K, bovine serum albumin; 53K, glutamate dehydrogenase; 12.4K, cytochrome c. Sample preparation and electrophoresis procedures are described in the text.

Fig. 2. Photograph of an SDS-polyacrylamide slab gel (10 cm long) after electrophoresis of whole brain homogenates of C57Bl/6J normal and quaking mutant mice. Arrow (→) indicates band (∿ 45K) not apparent in the mutant. Symbols: O, origin; N1, brain homogenate from normal mouse; N1', a 1:1 dilution of N1 with sample buffer (LSB-A--see text); Q1, brain homogenate from quaking mutant mouse; Q1', a 1:1 dilution of Q1 with sample buffer LSB-A; MW, molecular weight standards. Molecular weight standards: 130K, β-galactosidase; 94K, phosphorylase a; 68K, bovine serum albumin; 60K, catalase; 53K, glutamate dehydrogenase; 43K, ovalbumin; 37K, D-amino acid oxidase; 25.5K, histidine-binding protein J; 12.4K, cytochrome c. Sample preparation and electrophoresis procedures are described in the text.

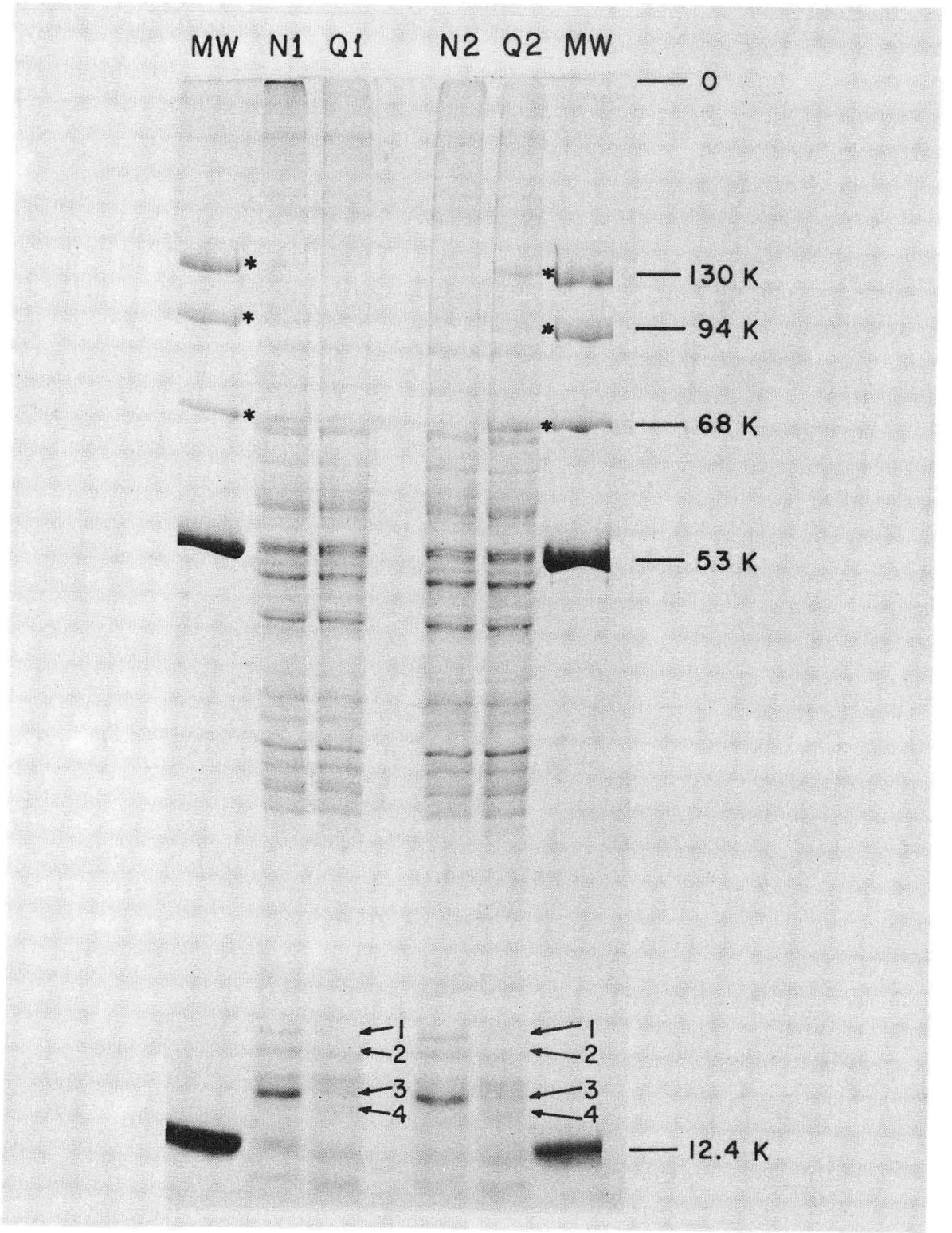

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Fig. 1.

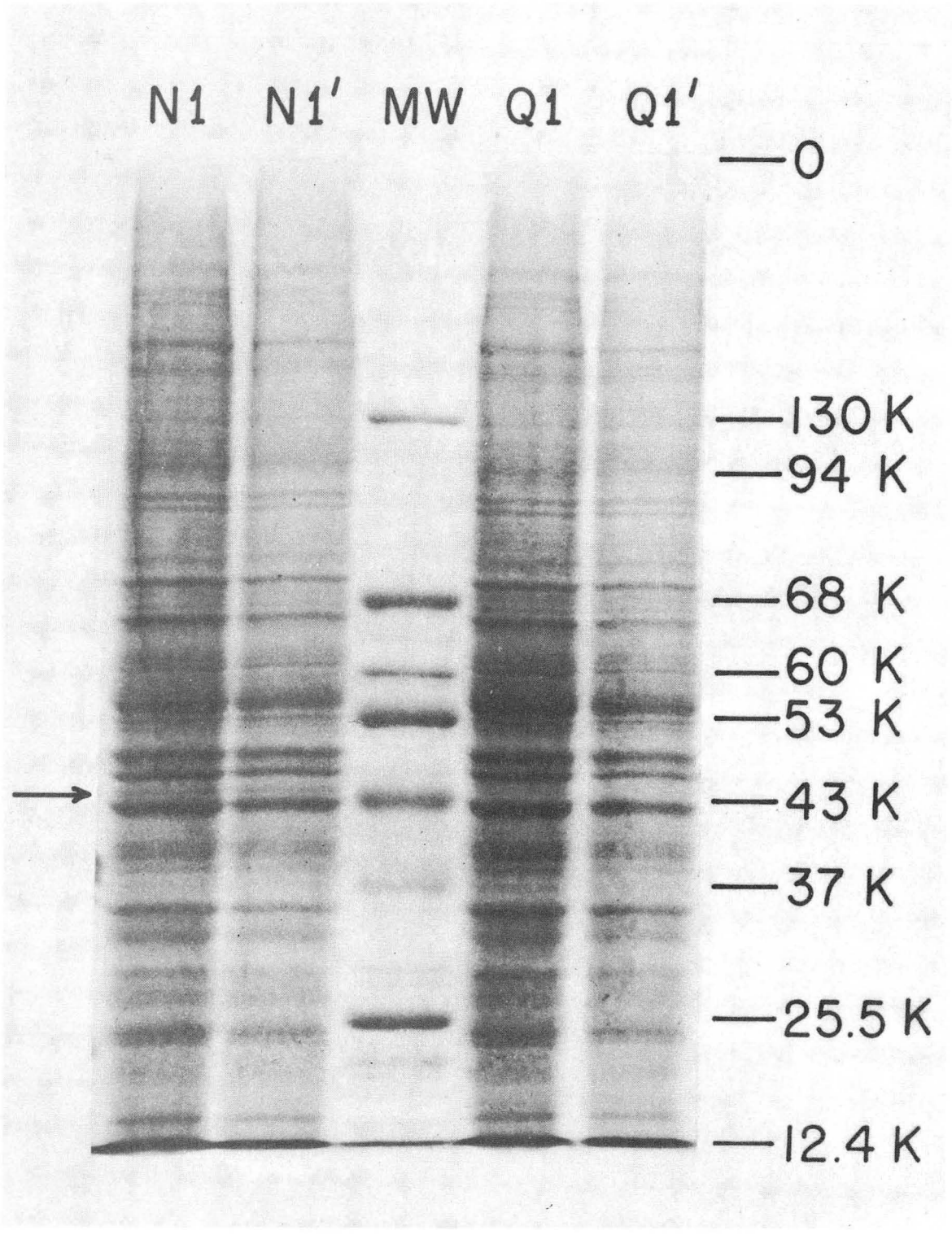

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Fig. 2.

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