Frequency of granulated metrial gland cells per microscopic field[a] of metrial gland

Day of gestation	Genotype of female					
	op/op			*+/op*		
	No. of mice	No. of implantation sites	No. of GMG cells/400× field[b]	No. of mice	No. of implantation sites	No. of GMG cells/400× field
10	3	6	35,38,41,42,44,53	1	4	42,43,49,58
14	2	4	53,57,60,64	1	2	64,70

[a] Viewed at 400× magnification; [b] each number represents the result from one implantation site.

mesometrial triangle, the site of GMG cell development. Thus it remains possible that macrophages may influence the differentiation of GMG cells or that uterine macrophages may respond to the same differentiation signals as GMG cells.

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Mechanoelectrical transduction, ion movement and water stasis in uromodulin

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Abstract. Mechanical movement of a column of the urinary glycoprotein uromodulin modulates an applied voltage. This change is a property of the glycoprotein and its interaction with the walls of the container and is related to its capacitance. The voltage modulation is not accompanied by changes in rotationally restricted water as has been reported for hyaluronic acid. Diffusion experiments with tritiated water also support the hypothesis that uromodulin acts as a water barrier, but allows ion movement.
Key words. Uromodulin; glycoprotein; hyaluronic acid; electret; electrical transduction.

Uromodulin (Tamm-Horsfall glycoprotein) is associated with the plasma membrane of the epithelial cells lining the ascending loop of Henle[1-3] in the human kidney, and an area of the distal convoluted tubule with morphologically similar cells[3,4]. It is also found in human urine. The glycoprotein has been shown to be present on both the luminal and basal plasma membranes[5]. Similar glycoproteins have been found in other placental mammals. The role of the glycoprotein is not known; it has no known enzyme activity or structural function, but it has been postulated that it acts as a water barrier[5]. The glycoprotein is found in that region of the kidney which is associated with salt reabsorption, where the ionic permeability is high but the water permeability is remarkably low[6,7]. Two other features of this region have been noted, firstly net chloride absorption proceeds against a transepithelial electrochemical gradient and is associated with a lumen-positive transepithelial voltage, secondly both chloride transport and the voltage gradient depend on the activity of basolateral membrane Na^+, K^+-ATPase.

Uromodulin has both hydrophilic and hydrophobic regions exposed to solvent water which will influence the hydrogen bonding of the water molecules surrounding the glycoprotein in ways different to those of bulk water; computer simulations have suggested this altered water region may extend at least 1 nm from a hydrophobic surface[8,9].

The consequences of such water with reduced rotational mobility have been reviewed[10].

During attempts to measure ion transport in uromodulin gels the mechanical modulations of the apparent value of the applied potential described in this paper were noted. Superficially similar observations have been made on hyaluronic acid/salt solutions by several workers[11-14] where electrical potentials developed between the ends of columns of hyaluronic acid solutions displaced by gentle pressure. The optical rotational dispersion properties of these solutions also changed[11] and the loss of a bound water component was measured[12].

The nature and significance of the modulations observed in uromodulin solutions will be discussed. The results were consistent with water stasis and ion movement in uromodulin; direct observations made with a model system are described which support this suggestion.

Materials and methods

1% w/v Uromodulin in 20 mM sodium chloride + 100 mM potassium chloride solution was placed in a Y-tube similar to that described by Barrett[11]. One arm of the Y-tube was arranged so that a positive air pressure could be introduced and maintained. The bottom arm of the Y-tube was sealed with a dialysis membrane and a measuring pipette was attached over this membrane. The stem of the Y-tube and the measuring pipette were filled with water. Measurements of electrical potential were made with a voltmeter (I.C.E. Minitest 80). The resistance of the measuring circuit was 18 kohm.

Silver/silver chloride electrodes, prepared by electrolytic chloridation at about 0.5 A/m^2 overnight in the dark, were used for measurements of the potential difference. Both electrodes of a pair were connected in parallel as the anode, with a silver cathode during electrolytic chloridation. Electrodes were prepared freshly each day, and kept in the dark throughout with precautions to prevent drying and mechanical damage. A battery (1.2 V) could be included in the circuit via a 2.5-ohm resistor, so that the open (left) arm of the Y-tube was positive.

Resistance measurements were made with the same voltmeter using both copper or silver electrodes, with identical geometry to the half cells. It was noted that when the silver/silver chloride electrodes became photoreduced readings could not be obtained; only with freshly prepared electrodes was the modulation observed.

Uromodulin was prepared from pooled male urine by the procedure described by Tamm and Horsfall[15,16].

Diffusion measurements were made in a two-chambered, perspex apparatus constructed so that the two chambers were separated by a removable membrane. The membrane was sealed by the machined faces of the two chambers which were lightly smeared with teflon lubricant (WGL teflon lubricant, Simpson Lawrence) to prevent possible water leakage around the membrane. The adjacent faces of the two chambers had matching 25 mm^2 holes which were covered by the membrane so that the only contact between the chambers was through the membrane. The membrane used in these experiments was dialysis tubing (Medicell International Ltd) which was prepared by boiling for 10 min to remove contaminants, and soaked in water overnight before use. Two magnetic stirrers ensured even mixing in both chambers which had a maximum working volume of 80 ml each.

Uromodulin was applied to the membrane as a gel (or viscous solution) made by suspending 30 mg of freeze dried uromodulin in 1 ml of sterile 20 mM NaCl and allowing it to dissolve by standing overnight at room temperature. The uromodulin layer was 4–5 mg/cm^2; such prepared membranes were used in a series of experiments with a gentle washing phase between measurements.

Results

1) Mechanical displacement. The uromodulin/salt solution was introduced into the Y-tube and left to equilibrate for 24 h so that a baseline for zero displacement could be established. The silver/silver chloride electrodes were dipped exactly 0.5 mm below the surface of the solution, and were realigned precisely at each displacement to maintain the exact geometry of the circuit. The right-hand column of the Y-tube was moved in 2-cm intervals by altering the air pressure in that arm and the change in potential was noted at set time intervals. When attempts to measure osmotic changes were being made

the time interval was 1 h; when only potential changes were measured the time interval was 10 min, although the effective change took place in less than 1 min (the shortest time taken to realign the electrodes).

Without the battery in the circuit no potential was measurable under any conditions or degrees of displacement of the solution. This is in contrast to the observations reported on hyaluronic acid solutions where a potential developed on displacement.

With the battery in the circuit the observed voltage was about 1 V at zero displacement, depending on the separation of the electrodes and other variables.

Figure 1 shows the effect of successive 2-cm displacement steps on the observed potential. There was an approximately linear relationship between displacement and the change in observed potential such that the observed voltage fell by about 150 mV at 8 cm displacement. This change is the same magnitude of change as that reported by Barrett [11]. The potential change recorded in figure 1 was made in a Y-tube with polythene walls. The same effect was observed in glass Y-tubes although the magnitude of the change was less, at about 100 mV. The magnitude of the potential change also depended on the diameter of the Y-tube, which was 0.3 cm in these experiments. Smaller tube diameters produced greater changes, but with lower reproducibility, while larger diameters showed smaller changes. Similar observations were made by Barrett in the hyaluronic acid system.

The overall resistance of the system was not affected by the displacements; if silver or copper electrodes were used in place of the silver/silver chloride half cells no change in potential was observed.

The changes in potential were not observed with the salt solution alone, nor if other biopolymers such as starch were used in place of the glycoprotein.

A second major difference between the uromodulin system and the previously reported hyaluronic acid system was in the water movement across the dialysis membrane. In the case of hyaluronic acid a volume change of 0.01 ml was observed with a displacement of 8 cm in a column of 1% hyaluronic acid of similar dimensions to the apparatus used in the experiments with uromodulin; this was interpreted by Barrett [12] as the adoption of a less entropic configuration by the hyaluronic acid when strained, releasing bound water and thus increasing the entropy of the water component of the solution. With solutions of uromodulin no change in the water component was detected; the sensitivity of the measuring system was such that 1 µl of water transferred across the membrane would have been detectable.

The effect observed with uromodulin seems to be dependent on an interaction between the glycoprotein and the wall of the vessel.

Many biopolymers exhibit electret behaviour [17] and while several sources of electret fields are known, in biopolymers two sources are important, dipoles and ionic space charges.

Figure 2 shows the maximum current observed at the start of the capacitance decay curve for uromodulin gels (or highly viscous solutions), a phenomenon which is

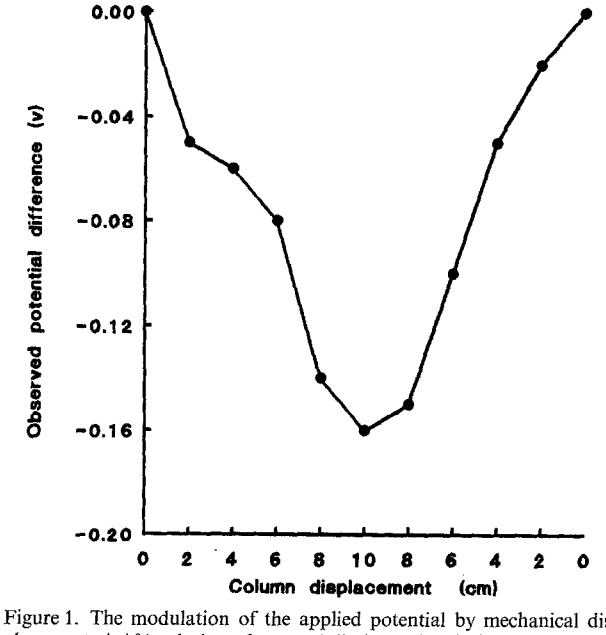

Figure 1. The modulation of the applied potential by mechanical displacement. A 1% solution of uromodulin in a salt solution consisting of 100 mM potassium chloride and 20 mM sodium chloride was introduced into a Y-tube with silver/silver chloride half cells in each arm, as described in the text. Displacement of the column in the right hand arm by air pressure resulted in the modulation of the observed potential of the system. The geometry and the resistance of the apparatus remained constant throughout the experiment.

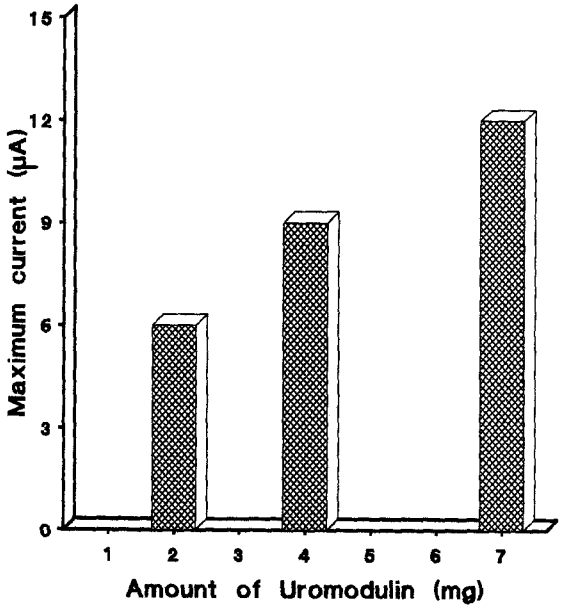

Figure 2. The maximum current observed at the start of the capacitance decay curve of uromodulin gels after polarisation. Samples of a 10% gel of uromodulin in salt solution were placed between pieces of preweighed filter paper and the sandwich introduced between the parallel faces of a capacitance measuring instrument. Values for corresponding controls without the gel were subtracted from the readings. The gels were charged from a 9 V battery for 10 s before readings were taken.

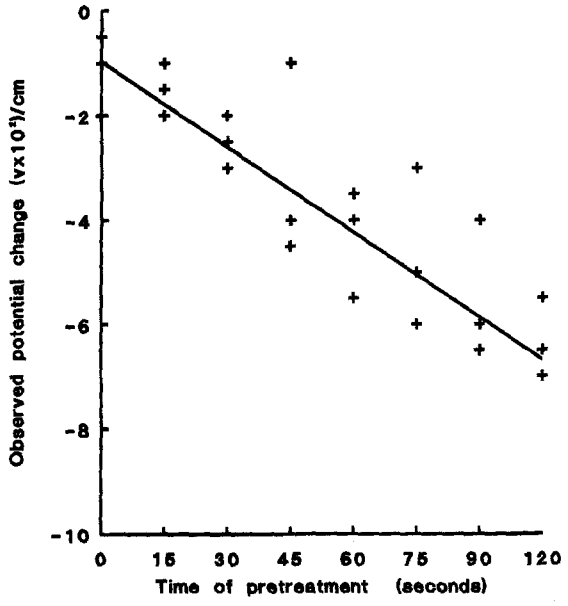

Figure 3. The effect of applied voltage on observed potential was measured in the Y-tube apparatus described in the text but with the addition of two electrodes for charging the solution. A 9 V battery was used to charge the solution for various periods of time before the electrodes were removed and the silver/silver chloride half cells re-introduced. Measurements were made of the observed potential when successive 2-cm displacements of the glycoprotein solution were made. Each point represents the average of three displacements (0–6 cm).

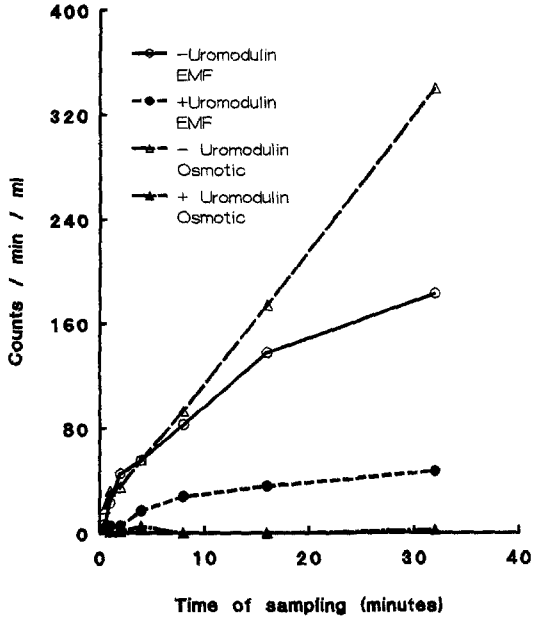

Figure 4. Increase with time of ^3H transport across membranes with and without uromodulin when an osmotic or EMF gradient is applied. Tritiated water was added to one chamber of a two-chambered apparatus and samples taken from the other, as described in the text. The data are corrected for the rates observed in the absence of the osmotic or EMF gradients.

characteristic of an electret. The gels are identical to the solution used in the Y-tube experiments except that the concentration of uromodulin is 10% w/v. Such solutions form gels on standing at room temperature for several hours, or more rapidly on warming to 40 °C. The amounts of gel shown in figure 2 were placed between two pre-weighed portions of Whatman filter paper No. 54, and the sandwich positioned between the parallel faces of a capacitance measuring instrument. The decay curves for each concentration of uromodulin were recorded. The values reported are the initial current readings corrected for the readings of blank controls of a filter paper-saline preparation. Charging of the preparations from a 9 V battery was maintained for 10 s. The capacitance effect is clearly related to the amount of uromodulin present.

Figure 3 shows the effect of pretreatment by an applied voltage to the Y-tube system. Two copper electrodes were also introduced into the open arms of the Y-tube and a 9 V battery was connected with the polarity in the same direction as the battery used in the measuring circuit. As the time of pretreatment increased so the average potential change per cm displacement increased, suggesting that the electret property of the glycoprotein was related to the ability to modulate the observed potential.

2) Water movement through uromodulin layers. Water movement was monitored using tritiated water. 10 µCi (in a total volume of 0.2 ml) of tritiated water was added to the right hand chamber and 0.1-ml samples were removed from the left hand chamber at measured time intervals and the radioactivity measured by liquid scintillation counting. The rate of diffusion through the membrane was measured for 32 min and the solutions were mixed and counted at the end of the experiment to determine the total counts added. Transfer rates were also measured when an osmotic gradient (left hand side 100 mM NaCl; right hand side 10 mM NaCl, pH 7), or an electrical gradient (4.5 V battery, l.h.s. or r.h.s. positive, 10 mM NaCl, pH 7) was applied.

The same series of measurements was made with the same membrane coated with uromodulin.

Figure 4 shows the result of the experiments using this simple system. The transfer of radioactivity as a result of an osmotic gradient is prevented when a membrane is coated with uromodulin, an observation which might be made with any lipophilic material, but when a proton gradient is applied (r.h.s. + ve) the passage of 'protons' (measured as the tritium-labelled equivalent), although reduced, is not prevented. Under the opposite field (l.h.s. + ve) a much smaller transfer of radioactivity was observed. The physical quantity of water, or protons, transferred under these conditions is small, the control value, without uromodulin, represents approximately 0.05 ml, while the increased transfer in an electric field through a uromodulin-coated membrane is equivalent to about 0.005 ml water. The transfer of protons under an electric field could also be detected as a pH change in the unbuffered solutions. Similar rates of transfer were observed with hydrostatic and pH gradients.

In a parallel series of experiments the transfer of chloride ions through identical uromodulin-coated membranes was observed when the radioactive species was $Na^{36}Cl$.

Discussion

Unlike the mechanical displacement of a hyaluronic acid solution the uromodulin system did not appear to generate a potential on displacement. The modulation of an applied potential by mechanical displacement of the glycoprotein does however exhibit many of the previously reported properties[11–13] and an explanation of the phenomenon could follow similar lines. It is suggested that the potential is due to a) an interaction of the glycoprotein with the walls of the container and b) the elasticity of the system.

As in the case of hyaluronic acid solutions it is likely that the molecular orientation of the bulk solution is influenced by the electrical potential, and that the mechanism by which displacement energy is stored is dependent on a change in entropy[18]. In the case of hyaluronic acid a decrease in the ionization of carboxyl groups on the acid was thought to promote the formation of cross-links in hyaluronic acid increasing the order of the polymer arrays, decreasing its entropy, and if thermodynamic equilibrium is maintained, increasing the entropy of the water component. In the case of uromodulin cross linking into gels of increased order involves bound divalent ions, such as Ca^{2+} and Mg^{2+}, and water entropy cannot be shown to change. These observations and the suggested interpretation is consistent with the hypothesis that the rotationally restricted water in uromodulin remains constrained even when ion movements occur, and it is possible that order changes in the gel result in ionic space charge variations, since the system is at thermodynamic equilibrium.

The interpretation of diffusion experiments with tritiated water is complicated by the ionisation of the tritiated species which can be represented:

$$THO + H_2O = TH_2O^+ + OH^- \text{ or } H_3O^+ + OT^-$$

(where T represents tritium).

This suggests the probability of the protonic form being radioactive because a tritium replaces a hydrogen is greater than the probability of the 'hydroxyl' being radioactive, which is confirmed by the relative transfer rates for radioactivity with the two opposing electrical fields; the 'reverse' (l.h.s. +ve) field reduces the transfer of radioactivity by a greater amount relative to the simple diffusion rate than the 'forward' field increases it. (Data not shown here.)

If the assumption is made that the increased transfer of radioactivity in an osmotic gradient is due to un-ionised water transfer and that the transfer in an electrical field is due to the charged species then the results indicate that water transfer is largely prevented by uromodulin under these conditions but ion transfer can occur. The complications of unknown ionisation under the electrical field, unknown relative rates of diffusion of ionised and un-ionised water and possible isotope effects prevent the interpretation of absolute (total) radioactivity transfer rates but the observed rates are consistent with the interpretation made above.

The conclusion that ions are transferred through a water impermeable, though hydrated, layer is strengthened by the observed transfer of chloride ions under identical conditions.

The lower rate of transfer through uromodulin-coated membranes and the non-linear rate observed may be explained by the proton conduction of the uromodulin layer being a rate limiting step. Proton conduction by proteins has been demonstrated[24], the transfer of radioactivity to the positive electrode compartment when the tritiated water is added to the negative side of the membrane suggests that the transfer of negatively charged ions is also possible, in agreement with the observation that chloride ions can move through uromodulin layers apparently without simultaneous water movement.

Measurements of the amount of uromodulin on cell surfaces in the human kidney have not been made, but measurements of the amount of the glycoprotein present on the surface of BHK 21C13 cells, which express the hamster equivalent of uromodulin, shows a variation between 6 pg / cell and 40 pg / cell during the cell cycle[19]. Uromodulin is a fibrous molecule of about 7×10^6 dalton[20] and a mean axial ratio of 133, corresponding to a rod 5600 Å by 42 Å[21]. These dimensions suggest that such a cell could be covered by uromodulin to a thickness of from 1 to 5 molecules, depending on the stage of the cell cycle and the assumptions made about the size of the cell and the distribution of the glycoprotein on the surface.

Hyaluronic acid is also present on the cell surfaces, and attention has been drawn to the relevance of the mechanoelectrical transduction process[11] to kidney function within the physico-chemical framework suggested by Ginetzinsky[22]. In this context the presence of uromodulin, with analogous mechanoelectrical properties to hyaluronic acid, except for the entropic change in associated water, on the cells lining the ascending loop of Henle is consistent with the high ionic permeability and low water permeability of the region compared to regions with only hyaluronic acid on the cell surfaces. The secretion of hyaluronidase under the influence of anti-diuretic hormone[22] by the nephron cells, where both macromolecules occur together in the surface molecular layers of the glycocalyx, could provide a mechanism to explain the loss of uromodulin into the urine.

These experiments taken together support the hypothesis that uromodulin acts as a water barrier, but allows ion movement.

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The effect of shock on blood oxidation-reduction potential

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Abstract. Oxidation-reduction (redox) potential measurements were made in the blood of rabbits subjected to hemorrhagic shock followed by treatment with a mild oxidizing agent (albumin). Control redox potential reading corrected for pH was -8.8 ± 1.3 millivolts (mV) in arterial blood (A) and -18.0 ± 2.0 mV in venous blood (V). This A-V difference indicated that hydrogen equivalents coming from muscle and other tissues were partially consumed in the lungs. A 20-mV drop on the V and a 13 mV on the A side was seen after shock. This did not fully return to control 2 h after return of the shed blood. Infusion of 2 g of albumin/kg/h raised the V redox potential to control, but it returned to untreated levels when the albumin was discontinued. The reductive load imposed on the animal by shock appeared to be large and not readily reversed by reperfusion or by the quantity of albumin given. Thus, it may be concluded that cellular respiration had not been adequately restored. This reductive load may impede recovery by suppression of cellular respiration and other cell and organ functions.
Key words. Hemorrhagic shock; oxidation-reduction potential; carbon electrode; denatured albumin; reductive load; oxygen radical challenge; reperfusion injury; thyroid function.

Acid-base balance, involving proton transfer, is described by the Bronsted theory and expressed by the Henderson-Hasselbach equation. It involves the hydrogen ion (H^+) (not to be confused with hydrogen), is measured as pH and is recognized as an important part of homeostasis in health and disease. In contrast, oxidation-reduction (redox) balance arising from ubiquitous biochemical redox reactions is equally important but less familiar. Redox balance involving electron transfer is described by the Nernst-Peters equation, and is measured as redox potentials or redox states. Hydrogen (electron, with proton) is transferred to carriers such as NADH, NADPH, sulfhydryls and others. This system, as it pertains to health and disease, has been reviewed by Shapiro [1].

According to Shapiro, redox balance, like acid-base balance, requires a normal range, with a rise defined as oxidosis and a fall defined as redosis. Like acidosis and alkalosis, oxidosis and redosis could be further classified as 'respiratory' or 'metabolic'. Oxygen availability is a respiratory determinant of redox states, so that an elevated pO_2 (hyperoxia) could give rise to respiratory oxidosis, and a low pO_2 (hypoxia) would result in respiratory redosis. Metabolic oxidosis is due to accumulation of oxidants, or depletion of reductants, and vice versa for metabolic redosis. These terms, proposed by Shapiro, have not been used commonly.

Redox potentials can be measured in blood and other body fluids at gold, plantinum or carbon electrodes against a reference (such as silver/silver chloride) electrode. A reflection of an overall tissue redox state is provided by the lactate/pyruvate ratio and 'excess lactate' as calculated by Huckabee [2]. Another expression of the general redox state in tissue may be seen in the ratio of oxidized to reduced pyridine nucleotides. Altered redox states occur in various pathological conditions, and