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A codiffusing system as a novel model for capillary wall charge selectivity

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Abstract

The electrostatic interaction associated with polyion–polyion interaction has been thought to be the basis for the differential transport of charged transport probes across the capillary wall. The charge of the transport probe may interact with the negative charges of capillary wall cell surfaces and the intercellular glycocalyx or extracellullar matrix. This study has set out to quantitate the exact nature of the polyion–polyion interaction through the theoretical and experimental study of the partition–diffusion of albumin in solutions of anionic polysaccharides (dextran sulfate or heparin) at concentrations up to 250 meq l^{-1} or at an average intercharge distance of 2.2 nm, as compared with solutions of uncharged dextran at the same volume concentration. The results demonstrate that the albumin partition–diffusion is exactly the same in dextran sulfate, heparin and uncharged dextran matrices of the same polymer volume fraction. These results confirm previous studies from this laboratory that the charge effect through polyion–polyion interaction under physiological conditions is negligible.

Keywords: Albumin partition-diffusion; Polyion-polyion interaction; Transcapillary transport

1. Introduction

The capillary wall normally consists of an inner layer of endothelial cells with an underlying basement membrane. Transcapillary transport of macromolecules is generally thought to occur in the spaces or fenestrated regions between endothelial cells and across the basement membrane. A number of studies have established that transcapillary transport of macromolecules appears to be influenced by the net charge on the molecule. This is particularly evident through comparisons of the transport of positively and negatively charged forms of the macromolecule. The difference manifested in transport is called charge selectivity. The capillary wall has a net negative charge due to the presence of anionic glycosaminoglycans, such as heparan sulfate, and sialoproteins in the basement membrane and on endothelial cell surfaces. It is rationalised that the charge on the macromolecule being transported via extracellular pathways interacts electrostatically with the fixed negative charge of the capillary wall, and that this influences transport. The aim of this paper is to examine and quantitate these electrostatic effects on the transcapillary transport of charged proteins.

Studies on the transcapillary transport of proteins and other macromolecules with a net positive charge have given variable results in terms of facilitated

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transport [1–5] or restricted transport [6,7] or both [2]. It seems difficult to separate the influence of coulombic interaction on transport from the indirect effects of the binding of the positively charged macromolecule to the fixed negative charges of the capillary wall [2]. The binding may result in complex structural and cellular changes that may influence transport.

A more reliable measure of the influence of capillary wall charge is in terms of its influence on the transport of macromolecules with a net negative charge, as binding will be negligible and coulombic interaction should result in exclusion by electrostatic repulsion. Theoretical treatments of this transport suggest that the major determining factor controlling charge selectivity will be the equilibrium partition coefficient at the capillary lumen (or space)–capillary wall interface [8,9]. This partition is commonly described by a Donnan-type model which, however, has not been established experimentally for multivalent macromolecules owing to the general difficulty of analysing partition coefficients for charged macromolecules in charged environments [9].

It should be stated that there is a paucity of direct experimental estimates of capillary wall charge concentration. Theoretical estimates have put the charge concentration as high as 100–160 meq l^{-1} to account for the apparent marked partitioning of albumin at the glomerular capillary wall (the charge interaction has been thought to account for partitioning of 100:1 or greater for albumin), whereas direct measurement has yielded much lower values of 7–15 meq l^{-1} (for review see [10]). To test the influence of polyion–polyion interaction on polyion partitioning, we have devised a transport system which utilises the transport of radiolabelled protein between two solution compartments joined by a shear-formed liquid boundary. Specifically, we examine the transport of albumin in model capillary wall systems that contain anionic polysaccharide at extreme charge concentrations of 250 meq l^{-1} and with an average intercharge distance as low as 2.2 nm.

2. Theory

In transcapillary transport across extracellular charged regions, it is generally thought that the charged transport probe is subject to three distinct biophysical processes. The entry of the probe into the membrane or capillary wall from the lumen of the capillary will be governed by an excluded volume partition coefficient which will have both non-electrostatic and electrostatic components. The latter will represent the polyion–polyion interaction that will be a major influence on transport charge selectivity. Once in the charged environs of the capillary wall, the probe will be transported down the chemical potential gradient until it reaches the exit point. At the exit point the probe will enter aluminal solution through partition forces that will be exactly the reverse of those governing entry of the probe to the capillary wall at the luminal interface.

For the model capillary wall system, we are primarily interested in the diffusional transport of albumin between an aqueous polysaccharide solution compartment (also referred to as a polysaccharide matrix compartment) and a solvent compartment. The comparison of the diffusional transport of albumin out of a dextran sulfate or heparin matrix should generate charge selectivity in a simple, controlled model system. The diffusion of albumin out of a polysaccharide compartment represents a model of the exit conditions associated with the transport of material out of the capillary wall at the aluminal interface. The process is the reverse of the ability to diffuse into a polysaccharide matrix, although the former is considerably easier to study for density–stability reasons. We define the components such that component 2 is albumin, component 3 is the polysaccharide matrix and component 1 is the buffer solvent.

In the diffusion cell, we measure the flux J of albumin, which is defined as

$$-J_2 = D_{app} \mathrm{d}c_2/\mathrm{d}x \tag{1}$$

where D_{app} is the apparent diffusion coefficient of albumin and $\mathrm{d}c_2/\mathrm{d}x$ is the molar concentration gradient of component 2. Expressing the fluxes of components 2 and 3 in phenomenological terms [11], we have

$$J_2 = L_{22}(\partial\mu_2/\partial x) + L_{23}(\partial\mu_3/\partial x) \tag{2}$$

$$J_3 = L_{32}(\partial\mu_2/\partial x) + L_{33}(\partial\mu_3/\partial x) \tag{3}$$

where L_{ij} are phenomenological coefficients and μ_i is the chemical potential of component i. Analysis for component 2 by equating Eq. (1) and Eq. (2) gives

$$-J_2 = D_{app}\mathrm{d}c_2/\mathrm{d}x = -L_{22}(\partial\mu_2/\partial x) - L_{23}(\partial\mu_3/\partial x) \quad (4)$$

so that

$$D_{app} = -L_{22}\mu_{22} - L_{23}\mu_{23} \quad (5)$$

where

$$\mu_{ij} = \partial\mu_i/\partial c_{ij} \quad (6)$$

To proceed to develop Eq. (5), we use algebraic expressions describing chemical potentials of the components as functions of composition [12]

$$\mu_2 = \mu_2^\circ + RT(\ln m_2 + bm_2 + am_3) \quad (7a)$$

$$\mu_3 = \mu_3^\circ + RT(\ln m_3 + dm_3 + am_2) \quad (7b)$$

where m_i is the molality of component i and b, d and a are constant coefficients that may be related to thermodynamic virial coefficients. Differentiating Eq. (7a) and Eq. (7b) with respect to m_2 and taking the approximation that $c_i \approx m_i$, then

$$\mu_{22} = RT(1/c_2 + b) \quad (8)$$

$$\mu_{23} = RTa \quad (9)$$

We now want to express the phenomenological coefficients in Eq. (5) in terms of binary frictional coefficients f_{ij} [13], where

$$-\partial\mu_2/\partial x = f_{21}(v_2 - v_1) + f_{23}(v_2 - v_3) \quad (10a)$$

$$-\partial\mu_3/\partial x = f_{31}(v_2 - v_1) + f_{32}(v_3 - v_2) \quad (10b)$$

where v_i is the velocity of i. The experiments are performed so that there is no volume change during the course of the experiments. This condition may be written as

$$\Sigma V_i c_i v_i = 0 \quad (11)$$

where V_i is the partial molar volume of i. Using Eq. (11) in Eqs. (10a) and (10b) and solving for v_2 and v_3, assuming $c_1V_1 \gg c_2V_2$ and $c_1V_1 \gg c_3V_3$ and equating to Eq. (2) and Eq. (3) gives

$$L_{22} = c_2(f_{31} + f_{32})/P \quad (12)$$

$$L_{33} = c_3(f_{21} + f_{23})/P \quad (13)$$

$$L_{23} = L_{32} = c_2f_{23}/M = c_3f_{32}/P \quad (14)$$

where

$$P = -f_{21}f_{31} - f_{21}f_{32} - f_{23}f_{31} \quad (15)$$

Substituting Eqs. (8), (9) and (12) and Eq. (14) into Eq. (5) gives

$$D_{app} = -RT[f_{31} + f_{32} + c_2(bf_{31} + bf_{32} + af_{23})]/P \quad (16)$$

Further simplification of Eq. (16) can be reasonably made by assuming that the nonideality coefficient of albumin (b) is relatively low. In multicomponent diffusion analysis, it has been demonstrated that the cross frictional interaction term f_{23} will be negligible in relation to the frictional term f_{21} [14]. We therefore assume that the cross frictional product $f_{23}f_{31}$ can be neglected, so that

$$D_{app} = RT[1 + (ac_3f_{32}/(f_{31} + f_{32}))]/f_{21} \quad (17)$$

using the Onsager relationship $c_3f_{32} = c_2f_{23}$.

If component 2 is distributed between a solution phase denoted by ′ and a matrix phase denoted by ″, then from equations similar to Eqs. (7a) and (7b)

$$\mu_2' = \mu_2'^0 + RT\ln m_2' \quad (18a)$$

$$\mu_2'' = \mu_2''^0 + RT(\ln m_2'' + am_3'') \quad (18b)$$

An equilibrium distribution is defined by the condition $\mu_2' = \mu_2''$, so that

$$\ln m_2'/m_2'' = am_3'' \quad (19)$$

This can be approximated in an alternative fashion such that the partition coefficient λ is governed by

$$\lambda = m_2'/m_2'' = 1 + am_3'' \approx 1 + ac_3'' \quad (20)$$

If the matrix is immobile, which will occur at the boundary of the diffusion cell corresponding to a mean matrix concentration of $c_3''/2$, then $f_{31} \to 0$. With Eq. (20) in Eq. (17) we get further simplification

$$D_{app} = RT\lambda/f_{21} \quad (21)$$

The significance of Eq. (21) is that the apparent diffusion coefficient will be governed primarily by the partition coefficient and the frictional interaction of albumin with the solvent. The partition coefficient will be determined by both electrostatic interactions and excluded volume interactions. It is also emphasised that Eq. (21) is applicable to a codiffusing

system of components 2 and 3; i.e., the thermodynamic interactions that occur in the mixture of 2 and 3 will be manifested in the parameter λ that governs the diffusional relaxation of both concentration gradients.

The fact that the interaction term a will be the same for a codiffusing system as compared with the partition coefficient with an immobile matrix has been thoroughly demonstrated for the interaction of albumin with Sephadex beads (cross-linked dextran) as compared with dextran in solution [15,16].

3. Experimental

3.1. Materials

Dextran T500 ($M_w \approx 500\,000$, where M_w is the weight average molecular weight) and dextran sulfate sodium salt ($M_w \approx 500\,000$) (sulfur content 17%) were obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Dextran sulfate sodium salt ($M_w = 39\,700$, sulfur content 14%) was from TdB Consultancy, Uppsala, Sweden. Bovine serum albumin, fraction V (BSA) was from Boehringer Mannheim GmbH Biochemica, Mannheim, Germany. Heparin (sodium salt, from porcine intestinal mucosa; lot14H0507) and sodium azide were from Sigma Chemical Company, St. Louis, MO, USA. Sodium boro[^{3}H]hydride (211 mCi mg^{-1}) was from Amersham International, Buckinghamshire, UK.

3.2. Methods

3.2.1. Preparation of tritiated albumin

[^{3}H]Albumin was prepared by the method of Tack et al. [17]. This reaction involves a reductive methylation associated with a brief exposure to formaldehyde and sodium boro[^{3}H]hydride. The labelled preparation was then separated from free label on a Sephadex G-25 PD-10 column and dialysed extensively against phosphate-buffered saline, pH 7.45. Other studies have demonstrated that this type of labelled albumin preparation contains > 95% monomer albumin [18].

3.2.2. Preparation of solutions

Solutions of dextran and dextran sulfate were prepared from dried preparations. In all cases, unless otherwise specified, phosphate-buffered saline (PBS) (NaCl 0.1369 M, KCl 2.68 mM, Na_2HPO_4 8.10 mM, KH_2PO_4 1.50 mM) containing 0.02% sodium azide was used as the buffer. Sodium azide was included to prevent possible bacterial degradation of [^{3}H]BSA during the diffusion period.

Solutions of PBS at various ionic strengths were also used. These were prepared by altering the concentration of NaCl in the solutions.

3.2.3. Determination of diffusion coefficients

Unidirectional diffusion coefficients of [^{3}H]albumin were measured in diffusion cells which consist of two cylindrical chambers that rotate to shear-form a sharp, free liquid boundary between the upper and lower solutions. This technique has been described in detail previously [19,20].

The total amount Q of labelled probe crossing the boundary during the diffusing time t is given by

$$Q^2 = A^2 C_0^2 Dt/\pi$$

where A is the cross-sectional area of the interface between the upper and the lower diffusion compartments in each cell, C_0 is the initial concentration of the probe in the lower compartment and D is the diffusion coefficient. The tritiated probe was placed in the lower compartment so that diffusion would take place in an upward direction. The diffusion studies were performed with tracer albumin and polysaccharide in the same starting phase for gravitational stability reasons. This initial condition is also particularly appropriate to study theoretically.

The diffusions were performed in such a way that there were four time points at 9, 18, 36 and 72 h studied for each diffusion system. For each time point there were generally eight experimental measurements. Experiments were carried out at a temperature of 22°C ± 1°C on a vibration-free bench.

After completion of the transport, the upper chamber contents were transferred to a counting vial. The chamber was then rinsed twice with 0.4 ml of distilled water. The rinsings were added to the sample. The entire 1 ml sample was counted for tritium content in a Wallac LKB liquid scintillation counter with a scintillant/sample ratio of 3:1. The scintillant used was OptiPhase "HiSafe" from Wallac Oy, Turku, Finland.

The diffusion coefficients were calculated using

the Line Fit function in the plotting progam SigmaPlot Version 5.0 from Jandel Scientific.

4. Results

4.1. Diffusion of albumin in model anionic polysaccharide matrices

Fig. 1 shows the variation of the amount of [^{3}H]albumin transferred from the dextran matrix to the upper chamber containing solvent as a function of the square root of time. These results demonstrate that the rate of transfer is represented by a constant diffusion coefficient (proportional to the gradient) with a correlation coefficient of 0.994. The feature of these results is that extrapolation would demonstrate that the diffusion coefficient describes the albumin transfer for all times up to and possibly including zero time. This means that the diffusion coefficient measured in this way will represent the intitial events associated with albumin transfer out of the dextran matrix, and therefore closely represent a stationary matrix-partition system.

Results in Table 1 demonstrate that albumin diffusion is increased by a factor of ≈ 2 in the presence of uncharged dextran at a concentration of 25 mg ml^{-1} in the lower compartment. Further stabilisation of the boundary with the inclusion of 5 mg ml^{-1} albumin in the lower compartment of the diffusion cell gave essentially the same result. The increase in the apparent diffusion coefficient is essentially a reflection of excluded volume effects on the partition coefficient of albumin in relation to dextran (see Section 5).

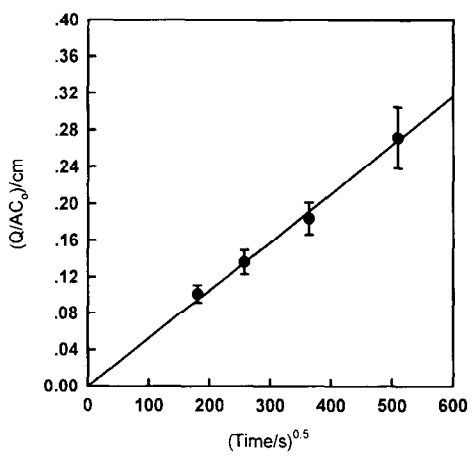

Fig. 1. Variation of the quantity Q/AC_0 (see Section 3.2), in units of cm, as a function of the square root of time for [^{3}H]albumin transfer from a 25 mg ml^{-1} dextran solution to solvent as measured in the diffusion cell. Values are means ± standard deviation ($n = 8$).

Table 1
Diffusion of [^{3}H]albumin from polysaccharide matrices contained within the lower compartment of the diffusion cell into PBS. The dextran sulfate used in most studies had $M_w \approx 500000$ unless otherwise stated

Lower compartment	$D_{app}/10^{-7}$ cm^2 s^{-1}	n [a]
5 mg ml^{-1} BSA	5.31 ± 0.50	96
25 mg ml^{-1} dextran T500	10.44 ± 1.10	96
25 mg ml^{-1} dextran T500, 5 mg ml^{-1} albumin	10.21 ± 0.85	32
50 mg ml^{-1} dextran sulfate	11.94 ± 0.86	64
50 mg ml^{-1} dextran sulfate, 5 mg ml^{-1} albumin	10.43 ± 0.51	32
50 mg ml^{-1} dextran sulfate ($M_w = 39700$)	14.56 ± 1.34	64
50 mg ml^{-1} heparin	10.67 ± 1.42	24
50 mg ml^{-1} heparin, 5 mg ml^{-1} albumin	8.60 ± 1.57	32

[a] The number of determinations n is equal to the number of different times analysed times the number of repeat experiments.

To examine the direct effect of charge on the partition–diffusion of albumin, the transport studies were made with dextran sulfate at a concentration of 50 mg ml^{-1}, which represents the same volume fraction of dextran as compared with dextran studied at a concentration of 25 mg ml^{-1} (this has been calculated on the basis of 17% sulfur content in the dextran sulfate in the form of substituted sodium sulfate). Dextran sulfate at 50 mg ml^{-1} also corresponds to a charge concentration of approximately 250 meq l^{-1}, which is considerably higher than that measured in capillary walls (see above). Albumin transport at this dextran sulfate concentration was essentially the same as that measured for the same volume fraction of dextran (Table 1). The albumin transport increased slightly for a lower molecular weight dextran sulfate matrix. The inclusion of 5 mg

ml^{-1} albumin in the lower compartment again did not make any significant difference. These results demonstrate that the charge of 250 meq l^{-1} had essentially no effect on the partition–transport of albumin, and the major factor governing the partition–diffusion value was the steric excluded volume effect. The albumin transport studies with heparin polysaccharide matrices were identical to those obtained with dextran sulfate matrices at the same concentration (Table 1).

The studies reported in Table 1 are associated with solutions that were made up with the same buffer (for convenience), so that they are not in thermodynamic equilibrium in relation to the simple electrolyte (NaCl) distribution. Thermodynamic equilibrium will result in the lowering of the NaCl concentration in the anionic polysaccharide matrix to approximately 0.125 M [21]. The influence of lowering the NaCl concentration on the transport of albumin is shown in Fig. 2. The effect is essentially negligible, as it is only at relatively low NaCl concentrations that small changes are seen. This also confirms the lack of electrostatic interaction on the transport of albumin in anionic polysaccharide matrices.

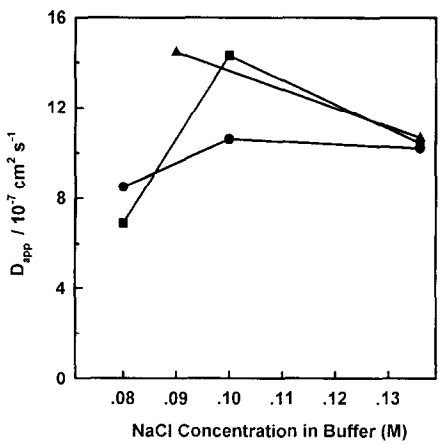

Fig. 2. Variation of the apparent diffusion coefficient D_{app} ($n = 32$) of [^{3}H]albumin as a function of the ionic strength of the phosphate buffer medium for diffusion from a 25 mg ml^{-1} dextran solution into PBS (●), diffusion from a 50 mg ml^{-1} dextran sulfate solution into PBS (■), and diffusion from a 50 mg ml^{-1} heparin solution into PBS (▲).

5. Discussion

The interaction between dextran and albumin is essentially an entropic excluded volume interaction without any significant enthalpic effects [22]. Eq. (21) would predict that the increase in albumin diffusion from a dextran matrix would be primarily determined by the partition coefficient which, in turn, would be described by excluded volume interactions. Laurent [15] and Ogston and Silpananta [16] evaluated the interaction coefficient in mass units (A^*) as 3.8×10^{-4}. We can convert this to the algebraic coefficient a in Eq. (20) such that $a = 10^{-3}A^*M_2M_3$, where M_2 and M_3 are the molecular weights of components 2 and 3 respectively. Using a concentration value of 25 mg ml^{-1} (as the diffusion coefficient is valid to times near zero time) we arrive at a predicted value of D_{app}/D^0_{app} of 1.665, where D^0_{app} is the diffusion coefficient of albumin (at 5 mg ml^{-1}, Table 1) in the absence of dextran. This predicted value is in very good agreement with the experimental value of 1.922 for dextran sulfate ($M_w \approx 500\,000$) calculated from the data in Table 1. The agreement is also good considering that we are utilising only second-order interaction terms. This close correspondence confirms the value of the partition–diffusion technique to achieve a quantitative measure of partition coefficients in macromolecular systems. The fact that there was essentially no difference between dextran, dextran sulfate and heparin suggests that the charge effect on albumin was negligible.

It is important to consider the extreme conditions under which efforts have been made to detect the putative influence of the electrostatic component of polyion–polyion interaction on excluded volume interactions. Bovine albumin has the highest valence of -18 of the albumins from different species [23] and this will maximise the potential for electrostatic interaction. Furthermore, construction of the matrix polyion solutions with 50 mg ml^{-1} dextran sulfate provides a very highly charged environment of approximately 250 meq l^{-1}. The average intercharge distance, assuming all saccharides are freely mobile, is approximately 2.2 nm. This is close to the intersaccharide distance along the polysaccharide chain of approximately 1 nm. The average interparticle (intermolecular) distance, based on hexagonal close

packing of spheres (assuming random coil conformation for the polysaccharides used), will be for 50 mg ml^{-1} heparin, dextran sulfate ($M_w = 39\,700$) and dextran sulfate ($M_w \approx 500\,000$) 8.7 nm, 13.8 nm and 31.7 nm respectively. When these average intercharge and interparticle distances are compared with the diameter of albumin of 7.2 nm, then it is evident that there is ample opportunity for significant interaction to occur at both the steric and the electrostatic level. The results would clearly indicate the marked dominance of steric interaction as compared with electrostatic interaction.

In the capillary permeability literature, discussion generally centres around the nature of the "pore" in the capillary wall and the nonhomogeneous distribution of charges associated with it. These pores have yet to be experimentally identified. In any case, with the extreme matrix concentrations used, we would suggest that distributions similar to the stochastic pore model would be covered by our model system. There is clearly an interaction of close proximity of albumin with the pores of our matrix model but this interaction is essentially only steric in nature.

These results confirm other studies in which albumin interaction with glycosaminoglycans or extracellular matrices in noncapillary systems has been interpreted on the basis of excluded volume effects alone and where charge effects were negligible. In studies of the equilibrium interaction of albumin and hyaluronan as measured by light scattering, Ogston and Preston [24] demonstrated that the excluded volume interaction was independent of ionic strength. Further, transport studies of albumin within polysaccharide matrices could be interpreted in terms of entropic–nonelectrostatic frictional interactions alone [25,26]. Maroudas and co-workers [27–29] demonstrated that albumin partitioning with slices of articular cartilage was directly related to the glycosaminoglycan content of the tissue and that the partitioning was a strongly decreasing function with increasing glycosaminoglycan content. These authors modelled their data quite satisfactorily on the basis of nonelectrostatic excluded volume effects.

The results of albumin partitioning in model systems studied here and elsewhere, together with studies performed in cartilage extracellular matrices, all point to the fact that charge effects associated with albumin–extracellular matrix (glycosaminoglycan) interaction are negligible. This has been confirmed in our own studies of transcapillary transport where, for both albumin and horseradish peroxidase, there was no charge selectivity once tubular reabsorption was inhibited [18]. Our previous work suggested that the Donnan partition could not account for the behaviour of interacting polysaccharide systems [9]. Although it has been previously proposed that charge effects would account for the partitioning of albumin at that glomerular capillary wall of the order of 100:1 or greater [10], the studies with albumin reported here now put into serious question the influence of the electrostatic component of polyion–polyion excluded volume interactions to generate any significant partitioning at all.

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References

[1] R.H. Adamson, V.H. Huxley and F.E. Curry, Am. J. Physiol., 254 (1988) H304.
[2] Y. Adal, L.M. Pratt and W.D. Comper, Microcirculation, 1 (1994) 169.
[3] J.A. Bertolatus and L.G. Hunsicker, Kidney Int., 28 (1985) 467.
[4] M.P. Bohrer, C. Baylis, H.D. Humes, R.J. Glassock, C.R. Robertson and B.M. Brenner, J. Clin. Invest., 61 (1978) 72.
[5] H.G. Rennke, Y. Patel and M.A. Venkatachalam, Kidney Int., 13 (1978) 324.
[6] M.A. Perry, J.N. Benoit, P.R. Kvietys and D.N. Granger, Am. J. Physiol., 245 (1983) G568.
[7] J.C. Parker, S. Gilchrist and J.T. Cartledge, J. Appl. Physiol., 59 (1985) 1128.
[8] W.M. Deen, B. Satvat and J.M. Jamieson, Am. J. Physiol., 241 (1980) F126.
[9] O. Zamparo and W.D. Comper, Biophys. Chem., 38 (1990) 167.
[10] W.D. Comper and E.F. Glasgow, Kidney Int., 47 (1995) 1242.
[11] D.G. Miller, J. Phys. Chem., 63 (1959) 570.
[12] E. Edmond and A.G. Ogston, Biochem. J., 109 (1968) 569.
[13] K.S. Speigler, Trans. Faraday Soc., 54 (1952) 1408.
[14] W.D. Comper, P.M. MacDonald and B.N. Preston, J. Phys. Chem., 88 (1984) 6031.

[15] T.C. Laurent, Biochem. J., 89 (1963) 253.
[16] A.G. Ogston and P. Silpananta, Biochem. J., 116 (1970) 171.
[17] B.F. Tack, J. Dean, D. Eilat, P.E. Lorenz and A.N. Schechter, J. Biol. Chem., 255 (1980) 8842.
[18] T.M. Osicka, L.M. Pratt and W.D. Comper, Nephrology, 2 (1996) 199.
[19] L.-O. Sundelöf, Anal. Biochem., 127 (1982) 282.
[20] M.-P. van Damme, W.D. Comper and B.N. Preston, J. Chem. Soc. Faraday Trans. 1, 78 (1982) 3357.
[21] R.P.W. Williams, Cartilage proteoglycans and osmotic flow, Ph.D. Thesis, Monash University, 1988.
[22] W.D. Comper and T.C. Laurent, Biochem. J., 175 (1978) 703.
[23] T. Peters, Adv. Protein Chem., 37 (1985) 161.
[24] A.G. Ogston and B.N. Preston, J. Biol. Chem., 241 (1966) 17.
[25] T.C. Laurent, I. Björk, A. Pietruszkiewicz and H. Persson, Biochim. Biophys. Acta, 78 (1963) 351.
[26] A.G. Ogston, B.N. Preston and J.D. Wells, Proc. R. Soc. London A, 333 (1973) 297.
[27] A. Maroudas, in M.A.R. Freeman (Ed.), Adult Articular Cartilage, Pitman Medical, Tunbridge Wells, 1979, p. 215.
[28] A. Maroudas, R. Schneiderman and O. Popper, in K.E. Kuettner, R. Schleyerbach, J.G. Peyron and V.C. Hascall (Eds.), Articular Cartilage and Osteoarthritis, Raven Press, New York, 1991, p. 355.
[29] J. McK. Snowden and A. Maroudas, Biochim. Biophys. Acta, 428 (1976) 726.