tants the requirements of training, research and academic medicine, and career balance can be met only by reductions in the numbers of units providing inpatient care. If paediatric services are contracted there would be a knock on effect on accident and emergency, obstetric, and other acute services. Fewer and larger units are needed if there is to be an economy of scale in terms of junior staff. These units also would need to be large enough to accommodate the workload for the whole population to be served. This is easy to say. Nevertheless, the difficulties associated with obtaining agreement to changes in the pattern of service or the closure of hospitals, even on grounds of quality and patient safety, must not be underestimated. The public and health authority members are concerned with the needs of local people who wish to keep their local units, not with the career aspirations of doctors.

It is very unlikely that there is the political will to change the service radically in order to meet the

medical manpower needs. If this sort of change is to be achieved there must be much greater public understanding and discussion of the issues in the training of doctors and the provision of a safe service to patients throughout the 24 hours.

The alternative to reducing the number of sites is to follow through the suggestion in *Plan for Action* that a safety net would not be possible at all sites. This could lead to a two tier system with first and second class hospitals and first and second class consultants.

We believe that when other health authorities and districts undertake a similar exercise the conclusions and the options available to them will be similar to ours. Surely the time has come for the government, the profession, and health authorities to reassess the proposals in *Plan for Action*.

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Scientific Tools in Medicine

Electrophoresis of proteins and nucleic acids: I—Theory

John D Hayes, Paul K Stockman

Electrophoresis is used extensively in both routine clinical chemistry and biomedical research as a rapid method to separate macromolecules. The high resolution of proteins or nucleic acids that can be achieved by electrophoresis makes it an indispensable tool in the research laboratory. The separation of complex mixtures of either protein or nucleic acid polymers is most conveniently achieved with electrophoresis; indeed, often the separations obtained by electrophoresis cannot be achieved by any other method. By contrast, the separation of smaller molecules such as amino acids, nucleotides, sugars, or drugs is best achieved by high performance liquid chromatography. Hence electrophoresis and high performance liquid chromatography are generally considered to be complementary in their applications.

Electrophoretic techniques have revolutionised the study of pathophysiological processes at a molecular level for reasons that include the following:

- Methods of high resolving power have been developed
- Methods are easily manipulated and optimised for any application
- Many samples can be processed simultaneously, thereby allowing direct comparisons between standards, controls, and unknown samples
- Most methods are quick to perform
- Sensitive detection methods have been described
- Suitable equipment is available commercially and is inexpensive
- All methods can be used for analytical or preparative purposes.

In this article we discuss the theory of electrophoresis, and in our next article we will consider in detail the more powerful techniques available and their clinical applications.

Theory

Electrophoresis refers to the transport of ions or charged macromolecules through a solution by an

electric field. Most biological macromolecules contain ionisable groups and under appropriate conditions (at the correct pH) will migrate in an electric field, but their movement is impeded by viscous drag. When a macromolecule migrates at a constant velocity during electrophoresis in free solution a balance exists between the electrical force and the viscous drag. The electrophoretic mobility of a charged particle under such circumstances in an insular medium can be described as:

$Eq = fv$, where E is electrical field, q is the charge on the particle, f is the frictional coefficient, and v is the constant velocity.¹ This equation can be rearranged as: $u = v/E = q/f$ to allow the mobility (u) of the macromolecule to be expressed as the velocity per unit field.

In practice, the situation is much more complicated because these equations assume that electrophoresis is performed in an inert medium that is not charged and is unaffected by the electric field.

The rate of migration of a macromolecule in an electric field, which depends on its size, shape, and charge, allows individual species to be distinguished and measured. The optimal separation of such macromolecules requires careful consideration of experimental conditions such as applied field, choice of buffer, and pore size of the support matrix as these variables can exert a considerable influence on the mobility of macromolecules during electrophoresis.

Choice of experimental approach

Figure 1 summarises the electrophoretic methods available. Some of the options available to the experimenter for selecting the most appropriate system for a particular application are outlined below.

NATURE OF SUPPORT MATRIX

Electrophoresis is carried out in a solution stabilised within a supporting gel matrix of polyacrylamide, agarose, cellulose acetate, or starch. Supporting matrices improve the resolution of proteins and nucleic acids by minimising the diffusion of the sample components that would occur in free solution and also

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FIG 1—Overview of electrophoretic methods for protein separation. PAGE=polyacrylamide gel electrophoresis; SDS/PAGE=sodium dodecyl sulphate polyacrylamide gel electrophoresis; IEF=isoelectric focusing

FIG 2—Continuous electrophoresis uses single buffer and supporting gel matrix for protein separation; discontinuous electrophoresis uses stacking gel for concentration and resolving gel for protein separation. Buffer in stacking gel has lower ionic strength and pH usually 2 units lower than buffer in resolving gel

reduce the convection disturbances that may arise as a consequence of heat generated during electrophoresis. The gels resolve proteins and nucleic acids of different sizes by acting as molecular sieves, allowing the free passage of smaller molecules while impeding the migration of larger molecules. Polyacrylamide or agarose are most commonly used for applications that require high-resolution separations. They differ appreciably in their filtration properties, agarose gels having a large pore size whereas the porosity of polyacrylamide gels can be varied to meet a wide range of requirements for separation.

Polyacrylamide gels

Polyacrylamide is the matrix of choice for separating proteins and small molecules of nucleic acid. It is chemically inert, easy to handle, and transparent. Polyacrylamide gels are formed from the copolymerisation of the acrylamide monomer and a bifunctional cross linking agent to form a complex uniform web-like polymer, the porosity of which depends on the concentration of the monomers. A variety of cross linkers have been described, but methylene bisacrylamide is most commonly used.

A ratio of 37.5:1 of monomer to cross linker is thought to produce a gel with optimal cross linking and good physical properties. As the relative proportions of acrylamide and bisacrylamide can greatly alter the mobility of certain proteins this represents an important experimental variable that should not be ignored.²

The main factor that determines the separation of polypeptides during electrophoresis is pore size of the polyacrylamide gel. Pore size is determined by the acrylamide concentration of the gel and can be mani-

pulated for optimal filtration. Two types of polyacrylamide gel can be prepared, and these differ in their applications.

Polyacrylamide gels of uniform acrylamide concentration are used to separate polypeptides of similar molecular weight and for routine fractionation of DNA fragments by size. The acrylamide concentration of these gels is chosen by experiment to give a pore size that gives optimal separation of the sample components. Gels of between 7% and 20% acrylamide are generally used for SDS/PAGE: those of 7% polyacrylamide have a large pore size and resolve peptides of 60-120 kDa most effectively, whereas those of 20% polyacrylamide have a smaller pore size and resolve polypeptides of about 10-30 kDa.

In polyacrylamide gradient gels the acrylamide concentration increases (and hence pore size decreases) in the direction of protein migration. Gradient gels (for example, 5-20% acrylamide) are fairly simple to prepare, and in contrast with gels of uniform acrylamide concentration they permit separation of polypeptides of a wide range of sizes without the need to optimise the acrylamide concentration. Gradient gels can be used to separate a complex mixture of polypeptides with a wide range of molecular weights in a single gel. Furthermore, sharp bands are produced as the polypeptides migrate into areas in which the pore size severely restricts their movement. Gradient polyacrylamide gels are also commonly used in analysis of DNA sequences.

Agarose gels

Gels of agarose, a linear polymer of D-galactose and 3,6-anhydro-1-galactose, have a large pore size and additional cross linkers are not required for their formation. Agarose gels are used to separate both nucleic acid molecules of sizes greater than several hundred base pairs and nucleoproteins. They are also used as a support matrix for isoelectric focusing, a procedure in which the impeding of the movement of proteins by the gel is undesirable. Gradient gels of agarose are not commonly used to separate proteins or nucleic acids.

CONTINUOUS AND DISCONTINUOUS BUFFER SYSTEMS

Electrophoresis can be performed under continuous conditions, in which the same buffer ions are present in the gel and the electrode solutions, or under discontinuous conditions, in which the buffer ions in the gel and electrode solution differ (fig 2). The discontinuous system can be exploited to improve dramatically the resolution of the final separation.

In zone electrophoresis the sample is put on the gel in a thin layer at the origin (point of application). When the electric field is applied the sample molecules migrate through the gel and separate according to their mobility as narrow bands or zones. The highest resolution of sample components is achieved when the volume of sample applied to the origin is as small as possible. Practical constraints usually prevent optimal resolution of dilute biological samples by zone electrophoresis.

Discontinuous buffer systems allow a sample to be concentrated more than 100-fold before it enters the resolving gel as a band only a few μm thick. They use a polyacrylamide stacking gel with low ionic strength and large pores, which is cast on top of the resolving gel in which the proteins are separated. A sample is applied on to the stacking gel, and when the electric field is applied the proteins migrate rapidly through this gel and are concentrated or stacked into a very sharp zone (fig 3). The discontinuity in the buffer ions causes a moving ion boundary to sweep up behind the proteins, which then stack. When the stack encounters the decreased pore size and increased pH

FIG 3—Electrophoretic concentration of proteins by a stacking gel

(by 2 units) of the resolving gel unstacking occurs and the proteins enter the resolving gel, where their rate of migration depends on their size and charge. The theory of discontinuous electrophoresis systems is explained by Ornstein¹ and Davis.¹

DISSOCIATING AND NON-DISSOCIATING CONDITIONS

Most proteins have complex globular shapes due to the folding of their polypeptide chains. While some proteins are composed of a single polypeptide chain, most are formed from several polypeptide chains. Under non-dissociating conditions, which maintain the native structure of the protein, proteins retain their native conformation and hence their functions; it is possible to visualise enzymes with special stains to detect their activity (zymogram analysis, see below). Under dissociating conditions, which dissociate proteins into subunits, only the primary structure is retained and individual polypeptides migrate independently of each other. The interpretation of the band patterns is simplest in experiments when dissociating conditions are used.

Agents that are used for dissociating proteins are the ionic detergent sodium dodecyl sulphate (SDS), which acts by binding to proteins and eliminating their intrinsic charge, and the non-ionic compound urea, which acts by disrupting hydrogen bonds and eliminating hydrophobic interactions. High concentrations (4-8M) of urea are required to denature proteins.

PREPARATION OF SAMPLES

In zone electrophoresis a dye which will migrate faster than the sample components is usually included with the sample and shows when the run is complete. Glycerol or sucrose (10%) are added to samples that are to be analysed by vertical slab or disc gel equipment to ensure that they are more dense than the electrode buffer.

When electrophoresis is to be conducted under non-dissociating conditions care has to be taken to prevent denaturation of the sample. If the electrophoresis is to be carried out with a continuous buffer system the samples can be diluted in water as samples with a low ionic strength will be subject to some stacking before they enter the resolving gel.

When dissociating conditions are to be used the protein samples are usually boiled for several minutes in sodium dodecyl sulphate to ensure denaturation and treated with 2-mercaptoethanol to reduce the disulphide bonds that cross link some polypeptides. Certain membrane associated transport proteins, however, show heat induced shifts in their electrophoretic mobilities that are presumed to be a consequence of changes in conformation. When membrane proteins are being analysed it is prudent to establish whether heating produces artefact changes in electrophoretic mobility.¹

DETECTION OF PROTEINS AND NUCLEIC ACIDS IN GELS

Many dyes have been used to stain proteins, including Amido black 10B, Bromophenol blue, Ponceau S, light green SF, and Coomassie brilliant blue G-250 and R-250, but direct staining with Coomassie brilliant blue R-250 is most commonly used to detect proteins in gels.⁶ Gels are soaked in a methanolic solution of the dye and acetic acid, which simultaneously precipitates and stains the protein. Excess dye is later removed by passive diffusion in a solvent solution.

Coomassie blue R-250 can be used to detect as little as 0.2-0.5 µg of protein, and the density of staining is quantitative up to about 15 µg of protein. Amido black 10B is 20-fold less sensitive than Coomassie blue R-250, being able to detect 5-10 µg of protein.⁷ Silver based stains⁸ and fluorescent stains⁹⁻¹² have also been developed to detect as little as 1-10 ng of protein, but these cannot be used to quantify proteins in gels and the staining procedures require carefully controlled conditions to achieve good results.

Densitometric scanning of stained gels allows the measurements of protein bands and accurate estimation of changes in the concentration of specific proteins. Figure 4 shows the examination of Ya, Yb, and Yc subunits of glutathione-S-transferase by SDS/PAGE. Glycoproteins can be visualised with the periodic acid Schiff stain.¹³

Proteins that have been resolved by electrophoresis can be detected immunochemically. Although methods have been described that allow specific proteins to be located within the resolving gel (a process called immunofixation), it is now common practice to transfer the resolved proteins to nitrocellulose paper before performing immunochemistry.¹⁴ This method is called western blotting and will be discussed later in this series.¹⁵

Nucleic acid fragments generated during sequence analysis can be labelled by including a radiolabelled base in the reaction mixture. These bases become

incorporated into the nucleic acid polymers and, after electrophoresis, can be detected in the gels by autoradiography. Double stranded DNA can be detected in gels under ultraviolet light after staining in an aqueous

solution of ethidium bromide; this method can detect as little as 1 ng of DNA.¹⁶

We thank Professor L G Whitby and Dr A C Robinson for critically reading this script, Mr I Lennox for preparing the artwork, Mr M Dirom for photography, and Mrs E Ward for secretarial assistance.

FIG 4—Above: SDS/PAGE of hepatic cytosol from normal rats (1) and from rats treated with phenobarbital (2) and of purified Ya, Yb, Yc glutathione-S-transferase from normal rats (3) and rats treated with phenobarbital (4). Gel was stained with Coomassie brilliant blue R-250 and calibrated by running proteins of known molecular weight. Below: Lanes 1 and 2 ((a) and (b) respectively) were scanned by densitometry to measure changes in relative concentration of protein

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ANY QUESTIONS

Is there any likelihood that regular exposure to emissions from an oil fired boiler could adversely affect natural secretions and cause body odour?

This is a curious question. Certainly infrared emissions—that is, heat—from an oil fired boiler could influence body odour. By raising the temperature more sebum, apocrine, and eccrine sweat would be produced, and a more rapid decomposition by bacteria might occur. The effect on apocrine sweat might be more noticeable than the effect on sebum or eccrine sweat, especially if the axillae are not washed daily, because rancid apocrine sweat is more noticeable and regarded by most observers as unpleasant. If this is allowed to collect and adhere to clothing, particularly clothing made of artificial fibres, after some days it may be difficult to wash out completely. Other emissions from boilers—exhaust gases, volatile oils—are not known to influence body secretions or body odour.—A B SHRANK, consultant dermatologist, Shrewsbury

Are there any long term side effects from laxatives containing magnesium?

Magnesium is absorbed from the gastrointestinal tract and excreted by the kidney. Small amounts may be absorbed from laxatives containing magnesium, but if renal function is normal this is of no consequence. In the presence of renal failure magnesium toxicity may occur. I am not aware of any other long term effects of laxatives containing magnesium, though prolonged excessive use of any laxative may result in electrolyte disturbances and laxative dependence.—LINDA BEELEY, consultant clinical pharmacologist, Birmingham