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Demonstration of a technique designed to examine the ultrafiltration coefficient of isolated mammalian glomeruli

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A plethora of techniques have been developed to examine glomerular permeability. Whole animal and whole organ examinations require assumptions about the number of nephrons per organ in order to garner information about single nephron permeability, and necessarily examine contributions of all nephron segments. Recent advances in the availability of glomerular epithelial and endothelial cells have facilitated the examination of the permeability characteristics of monolayers of the cells that contribute to the glomerular filtration barrier [1], although there are significant differences between these preparations and the intact glomerular filtration barrier. Two techniques permit the examination of single nephron glomerular permeability *in vivo*. Cannulation of early convolutions of proximal tubules [2] allows assessment of the rate of fluid filtration by glomeruli, and glomerular capillary hydrostatic pressure. Direct glomerular capillary micropuncture [3] permits measurement of all of the parameters required for calculation of glomerular ultrafiltration coefficient (L_pA). However, the requirement for surface structures directly accessible to micropuncture limits the number of animals to which these techniques can be applied.

The rate of transglomerular fluid flux in response to an oncotic pressure gradient, applied to isolated glomeruli *ex vivo*, can be used to calculate glomerular L_pA [4]. In this way, glomerular L_pA can be assessed in a wide range of species, independent of haemodynamic and circulating influences. We demonstrate a refinement of the original description of this technique.

Adult male Wistar rats were humanely killed. Bilateral nephrectomy was performed immediately, and glomeruli were isolated by filtering superficial renal cortical tissue through a series of metal sieves, using mammalian Ringer solution containing 1% bovine serum albumin (BSA). Intact decapsulated glomeruli were individually loaded onto a suction micropipette, and held within a glass capillary tube with rectangular cross section, placed above the objective of an inverted microscope. Glomeruli were then exposed to a flowing perifusate of 1%BSA (in Ringer solution) at 37°C. Activation of a remotely-operated tap permitted rapid switch of the perifusate to a solution (flowing at an identical rate) containing 8% BSA (in Ringer solution), thereby generating a transglomerular oncotic gradient. Consequent transglomerular fluid efflux caused a reduction in glomerular volume, which was recorded on videotape. Off-line analysis of recorded glomerular images permitted calculation of glomerular area in each video field (recorded at 0.02s intervals). Area measurements were transformed into glomerular volumes by assuming sphericity. For each glomerulus, a plot of glomerular volume versus time since perifusate switch was generated. Application of a linear regression line to the initial (>0.04s and <0.1s) period of decreasing glomerular volume permitted calculation of the initial rate of change of glomerular volume. The quotient of the slope of this regression line (nl s^{-1}) and the applied oncotic gradient (mmHg) permitted calculation of the glomerular L_pA (nl min^{-1} $mmHg^{-1}$). The mean L_pA of control glomeruli, obtained using this technique, compares favourably with the only report of L_pA values from Wistar rats obtained by direct glomerular capillary micropuncture [5] (mean±S.D. L_pA: oncometry 1.07±0.53, n=51; micropuncture 1.8).

In parallel to this estimate of L_pA, the relative volume change in each glomerulus at each time point can be calculated. By combining these measurements from a population of glomeruli, a plot of the mean relative volume change against time since perifusate switch can be generated. A linear regression line applied to the first 0.08s after perifusate switch was used to calculate the initial mean rate of transglomerular fluid efflux in response to a known oncotic gradient ($y = -0.398\pm0.023x + 1$ (mean±S.E.M.)). The slopes of these lines can be used to compare mean rate of transglomerular fluid efflux in populations of glomeruli exposed to different conditions. There was no significant difference between the slopes of these lines from five independent control populations of glomeruli (all $p>0.05$, one-way ANOVA with Bonferroni correction).

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Three dimensional reconstruction of glomeruli clearly shows the relationship between the podocytes and the three newly defined urinary spaces of the glomerulus

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We recently discovered that the well described singular urinary or Bowmans space within the Bowmans capsule could be split into three urinary spaces on an ultrastructural basis (Neal, 2003, 2005). Some of the fluid which filters across the glomerular filtration barrier (GFB) must enter a restrictive space under the podocyte (subpodocyte space, SPS) before entering the other urinary spaces. An anastomosing system of channels between the podocytes or interpodocyte spaces (IPS) are positioned in the glomerulus to drain filtered fluid from the centre to the edge of the glomerular capillary tuft and then into the shell like peripheral urinary space (PUS). The peripheral urinary space is very like the originally defined Bowmans space of 1842.

Although we have measured, defined and described the subpodocyte and the interpodocyte spaces using reconstruction from ultrathin serial sections (Neal, 2005) we have not shown fully rotatable 3-dimensional reconstructions of the inter-relationships between the urinary spaces the podocytes and the glomerular capillaries, which we demonstrate here.

Kidneys from humanely killed rats were either fixed with glutaraldehyde by immersion (n=3, renal arterial pressure of 0mmHg) or perfusion after a flush of the kidneys with Ringer

solution (n=5, renal arterial pressure of 100mmHg throughout perfusion). Kidney pieces were postfixed, dehydrated, embedded and serial ultrathin sections of glomeruli were cut (1-2 glomeruli from each rat). Regions of the capillary tuft were reconstructed from electron micrographs using Reconstruct software (version 1.0.3.9 John C Fiala).

The reconstructions show the close apposition between the podocytes and the glomerular capillary barrier. They clearly show the subpodocyte space and the anchoring processes which tether the podocyte to the glomerular basement membrane. With suitable highlighting the much more substantial anchoring processes of the perfusion fixed glomeruli can be seen in comparison with the immersion fixed glomeruli. Within the confines of the subpodocyte spaces the routes for movement of filtered fluid can be clearly seen along with exits from the SPS and routes through the interpodocyte and peripheral urinary spaces.

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