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TECHNICAL NOTE

Analysis of efferent arteriole serum protein by gradient gel electrophoresis

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The complete understanding of single nephron glomerular hemodynamics (single nephron plasma flow and arteriolar resistances) requires the calculation of single nephron filtration fraction (SNFF). This calculation depends on an estimate of the protein concentration in efferent arteriole serum. After the measurement of protein concentration, colloid osmotic pressure also can be estimated. This pressure is necessary to determine the net filtration pressure or the glomerular capillary ultrafiltration coefficient (K_f).

Currently two methods are available for the micro-analysis of the total protein concentration in serum sampled from the efferent arteriole. A microadaptation of the Lowry method [1] was described by Brenner et al [2] in 1969. The same laboratory subsequently developed a fluorometric method [3]. We wish to describe an additional method that can be adapted to the small volumes of serum obtained from the efferent arteriole. The method is gradient gel electrophoresis (GGE) as described by Ruchel et al [4]. Such gels are contained in 5 μliter volume capillary tubes and the sensitivity is such that 10^{-8} g of protein per sample can be detected.

Gradient gel electrophoresis

We have modified the GGE technique to analyze 5 nl of undiluted serum. Efferent arteriole samples or standards prepared from rat serum are taken up directly from the collection pipet into a constant volume pipet of approximately 5 nl. A small layer of oil is first allowed to enter the pipet so that oil covers the top of the sample to prevent evaporation during the volume measurement. The sample is immediately transferred to a layer of saline which has been placed on the polyacrylamide gel to within 5 mm of the end of a microcap. Gradient gels are prepared the afternoon prior to use in a 5 μliter volume microcap (Drummond Scientific Co.) as has been described previously [5]. Solutions of acrylamide, buffer and ammonium persulfate are added to the microcaps to provide a gradient of 4 to 40% polyacrylamide. The microcaps are used as purchased without additional cleaning. Approximately 60 gels can be prepared in 30 minutes. Handling of the microcaps during the sample filling procedure is facilitated by a mounting device consisting of a turntable with peripheral vertical holes containing plastic adapters to hold the microcap [6]. The microcap and constant volume sample pipet are viewed through a horizontally adjusted dissecting microscope (Bausch and Lomb Stereo Zoom Variable Pool, #31-26-94). The sample pipet is held in a Leitz micro instrument holder (#52-145) attached to a micromanipulator (Prior Code 22) [6] in order to position the tip of the pipet in the end of the microcap.

Electrophoretic separation of protein is started immediately after sample application using a specifically designed power supply (E. Schutt, Gottingen, FRG) [7] at a constant voltage of 70 V, exhibiting an initial current of 100 to 120 μAmp. Separation is completed after 50 minutes. Quantification of gels has been described previously [6]. Fast green is the stain of choice and the gels are scanned with Joyce-Loebl double beam microdensitometer (Model MK III CS). An integrator unit quantifies the gel scan and yields a linear relationship between integrator counts and total protein or albumin concentration. Approximately 12 gels can be scanned/hour.

Preparation of efferent arteriole sample

Collection of efferent arteriole blood follows the recent guidelines developed by Hughes and Ichikawa [8]. After sampling, the tip of the collection pipet is sealed with nail polish applied to the outside surface. The shaft of the pipet is cut off so a length of approximately 3 cm remains including the tip. The pipet is then inserted into a 4.5 cm length hematocrit capillary tube which has a slight constriction approximately 2.5 cm from one end. The shoulder of the collection pipet tip therefore lodges against the constriction so the pipet is firmly secured. The clot is allowed to form and retract for 30 to 60 minutes. The hematocrit tube is placed in a 400 μliter microcentrifuge tube and the sample is centrifuged for 5 seconds in a Beckman microfuge B. The collection pipet is then broken off about 3 mm above the serum. The tip is inserted into a mound of plasticine placed on top of the plastic adapters of the mounting device (see above). A shallow layer of oil remains on top of the serum sample and the top of the 5 nl constant volume pipet passes through this layer to reach the sample. All manipulations and viewing are done with the manipulator and microscope described above.

A standard curve (Fig. 1) for total protein concentration versus densitometer integrator counts was determined using rat serum. The serum was initially concentrated to 75 percent of

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Table 1. Multiple analyses of an efferent arteriole serum sample

	Efferent arteriole					Arterial				
	Total Protein	Albumin	LMW	Albumin	HMW	Total Protein	Albumin	LMW	Albumin	HMW
	g/dl			%		g/dl			%	
	6.5	3.2	3.1	49.5	47.4	5.5	2.6	5.3	46.3	48.4
	6.5	3.2	1.7	48.4	49.9	4.9	2.6	2.9	53.0	44.0
	5.6	3.0	3.6	53.6	42.7	5.5	2.6	4.6	47.4	47.9
	6.4	3.1	3.2	47.8	49.0	5.2	2.5	4.2	48.2	47.6
Mean	6.3	3.1	2.9	49.6	47.2	5.3	2.6	4.2	48.7	47.0
SD	0.5	0.10	0.8	2.6	3.2	0.3	0.05	1.0	2.9	2.0
SE	0.2	0.05	0.4	1.3	1.6	0.1	0.03	0.5	1.5	1.0

Abbreviations are: LMW, low molecular weight; HMW, high molecular weight.

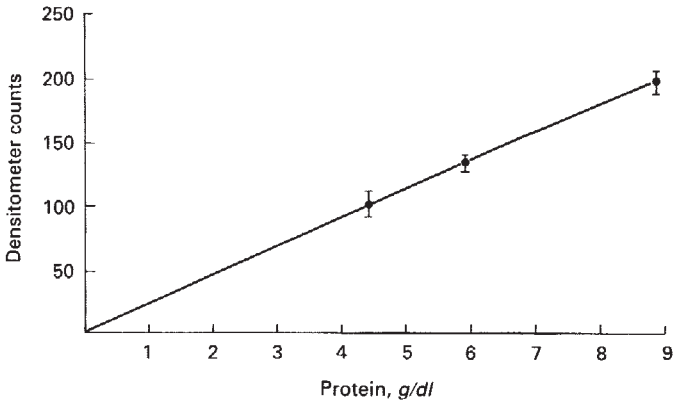

Fig. 1. *Standard curve obtained with a micro gradient gel electrophoresis method.* Densitometer counts are plotted against protein concentration (g/dl). Each point represents the mean ± SD for six standard samples.

original volume in an Amicon Centricon microconcentrator (#4208, 30,000 molecular wt cutoff). Total protein concentration determined by the Kjeldahl method [9] was therefore approximately 9 g/dl. Dilutions (1:1.25 to 1:2) of this serum were used to produce the standard curve using 5 nl samples (Fig. 1). The curve is linear over the range of protein concentration found in efferent arteriole serum. The standard deviation expressed as a percent of the mean concentration of each standard ranged from 4.7% to 10.4% (8.9 g/dl = 4.7%, 5.9 g/dl = 5.6%, 4.4 g/dl = 10.4%). A standard curve is prepared for each series of electrophoretic analyses.

Electrophoretic scans of efferent arteriole and arterial serum are presented in Figure 2. The total protein concentration (g/dl) was 6.9 and 5.2, respectively. Hence the filtration fraction in this pair of samples was 0.25.

Table 1 presents data from multiple analyses of one efferent arteriolar serum sample and simultaneously obtained femoral arterial sample. Sample volume was 5 nl. Total protein and albumin concentration are listed as well as the fractional protein composition of the samples. Low molecular weight (LMW) and high molecular weight (HMW) denotes proteins with molecular weight less than and greater than albumin, respectively. The calculated filtration fraction is 0.16 when either total protein or albumin concentrations are used.

This method represents another option for the analysis of

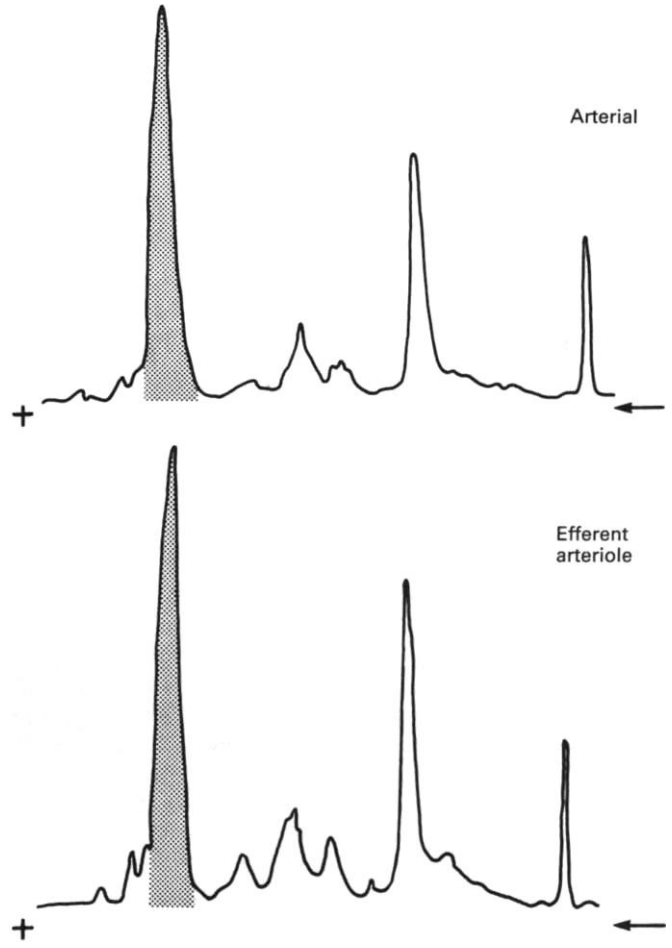

Fig. 2. *Electrophoresis analysis of arterial and efferent arteriole serum.* Direction of migration is from right to left. The stippled band is albumin. High molecular weight and low molecular weight bands are to the right and left of the albumin band, respectively.

efferent arteriole serum protein concentration. The sample size required (5 nl) and sensitivity are comparable to previous methods that have been used [2, 3]. The electrophoresis method has the advantage that minimal sample handling is required and the measurements of small volumes of reagents are not necessary. Since this is an ultramicromethod, reagent costs are

minimal (<$100/yr). In addition information about albumin concentration and fractional protein composition can be obtained. This allows a calculation of colloid osmotic pressure using the albumin and high molecular weight globulin concentrations and the Landis-Pappenheimer equation that includes terms for both concentrations [10]. When the albumin to HMW ratio deviates significantly from 1 as it does in the nephrotic syndrome, total serum protein concentration cannot be used to calculate colloid osmotic pressure.

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