Apparatus for Percoll Microgravimetry Determinations in Experimental Brain Edema

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A microgravimetry apparatus is described which utilizes osmotically-balanced Percoll gradients produced by a computer-driven low pressure gradient system. A plexiglass reservoir was designed to minimize parallax artifacts while allowing repeated measurements and easy retrieval of groups of 20 samples. This system provides inert and nontoxic gradients with profile flexibility, reproducibility, and longevity when used for the routine gravimetric measurement of edema in 30-mg central nervous system tissue fragments. (Stroke 1987;18:661-664)

Microgravimetry is a technique for the quantification of tissue water content by measurement of its equilibrium position (flotation point) in a liquid gradient. Quantification of central nervous system (CNS) tissue edema is required for a variety of investigations, including correlative studies on the relation of proton nuclear magnetic resonance (NMR) relaxation times and brain edema. Many studies have measured specific gravity (SG) on the kerosene/bromobenzene gradient established by Nelson et al., but problems inherent to possible toxicity and extraction of tissue components have led to the investigation of other gradient materials. Tengvar and colleagues have reported the use of Percoll-containing gradients with NaCl and sucrose for osmolar balance. This paper reports the use of Percoll gradients in a microgravimetry system consisting of computer-driven low pressure pumps and a specially designed reservoir. This system provides a number of advantages over established methods, such as wet/dry measurements, vacuum drying, or kerosene/bromobenzene gravimetry, including reduced toxicity, gradient reproducibility, flexibility of composition, and accuracy of measurements. The relation between microgravimetric values determined on this aqueous Percoll gradient and wet/dry ratio measurements were verified using a vasogenic model of cerebral edema.

Materials and Methods

Apparatus

A compact rectangular tank was constructed of 5 mm (front and back) and 8 mm plexiglass (sides and base) having exterior dimensions of 230 x 35 x 275 mm (w x d x h). The reservoir dimensions were chosen to allow the simultaneous measurements of 20 samples and easy sample retrieval. Particular care was taken to prevent scratching or marring the front and rear tank sides during assembly. To minimize the parallax artifact in measurements of tissue flotation position, a photopositive grid (with 0.1-in. graticule) was inserted between a mirror and the back of the tank (Figure 1). A tight-fitting plexiglass top prevented external contamination.

Gradients were formed by mixing heavy and light solutions (see below) in appropriate ratios using an ISCO (Lincoln, Neb.) low pressure gradient system. The apparatus consisted of an Apple computer interfaced by an Apple Super Serial Card (RS 232) to a serial interface and two WIZ (ISCO model RP) pumps. The computer was programmed to deliver a gradient divided into numerous linear subsections to allow the tailoring of the gradient to optimize the SG regions of interest. This is a very useful feature as any gradient profile can be reproducibly prepared from only 2 starting solutions. The gradients (800 ml volume) were prepared at a flow rate of 10 ml/min to avoid excessive mixing in the tank during formation. The output from the 2 pumps was joined in a Y connector and mixed on a 10 cm long glass column filled with 3 mm glass beads.

Solutions

The 2 solutions used for all gradients were prepared according to Tengvar et al. Both heavy (SG = 1.065) and light (SG = 1.020) solutions contained 0.125 M sucrose and 0.15 M NaCl. A stock Percoll solution was prepared by mixing 1.25 M sucrose and 1.5 M NaCl solutions in a 10:1 ratio with Percoll (SG = 1.310, 0.005 g/ml). Two hundred milliliters of this stock Percoll solution was then diluted to 500 ml with 0.15 M NaCl/0.125 M sucrose to prepare the heavy solution. The light solution contained no Percoll. Osmolarity of the light and heavy solutions were 330 and 395 mosm, respectively. All solutions contained 0.05% sodium azide to prevent bacterial contamination.

Gradient Sampling and Osmometry

Gradients were sampled using a 0.100-ml Hamilton model 1710 gastight syringe with a polyethylene tube extension to reach the tank bottom. Osmolarity was
determined on a 0.025-ml aliquot with a Micro-osm-ette (Precision Systems, Sudbury, Mass.) calibrated with freezing point depression standards (Advanced Instruments, Inc., Needham Heights, Mass.). A computer program was written to calculate SG from tissue flotation depth. Tissue fragments were retrieved from the tank using long (20-cm) Pasteur pipettes.

**Calibration**

Combinations of solutions varying in SG were prepared by mixing measured volumes of heavy and light solutions. SG was obtained for each mixture utilizing 2 American Society for Testing Materials (ASTM) hydrometers having SG ranges of 1.000–1.050 and 1.050–1.100 (ERTCO, Inc., New York, N.Y.). Calibrations were duplicated.

**Edema Model**

A vasogenic brain lesion was produced by injection of 1 ml/kg of 2 M NaCl by the right internal carotid artery of anesthetized rabbits. These procedures conformed to guidelines of the University Hospital and University of Western Ontario Councils on Animal Care. After injection, the animals were rapidly killed by exsanguination, and the dissected cerebral hemispheres were divided into 18 sections.

**Wet/Dry Ratios**

Wet/dry ratios (W/D) were obtained by comparing the weight of tissue before and after 96 hours drying at 65°C. Tissues were considered dry when subsequent measurements at 24-hour intervals varied by <2%. Samples were weighed on a Mettler AE100 (Zurich, Switzerland) electronic balance.

**Hemoglobin Determination**

Hemoglobin concentrations were measured spectrophotometrically on 10% ammonium hydroxide extracts of tissue fragments used for microgravimetry.

**Results**

The relation between SG and osmolarity for the mixtures of the 2 solutions provided the basis for gradient calibrations. The results of the SG calibration using ASTM standard hydrometers is shown in Figure 2. The parameters were slope $m = 0.6709$, intercept $b = 0.999$, with a correlation coefficient $r = 0.99$. Thus, calibration of the tank depth with osmolarity yields precise SG values following mathematical conversion using these values. Figure 3 illustrates the relation between tank depth and the solution osmolarity and SG for a typical gradient. In this case, the slope was 0.843, y-intercept = 316.5, with $r = 0.99$. The values from this calibration curve were then used to assign SG values to tissue portions.

Typically, 30-mg pieces of CNS tissue were gently placed at the top of the gradient. These tended to reach an equilibrium position within 60 seconds and remained there for up to 24 hours (longest noted). The remarkable consistency of the readings on the osmolarity-balanced gradient makes accurate timing of the readings unnecessary. The expected linear relation between tissue W/D and SG for water content analysis in cerebral vasogenic edema was observed. Figure 4 depicts the relation between tissue W/D and microgravimetric analysis for samples from the brain of a rabbit injected with NaCl. The samples used for microgravimetry were 30-mg fragments of the 120-mg brain sections used for W/D measurements. The small sample achieves a rapid and stable equilibrium in the gradi-
ent and allows more extensive sampling compared with the larger samples needed for W/D measurements.

In the past 12 months numerous reproducible and robust gradients have been poured (18 in all). Although a few lasted 4 weeks, extensive use was accompanied by gradual fogging of the depth of interest, presumably due to contamination by residual cerebrospinal fluid. Repeated calibrations, however, revealed that gradient profiles were very stable despite being repeatedly used for determinations of 30–50 samples per day. Each gradient can therefore measure approximately 500 samples. The gradient profile would be expected to change if bacterial growth metabolized the Percoll or sucrose, or if sample retrieval stirred up the gradient. To increase the accuracy of some determinations, an expansion of the SG region of interest (1.04–1.06) was performed using the gradient program (results not shown).

Because of the known influence of blood on the SG of brain tissue it is essential that brain hemoglobin content be accurately determined. Hemoglobin determinations were extremely low and uniform in the brain samples from exsanguinated experimental animals. Brain samples can be considered essentially blood-free in these studies as the hemoglobin content was 0.1–0.4 μmol.

Discussion

Microgravimetry is an excellent and reproducible means to assess cerebral tissue water content. The technique described in this paper is ideally suited to studies that use tissues for subsequent studies such as hemoglobin determinations, histopathologic analyses, and enzyme or transmitter assays. The absence of difficulties with histopathology or hemoglobin analysis after microgravimetry attest to the inert nature of the medium. In addition, the Percoll-sucrose-NaCl gradient is osmotically balanced to prevent water transfer, which would introduce a significant error in gravimetric determinations. This mixture has many advantages in the laboratory over the commonly used kerosene/bromobenzene combination, including reduced toxicity, flammability and fumes, and difficulties with disposal.

The low-cost apparatus and computerized microgravimetry system described here provides reproducibility between gradients and flexibility to tailor gradient profiles to individual SG regions using the same heavy and light solutions. This is particularly important when more than one experimental routine or model is being used. In our laboratory, microgravimetry is routinely used to assess cerebral edema in experimental allergic encephalomyelitis, experimental epilepsy, and vasogenic edema. The characteristics of edema in these models are different, and analysis requires both reproducibility between repeated gradients and flexibility of profiles for optimizing SG determination. In addition, the design of the tank increases the accuracy of individual determinations by minimizing parallax artifacts.

The advantages of this system are 1) excellent stability and longevity permitting repeated use, 2) flexibility in gradient profile to optimize measurements, 3) minimization of parallax artifact, 4) low cost, 5) ease of sample retrieval, 6) ease of calibration, 7) compact size, 8) low toxicity, 9) inert medium to allow subsequent analyses, and 10) small sample size.

This microgravimetry system provides an accurate, reproducible, flexible, and resilient system for the measurement of CNS tissue water content, which is applicable to a variety of different brain edema models.

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