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Continuous Preparative Gel Electrophoresis

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An apparatus is described for continuous electrophoresis in polyacrylamide gel. Experiments may be run for 10 days or longer. Protein loads may be 1 g per day or more, and there are no obvious obstacles to scaling up. A revised classification of electrophoretic processes is required.

Electrophoresis is widely used as a separative technique, particularly in protein chemistry. In some respects gelatinous media have an advantage over others as support media for electrophoresis, perhaps most obviously when a gel matrix of molecular dimensions is employed, allowing the simultaneous use of two properties for separation of the particles of interest—size and surface charge density. Several devices have been described for preparative gel electrophoresis showing that there is widespread interest in the possibility of applying this powerful analytical technique on a larger scale (1–6).

The idea of continuous operation is always attractive for preparative work. Continuous electrophoresis was introduced over 20 yr ago, but it has hitherto been confined to media readily permeable to water (7) or to conditions of free solution (8–14) where subtle and fascinating approaches have been used by many workers to prevent convective mixing. This paper describes an apparatus for continuous electrophoresis in a gel medium, not freely permeable, commencing with an account of the principle upon which such a device must depend, and also indicates how this relates to other techniques in a general classification of electrophoretic processes.

Consider a strip or slab of gel which is transported continuously in a direction parallel to one of its faces. (By "face" we mean to include the narrow face or edge of a thin rectangular slab.) If a sample solution is applied continuously to this face and if an electric field is applied continuously in a direction at a right angle to the direction of transport,

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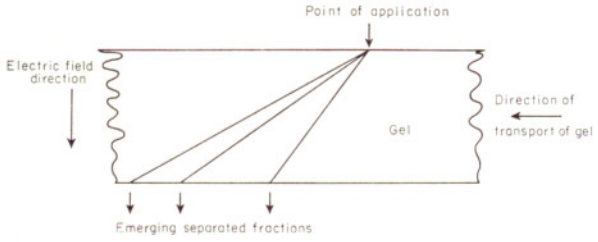

FIG. 1. Principle of continuous preparative gel electrophoresis.

then the species of interest in the sample are simultaneously subjected to two displacing influences. Firstly, and equally for all, there is the transport of the gel slab. Secondly, there is the influence of the electric field which will produce migration at a velocity characteristic of each individual species. Each species will thus follow a path which is the resultant of the effect of the two influences, and, supposing that all migrate in the same direction in an electric field, each will emerge at a characteristic point on that face of the slab which is opposite to the face where the sample was applied, in the axis of the electric field (Fig. 1)—effecting a continuous separation process. It remains to consider how the continuously transported slab may be realised in practice and how the emerging, separated fractions may be collected.

Short of arranging for continuous formation of a slab of gel one can only consider a slab wrapped round on itself to form an endless belt. In the apparatus to be described this takes the form of a vertical hollow cylinder, giving a superficial resemblance to certain devices for continuous chromatography. Indeed, there is a degree of correspondence of principle. The design of the present apparatus, however, was not approached from a knowledge of continuous chromatography and the principle is capable of realisation in forms other than that of a vertical hollow cylinder (see Discussion).

The method used for collecting fractions is based on the work of Hjertén, Jerstedt and Tiselius (3). Fractions emerge from the gel into a packed bed of agarose beads in buffer solution and are aspirated continuously via a series of filters distributed evenly around the circumference of the apparatus.

One further significant point of principle is the mode of application of sample. A simple solution disperses too readily and would not give a sharp origin point; sucrose solutions were found no better in this respect. The desired physical properties are attained by the use of a polyacrylamide solution (not cross-linked as in a gel) which is mixed with the sample solution before use. This disperses from the origin point very

slowly—effectively not at all before the species of interest have migrated into the gel—and is removed along with undesirable particulate matter, polymeric complexes, etc., once its function has been fulfilled, by a combination of scraping and aspiration.

Construction and Function of Apparatus. The core of the apparatus consists of two matching sets of concentric cylinders—the upper and lower sections—fashioned from "perspex" tubing of 3-mm thickness, 14.6 cm internal diameter and 12.6 cm external diameter respectively for the outer and inner walls. Struts are placed between the outer and inner perspex walls so as to make each part of the apparatus a rigid unit (Fig. 2). In use these are placed one above the other with circular faces in contact. Where the two sections of the apparatus meet, the perspex faces are ground flat, allowing a watertight joint (with the aid of a little silicone grease).

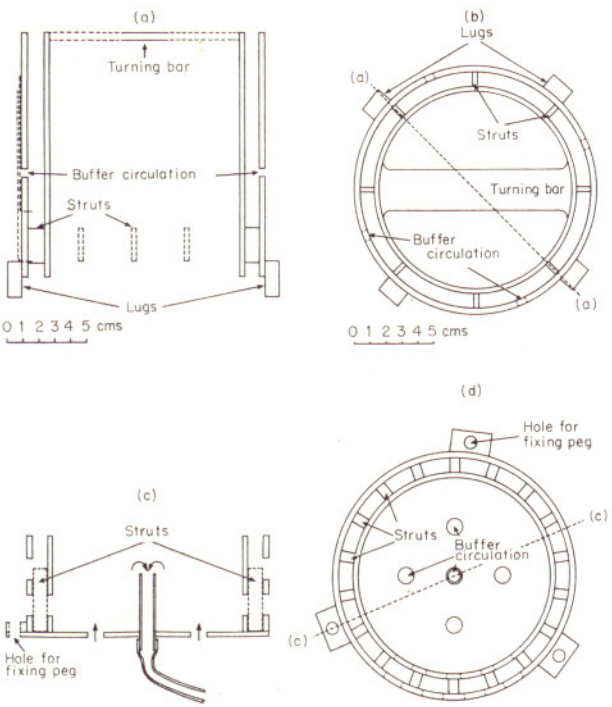

FIG. 2. Construction of electrophoresis apparatus proper. (a) Upper section elevation. Note sensing electrodes spaced 3 cm apart in gel, accessible to a voltmeter by terminals at the upper end of the section. (b) Upper section, plan view. (c) Lower section, elevation. Arrows show circulation of buffer for mixing and cooling. (d) Lower section, plan view. Perforations in inner and outer walls are indicated in only a few compartments.

The upper unit is provided at its lower edge with four lugs which fit over the rim of the lower unit and locate the upper unit unequivocally. At its upper end a turning-bar is provided which engages with the shaft of a slow-rotating motor. The whole upper unit is thus made to rotate on the lower, one revolution in 24 hr. A higher speed may be desirable in some applications. The lower unit is fixed by slotting it over pegs glued to support blocks on the floor of the buffer tank, in which the complete unit stands. The lower electrode is secured around the perimeter of this tank, which therefore serves as the lower electrode compartment (Fig. 5).

In use, the lower part of the upper section (between inner and outer walls) is filled with polyacrylamide gel. The lower section is packed with agarose beads. Gel and agarose are in direct contact. The upper electrode is supported on a framework (Fig. 3), clamped so as to hang within the cavity of the upper section (between inner and outer walls). This cavity, therefore, forms the upper electrode compartment. The electrode assembly does not rotate with the upper section: there is no need for it to do so, and it bears the sample delivery system which must remain stationary. Alternative future designs, however, may have the electrode fixed to the wall of the rotating upper section. The electrode is connected by an insulated lead to a terminal at the top of the framework.

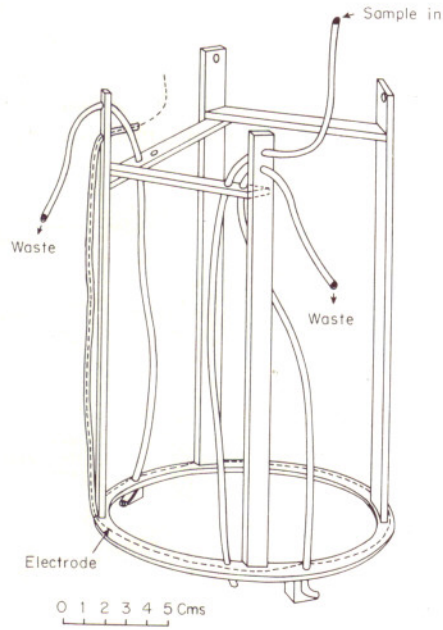

FIG. 3. Upper electrode assembly.

Around the periphery of the lower section are a series of holes in the outer wall, 1.25 cm in diameter, centered 2.1 cm from the top of the section. These accommodate filters consisting of porous plastic (Vyon sheet, 0.1 in. (2.5 mm) thick, Porvair Ltd., Kings Lynn, Norfolk, U.K.) 0.7 cm in diameter carried by perspex filter holders (Fig. 4). Buffer is aspirated through these, carrying in solution the separated fractions. Near the bottom are corresponding sets of holes in the inside and outside walls, 1.25 cm in diameter and centered 1.7 cm above the bottom of the cavity of the section. Each is occluded by porous plastic sheet (Vyon sheet, as above). In the case of the outside series of holes discs of the porous plastic are forced in on top of pieces of dialysis membrane. The outer series of holes serves to carry electrophoretic current, and the inside series permits the flow of buffer to be aspirated (Fig. 4). Any protein which may escape past the filters migrates down to the dialysis membrane where it is arrested, probably precipitated, and certainly does little harm.

The filters are connected by flexible tubes to a Watson-Marlow "delta" pump (Watson-Marlow Ltd., Falmouth, Cornwall, U.K.) driven by a type D/K 40 S motor, giving approx 3.8 ml/hr per channel with 1 mm i.d. pump tubing. This rate has proved adequate but could be varied as indicated below. In the present version, there are 18 aspiration points, occupying 18 of the 20 channels of our pump. From the pump, further flexible leads carry the separated fractions to collection receptacles—here boiling tubes of 150-ml capacity in a specially made rack, packed in ice, and enclosed in an insulated box. Buffer is continuously dripped into the buffer tank to replace that lost by aspiration of fractions.

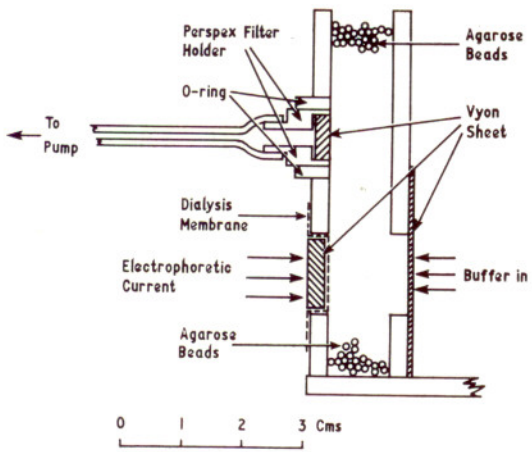

FIG. 4. Detail of lower section. Agarose beads are shown only at top and bottom, but in fact fill the entire cavity of the lower section.

The pumping rate can be varied in discrete steps by using other tube sizes or a different type of motor. The manufacturers do not recommend the use of more than 10 pump channels with the D/K 40 S motor, but in practice there has been no trouble, using the narrow pump tubing. Up to 50 channels can be handled by a single motor of the slower types, at a maximum pumping rate of 6 ml/hr per channel (using 5 mm i.d. tubing). Alternatively, and perhaps most conveniently, the pump can be driven by a continuously variable motor (Watson-Marlow MHRE), which also will handle up to 50 channels. Figures 5 and 6 provide, respectively, a schematic and an actual view of the assembled apparatus.

Buffer circulation and temperature control. The upper electrode compartment is necessarily small in this apparatus, so that a brisk flow of fresh buffer through this compartment is mandatory. This is conveniently done by means of a closed-circuit system and is combined with cooling. Buffer is withdrawn from the inner buffer compartment by means of a circulating pump and forced into a heat exchanger, consisting of Wolff Bottle immersed in coolant. (A more efficient heat exchanger would certainly be preferred.) From the heat exchanger, buffer is returned via a lagged pipe to the upper electrode compartment and thence to the lower electrode compartment (buffer tank) via several holes drilled round the periphery of the upper section, a little above the upper elec-

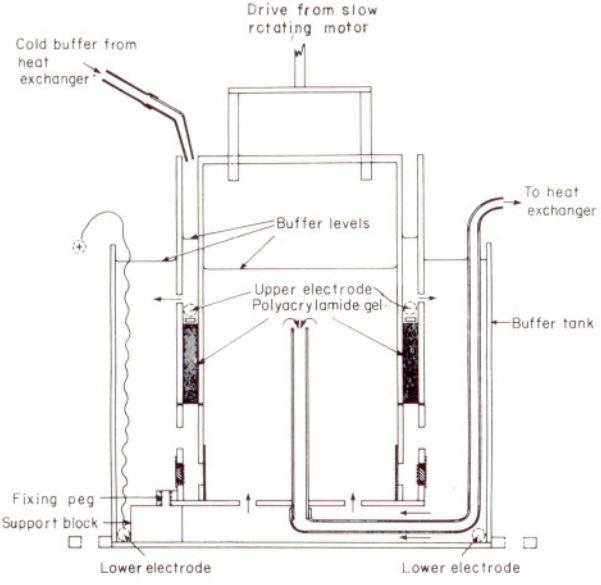

FIG. 5. Vertical section of assembled apparatus. Arrows show buffer circulation for mixing and cooling. Detail of aspiration system omitted. Lugs omitted. The electrophoresis unit proper is raised on blocks above the floor of the buffer tank.

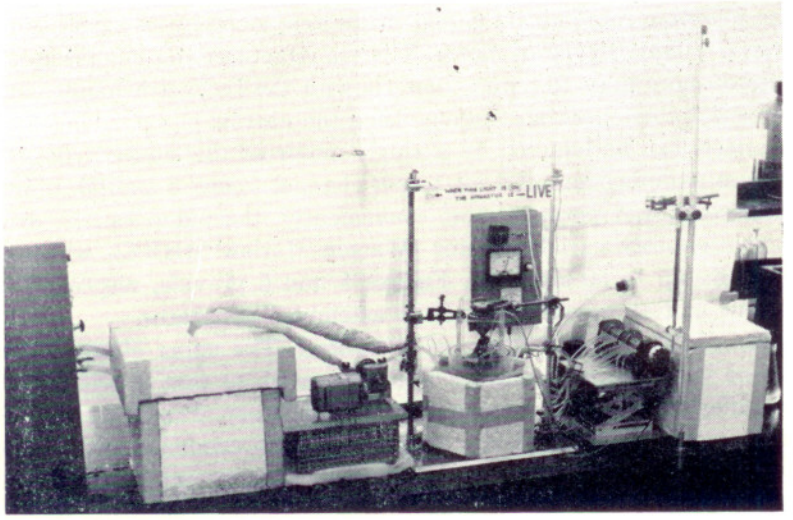

FIG. 6. General view of apparatus in operation. From left to right: "Chiller–circulator," heat exchanger (in polystyrene box), buffer circulation pump, electrophoresis unit (in polystyrene box)—with power supply behind, multichannel aspiration pump, fraction collection rack (in polystyrene box) with, above, supported by clamps, the buffer reservoir and sample reservoir (burette).

trode. From the lower electrode compartment, buffer returns to the inner buffer compartment by channels alongside the suction pipe. The heat exchanger is cooled by an ethylene glycol–water mixture circulated from a "Chiller-Circulator" (Churchill Instrument Co., Perivale, Middx, U.K.). Any other convenient refrigeration device may be employed and, in the event of mechanical breakdown, the tank may be filled with ice.

Heat exchanger, electrophoresis apparatus proper, and fraction collection rack are all lagged with expanded polystyrene, allowing operation at normal ambient temperatures. We would like to work at lower buffer temperatures than to date, chiefly because of evidence that swelling of acrylamide gel can be held to an extremely low level at 0–4°C (Boyde, unpublished). This might be achieved by working a cold room (which would also avoid difficulties over keeping the sample reservoir cold), but the practical difficulties and the increased risk of electric shock argue against this.

Packing agarose-bead bed. The electrophoresis unit, less upper electrode, is established in the buffer tank, on a level surface, and water poured in up to the level of the top of the lower section. The joint between the upper and the lower sections should have been lightly and evenly greased. The agarose beads used to date have been Sepharose 2B

(Pharmacia). The suspension is diluted by adding one-tenth volume of water and 250 ml is poured into the cavity between the walls of the cylinder. The level comes above the top of the lower section, but water is lost through the porous plastic. After allowing a little time for settlement, water is withdrawn from the buffer tank, little by little, until the layer of agarose is flush with the top of the lower section. The upper section is then very carefully removed.

Casting gel for electrophoresis. An ancillary piece of apparatus is required here—a raised ring or solid cylinder with a flat upper surface and of dimensions suitable to occlude the lower end of the interwall cavity of the upper section, without interfering with the downward-projecting lugs. The occluding ring is placed on a flat surface, its upper surface covered with a greased rubber gasket and the upper section placed in position on top of this, lightly weighted to hold it in place. The gel-casting solution is made up as follows:

Acrylamide 9.5 g, *N*,*N*′-methylene *bis* acrylamide 0.5 g (both from Koch-Light Laboratories, Colnbrook, Bucks., U.K.) and ammonium persulphate 200 mg (Analar, BDH Ltd., Poole, Dorset, U.K.) are dissolved in water to a final volume of 200 ml. To this is added 0.2 ml *N*,*N*,*N*′,*N*′-tetraethylenediamine (BDH, Poole, Dorset, U.K.). After mixing, the solution is carefully poured into the inter-wall cavity of the upper section, and left to set for 1 hr. This gives a gel of height 5 cm. The volume of gel casting solution can readily be varied to give gels of different heights. The lower limit is set by the necessity to cover the tops of the struts. The upper limit is normally set by the buffer-circulation holes, though these can be occluded with bungs and a higher series of holes employed. Gel strength and composition can also be easily varied. It is not necessary to exclude air from the polymerising gel. Provided ambient temperature is high enough and the apparatus accurately levelled the procedure described gives reproducibly a strong gel with a smooth, level, upper surface.

Assembly and pre-run. The pre-run serves several functions—to remove catalyst molecules from the gel, to introduce the electrophoresis buffer to the gel, to bring the whole system to the condition of temperature, electric field, buffer flow, etc., which will be used in the experiment and to test out all systems before risking any sample.

The upper section, complete with gel, is gently freed from its casting base, the lower perspex edges lightly greased (Silicone compound M.S. 4 has been used. This is prepared for M.S.E. Ltd. by Robert Blackie Ltd., London S.E. 14, U.K. Other silicone greases may well be suitable). The section is then put in place on the lower section and twisted to and fro to ensure a good seal and to spread the grease evenly. It has been found use-

ful to suspend weights of about 500 g from the turning bar to reduce the risk of the upper section becoming detached. Distilled water and buffer concentrate are now added to bring the level of the selected buffer up to 1 cm from the top of the buffer tank. The upper electrode assembly is lowered into place and clamped, taking care that it does not interfere with the turning of the upper section. The drive motor is brought down to engage with the turning bar, and clamped into place, making sure that the drive is correctly centered (otherwise the turning of the upper section will be jerky, or it may even become completely detached). The buffer delivery pipe is clamped so as to deliver into the upper electrode chamber. After making electrical connections, all systems are set going—refrigeration unit, buffer circulation pump, electrophoresis power supply, slow rotating motor, multichannel pump, and buffer replenishment supply. Temperature and voltage settings should be those to be used in the actual experiment.

With a pre-run done in this manner, the unwanted catalysts from the gel are only diluted, not completely removed. It would doubtless be possible to remove them by dialysis through the upper electrode chamber, or by providing for a different system of buffer flows during the pre-run. The total buffer capacity of our system is over 8 liters, however, so that the catalyst molecules are diluted 40-fold—probably sufficient to prevent them from being a nuisance.

The pre-run is normally continued overnight to ensure achievement of stable conditions.

Preparation and application of sample. A polyacrylamide solution is prepared as follows: 20 g acrylamide, and 200 mg ammonium persulphate are dissolved in water to a final volume of 200 ml. To this is added 0.2 ml *N,N,N′,N′*,-tetraethylethylenediamine, and after mixing the whole is poured into a 1-liter beaker and left 1 hr to set. The catalysts are now removed by dialysis. The beaker is filled with water, left for 1 hr, then the water poured off and the beaker refilled. This is done three times in all. The solution may be kept indefinitely. It has proved difficult to produce polyacrylamide solution of consistent viscosity: we are not sure why; possibly this is a matter of variation between different batches of reagents. Fortunately, the system can tolerate considerable variation in viscosity—flow rate is readily controlled. As a rough guide, the polyacrylamide solution should be such that if 50 ml is placed in a 100-ml measuring cylinder and this is inverted, it reaches the orifice in about 30 sec. Recently, we have used the dialysed 10% polyacrylamide directly. Formerly it was necessary to dilute the solution by stirring in an equal volume of water—a tedious job; the introduction of bubbles must be kept to a minimum as they are lost very slowly.

A predetermined amount of the polyacrylamide solution is weighed out

into a stoppered cylinder and to this is added an equal volume of the sample, prepared as a protein solution of 5%. The two are mixed *by very gentle inversion only*. Air bubbles are fatal unless very few in number: they are easy to introduce and difficult to remove (though high-speed centrifugation might do it). The mixing process takes about 30 min. A mechanical device would no doubt be useful for this job.

The sample reservoir consists at present of a burette, attached to a length of 2 mm i.d. flexible tubing. Rate of sample flow is controlled by raising or lowering the reservoir. It is very convenient to be able to monitor rate of sample flow by the burette—but a tap-funnel suspended from a spring balance might be better.

The prepared sample solution is poured into the reservoir (burette)—again a task requiring patience—and allowed to run down the flexible sample delivery tube to its end before the tube is placed in position. To do this, the electrophoretic current must be switched off and the upper electrode assembly raised so that the tube may be threaded through its guide holes (Fig. 3). The electrode assembly is then again lowered into position, and clamped so that the top of the sample delivery tube is about 1.0 mm above the surface of the gel. When sample flow is switched on, the viscous solution should flow out forming a mound 1–2 cm in length within a few minutes. Flow rates of up to at least 2.0 ml/hr have been found satisfactory.

The exhausted polyacrylamide solution (free of sample) is scraped up from the upper surface of the separative gel by means of "shoes" attached to the upper electrode assembly, and aspirated via the multichannel pump—occupying the remaining two channels. Two shoes are used, placed to scrape up polyacrylamide 6 and 22 hr after the application of sample (Fig. 3). It is not yet possible to say whether this gives improved results.

EXPERIMENTAL

We present here the results of one particular run, in which two completely different separations were attempted—first bovine serum albumin and human hemoglobin, over a period of 48 hr, then, after a brief interval, the oligomeric forms of horse spleen ferritin, again over a period of 48 hr. Following this, the run was kept going without sample for 9 days (total 15 days) and the condition of the electrophoresis gel examined at the end of that period. The results obtained on this occasion are representative.

For run A, the sample was prepared as follows: human bank blood was centrifuged and the packed red cells washed three times by resuspending in 1.2% sodium chloride and again centrifuging. The resulting washed red cells were hemolysed by resuspending in 4 volumes of distilled water and this solution cleared by centrifugation at 100,000*g* (MSE

SS50, rotor No. 59113, 30,000 rpm for 2 hr). The hemoglobin concentration was measured by diluting and measuring the absorbance of the solution at 540 nm and was found to be 7.34 g/100 ml. This solution was mixed in equal parts with a 5% solution of bovine serum albumin, obtained by dilution of a 30% solution (as supplied for immunological testing by Poviet Producten, N.V., Amsterdam, Netherlands). Of the resulting solution (3.67% hemoglobin, 2.5% albumin), 22 ml was mixed with 31 g dialysed 10% polyacrylamide.

For Run B, 10% horse spleen ferritin, as supplied by Koch-Light Laboratories Ltd. (Colnbrook, Bucks, U.K.) was diluted to 4% and of this solution 20 ml was mixed with 21.4 g dialysed 10% polyacrylamide. Regrettably, the ferritin solution was not centrifuged before use.

Details of the electrophoresis conditions are given in the legend to Fig. 7. Run A shows good separation of hemoglobin and albumin. There is some "overlap," but this may be due in part to heterogeneity of the hemoglobin. Hemoglobin was estimated in eluted fractions by determining absorbance at 540 nm, and albumin by absorbance at 280 nm—corrected for the hemoglobin content. A_{280} due to hemoglobin was taken as $A_{540} \times 0.282$. Albumin was taken as A_{280} (corrected for hemoglobin) $\times$ 140 (mg/100 ml). Because of the means used to estimate albumin, the appearance of "albumin" in fractions 7–12 may be due to experimental error either in this experiment, or in the factors used for calculations.

Qualitative electrophoresis of the separated fractions confirms the good separation achieved (Fig. 8). There is evidence of heterogeneity within the main hemoglobin band, but it has not been possible to pursue this further in respect of the particular blood donor concerned. Recovery figures, based on collections A 2 and A 3 and on the average sample flow rate, were albumin 83%, hemoglobin 75%.

Run B shows clear separation of the monomeric ferritin. Ferritin content of separated fractions was estimated by absorbance at 280 nm—chosen for sensitivity, but perhaps unwisely since the apoferritin always present in ferritin preparations also absorbs at this wavelength. Its electrophoretic mobility differs slightly from that of ferritin, so that the results may be slightly blurred. Ferritin dimer does not appear clearly in B 1, not having had time yet to emerge, but is clearly shown in B 2 and B 3 in fractions 12–16. Trimer was not detected on this occasion. The identifications given above were confirmed by qualitative electrophoresis (Fig. 9), which shows also that a small amount of monomer is present in fractions 12–16. Figure 7 shows 280-nm absorbing material present in all fractions, which is very probably ferritin monomer and may have appeared either because of escape of ferritin past the aspiration points or because of elution of monomer from ferritin aggregates deposited

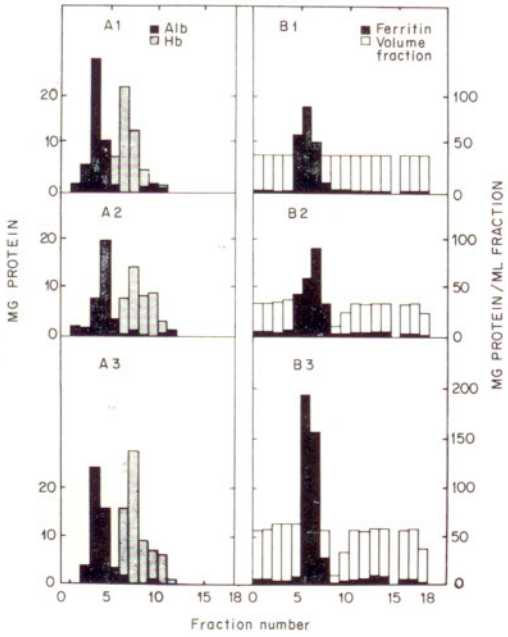

FIG. 7. The graphs show total protein content of individual fractions determined as stated in the text. (But for run B divide figures from graph by 10.)

For Run A conditions were as follows: 50 mM sodium borate buffer pH 8.4. Potential applied—100 V (anode end down), giving approximately 140 mA and a gradient of 5 V/cm on the gel itself. Temperature of buffer flowing into upper electrode compartment 6.7–8.5°C. Temperature of outer electrode compartment 6.5–8.5°C. Sample applied at about 45 cm head, giving a flow rate of 0.43 ml/hr. Collections made as follows: A1, 21 hr; A2, 30 hr; A3, 44 hr, after applying sample.

For Run B conditions were as follows: 50 mM sodium borate buffer pH 8.4. Potential applied 140 V, giving approx. 230 mA and a gradient of 7 V/cm on the gel. Temperature of upper electrode compartment 8.5–11°C. Sample applied at approx 60 cm head, giving a flow rate of 0.20 ml/hr. Collections as follows: B1, 20 hr; B2, 28 hr; B3, 42 hr after applying sample.

The run continued for a further 9 days under the same electrophoresis conditions. At the end of this time, the gel temperature was 14.3°C—presumably it had been near this temperature throughout both runs.

Fraction 1 is that collected from the elution point immediately below the sample application point; remaining fraction numbers count round in sequence in the direction of rotation.

Elution point 15 yielded nothing throughout the experiment (blocked filter). The other elution points worked well to begin with, but Nos. 9 and 10 began to fail during Run B—presumably due to partial blocking of the filters. This run was carried out with an early design of aspiration filter of smaller area and more liable to blockage than the present design.

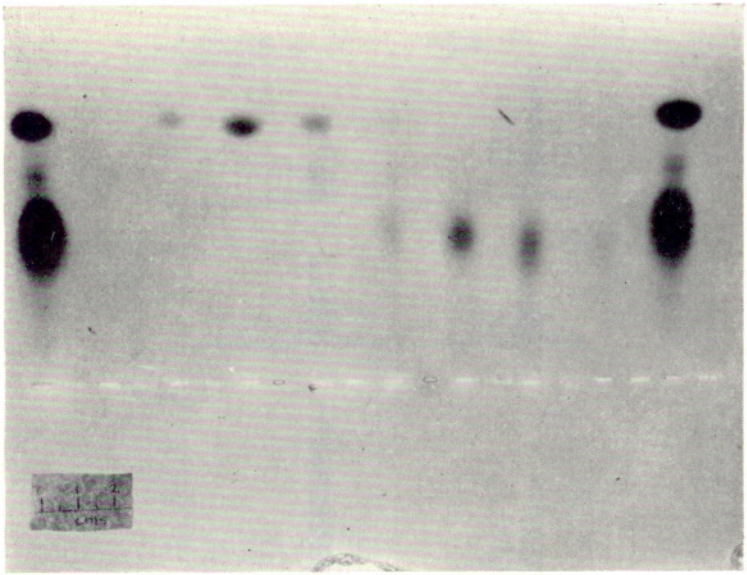

FIG. 8. Results of run A1. Qualitative electrophoresis of eluted fractions, concentrated by dialysis against Aquacide (Calbiochem). From left to right: bovine serum albumin–hemoglobin mixture (BSA/Hb), fractions 3–5 (showing appearance of albumin dimer), 6–9, BSA/Hb. Electrophoresis in polyacrylamide slab, and staining for protein, as described earlier (17,18).

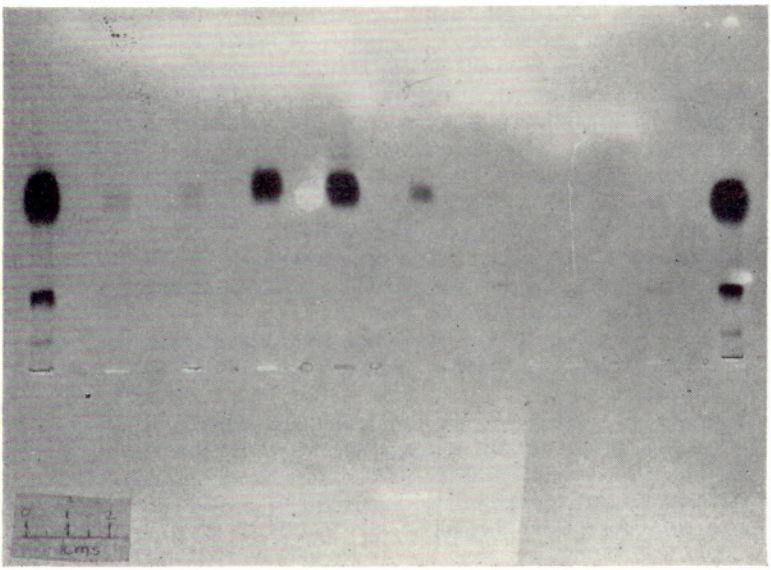

FIG. 9. Results of run B2. Methods as for Fig. 8. From left to right: original ferritin, fractions 1, 2, 6–8, 12–14.

all round the top of the separative gel. Recovery, calculated as for run A, was 92%.

At the end of Run B, the separative gel was stained brown all around. This is attributed to the presence of aggregates, as indicated immediately above, and could presumably be eliminated by preliminary high-speed centrifugation.

At the end of Run B, the separative gel was not appreciably swollen, but the electrophoresis was continued for a further 9 days (without sample) and, at the end of this time, there was marked swelling of the top (cathode end) of the gel, although not to the point of fragmentation. There is reason to suppose that the use of lower temperatures would delay the onset of serious swelling even further.

DISCUSSION

Electrophoretic experiments may be classified primarily according to whether they are of "microscopic," "boundary" or "zone" type and then according to whether they are conducted in free solution or in a supporting medium. Preparative processes are necessarily of the zone type, and may be further subdivided according to whether they are discontinuous (1-stage, with automatic elution; or 2-stage, in which elution of fractions is a separate operation) or whether they are continuous in operation. As indicated above, apparatus for continuous preparative electrophoresis is available both for free-solution and for supporting media, but until now has not been available for use with media which also permit molecular sieving effects. An additional entry must, therefore, be made in the classification given earlier (6) (see Table 1). It should, perhaps, be emphasized here that the definition adopted of the term electrophoresis is that given earlier (6)—"measurements or separations based specifically on migration velocity in an electric field"—and thus excludes techniques which employ similar apparatus but slightly different principles (isoelectric focusing, isotachophoresis) or additional physical principles (e.g., electrodecantation, field-flow cataphoresis, etc.), but includes electrophoretic separation techniques in which the supporting medium plays a significant part.

The endless belt of separative gel need not take a cylindrical form. The present form of apparatus may be likened to the Edison "Phonograph": it is also possible to build an apparatus analogous to the "Gramophone." Here a rotating hollow-centered disc of gel makes wiping contact with other (stationary) discs lying in the same horizontal plane inside and outside the rotating disc. At the inner and outer limits, respectively, of the stationary gel discs are collection chambers separated from each other by radial walls and limited on their "electrode" side by sheets of dialysis membrane. At the centre and at the periphery of the

TABLE 1
Classification of Electrophoretic Processes (Reference Numbers)[a]

Type \ Medium			Free	Supporting medium	
				No molecular sieving	Molecular sieving
Microscopic (analytical)			19		
Boundary (analytical)			20	21	
Zone electrophoresis	Analytical			22	23 24
	Preparative	Two-stage	25 26	27	28
		Single-stage (a) flow elution		29	1 5
		Single-stage (b) intermittent elution			2 6
		Continuous	8, 9	7	This paper

[a] Note: (i) References given are exemplary only and do not necessarily indicate either priority of principle or the best available technique. (ii) Gel techniques occur in both the middle column and the last. (iii) Many techniques could be applied to supporting media other than those for which first described.

apparatus would be the electrode compartments. This alternative design may well prove superior for small-scale work. Sample would be introduced continuously into a circular groove in the rotating disc. Means would be required to prevent electrodecantation: quite possibly the viscous polyacrylamide solution used in the present work would be suitable for this purpose also. The major advantage of this arrangement would be the possibility of collection of species migrating in both directions from the origin slot. For the use of the present apparatus it is essential that all the significant species should migrate in the same direction (downwards). One can conceive of arrangements permitting double-ended operation with the vertical cylinder configuration, but none seem likely to be really practicable. Still other geometrical arrangements of the endless belt are conceivable, but perhaps less likely to be of service.

Several other recently described electrophoresis devices are cylindrical

in form with an annular separative chamber. There, however, the resemblance ends. The apparatus of Jovin, Chrambach, and Naughton (5), and others apparently based upon it, are for single-stage discontinuous preparative gel electrophoresis. The hollow cylindrical form is adopted for convenience and to assist in providing symmetrical cooling. The cylinder does not rotate in these designs, nor does it in the apparatus of Ferris *et al.* (15) which is for analytical electrophoresis of multiple samples. In other apparatuses (10,11,13,14), rotation of the cylindrical electrophoresis bed plays an important role, but is essentially for stabilisation in a system for free-solution preparative electrophoresis. Similarly with the apparatus of Hjertén (16), in which the chamber, though cylindrical, is not annular.

There remain some difficulties with the present apparatus. We observe repeatedly that the elution points of some particular protein may vary as much as ± 0.5 aspiration point during the course of a run (i.e., $\pm 10°$ of arc). The most plausible explanation we can assign to this phenomenon is variation in the electrophoretic field strength with time. We have been obliged to use rather crude equipment for providing the electrophoretic potential—without true voltage or current stabilisation—but even so the degree of variation is surprisingly large. The problem may be eliminated when we can use a stabilised power supply of sufficient capacity, particularly if this is controlled from remote sensing electrodes. Spatial variation of the electric field should be less serious, since it should not cause the elution point to change from time to time.

Slow-moving species may be carried around through more than one revolution, and thus be eluted along with faster migrating material. In the final analysis, this is a weakness inherent in the use of an endless belt: it may be minimised by choosing appropriate conditions for a particular assay and in general by the use of a longer "belt." For a machine resembling the present one, this would mean using a much larger diameter cylinder. Its linear circumferential rate of movement would be similar to what is used now, but its angular velocity would be correspondingly less. There would be many more aspiration points, arranged at linear intervals similar to what is used at present. The alternative of using much higher electric fields and therefore higher migration velocities, may be impractical because of the requirement to keep the acrylamide gel cold. We cannot emphasize too much that the gel itself must be at less than 10° if swelling is not to become a problem over a period which may be 30 days or more. Probably, even lower temperatures would be advantageous.

Other authors have had trouble with swelling of polyacrylamide gels used for preparative electrophoresis. Our experience so far suggests that

this is chiefly because the actual temperature of their gels was greater than they supposed, that is, they were using too high a current for their conditions, though buffers vary considerably in their potentiality for causing swelling.

Scaling up would be necessary to bring the apparatus into the true commercial range. A modest increase in scale should be possible with apparatus very like the present one and an increase in capacity by 10–100-fold might be possible using the principle of face cooling (6). The limiting factor, if polyacrylamide gel is to be used, is in holding the gel temperature low enough, in the center of the slab, to prevent serious swelling. The apparatus, or a similar one, may find uses with other types of support medium.

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