# Regulation of Cell Volume by Glycosaminoglycans

Jelena Joerges,[1] Tobias Schulz,[1] Jeannine Wegner,[1] Udo Schumacher,[2] and Peter Prehm[1*]

[1]Institute of Physiological Chemistry and Pathobiochemistry, Muenster University Hospital, Waldeyerstrasse 15, D-48149 Münster, Germany

[2]Institut für Anatomie und Experimentelle Morphologie, Universitätsklinikum Hamburg-Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany

## ABSTRACT

Cell volume is regulated by a delicate balance between ion distribution across the plasma membrane and the osmotic properties of intra- and extracellular components. Using a fluorescent calcein indicator, we analysed the effects of glycosaminoglycans on the cell volume of hyaluronan producing fibroblasts and hyaluronan deficient HEK cells over a time period of 30 h. Exogenous glycosaminoglycans induced cell blebbing after 2 min and swelling of fibroblasts to about 110% of untreated cell volume at low concentrations which decreased at higher concentrations. HEK cells did not show cell blebbing and responded by shrinking to 65% of untreated cell volume. Heparin induced swelling of both fibroblasts and HEK cells. Hyaluronidase treatment or inhibition of hyaluronan export led to cell shrinkage indicating that the hyaluronan coat maintained fibroblasts in a swollen state. These observations were explained by the combined action of the Donnan effect and molecular crowding. J. Cell. Biochem. 113: 340–348, 2012. © 2011 Wiley Periodicals, Inc.

KEY WORDS: HYALURONAN; CHONDROITIN SULPHATE; HEPARIN; CELL VOLUME; EXTRACELLULAR MATRIX; DONNAN EFFECT; MOLECULAR CROWDING

Cell volume serves as signal for a wide variety of responses including proliferation, migration, apoptosis, oncotic necrosis and transepithelial transport [Hoffmann et al., 2009]. It is regulated by the intracellular content of osmotically active compounds and the extracellular tonicity, because the plasma membrane is highly permeable to water. Normally cells counteract swelling by KCl release and shrinkage by KCl gain. The ratio of intra- and extracellular $K^+$ concentration in turn determines the membrane potential, another critical parameter of cellular metabolism. Glycosaminoglycans exert a strong osmotic pressure and often concentrate locally on the cell surface by receptor binding. High molecular weight hyaluronan has exceptional properties in particular, as it displays molecular crowding which in turn pushes other macromolecules from its hydration volume due to steric exclusion. This potentiates the osmotic pressure [Laurent and Ogston, 1963]. While many studies investigated the osmotic behaviour of glycosaminoglycans in solution [Ogston, 1966; Maroudas, 1975; Maroudas and Venn, 1977; Urban et al., 1979; Maroudas and Bannon, 1981; Urban and Maroudas, 1981; Maroudas et al., 1985; Reed and Rodt, 1991; Peitzsch and Reed, 1992; Knepper et al., 2003], their influence on the cell volume has been neglected so far. The precise effect of exogenous glycosaminoglycans on the cell volume has to be determined experimentally, because the three parameters cell volume, intra- and extracellular ion distribution and membrane potential exhibit a complex relationship [Fraser and Huang, 2004]. Recently, we demonstrated that high molecular weight hyaluronan has a profound depolarising effect on the membrane potential [Hagenfeld et al., 2010].

The role of hyaluronan in regulating cell volume and tissue hydration is of particular interest, because it has such an enormous hydration volume. Hyaluronan is synthesised at the inner side of plasma membranes [Prehm, 1984] and exported by the ABC transporters MRP5 from fibroblasts [Schulz et al., 2007] and CFTR from epithelial cells [Schulz et al., 2010]. Hyaluronan export from fibroblasts is inhibited by intracellular cGMP which also acts as a vasodilator [Carvajal et al., 2000], and mediator of tissue hydration [Metry et al., 2001].

## MATERIALS AND METHODS

### MATERIALS

Bacterially derived hyaluronan with an average molecular weight of $1.4 \times 10^6$ Da [Shiedlin et al., 2004], hyaluronidase from testis or *Streptomyces* and other chemicals were from Sigma Chemical Co.

## CELLS AND CELL CULTURE

Epithelial breast cancer cells HMT3552 [Jojovic et al., 2002], human embryonic kidney cells (HEK) [Hagenfeld et al., 2010], primary human skin fibroblasts cultures from wild type and MRP5 deficient mice [Schulz et al., 2010] have been described. Cells were grown in Dulbecco's medium supplemented with streptomycin/penicillin (100 units of each/ml), 10% fetal calf serum; else they were grown in serum-free Quantum medium supplemented with streptomycin/penicillin (100 units of each/ml), kanamycin (100 units/ml) on 96-well microtiter plates.

## DETERMINATION OF THE CELL VOLUME

Relative changes in the cell volume were determined by the fluorescent calcein acetoxymethyl ester (calcein-AM) method [Capó-Aponte et al., 2005] with slight modifications. Cells were grown in 96-well plates and the original medium was replaced with serum-free Quantum medium containing $10\,\mu M$ calcein-AM for 60 min at $37^{\circ}C$. Cells were then washed twice and incubated with Quantum medium as control and Quantum medium containing varying concentrations of glycosaminoglycans and hyaluronidase. Initial readings were taken for 5 min at intervals of 30 s, then after every 5 min until a steady state was reached. Changes in fluorescence were monitored from the bottom of the wells at excitation and emission wavelengths of 485 and 525 nm respectively. Prior to every experimental run, initial fluorescence values were taken for every well. These were set to 1 for subsequent calculation of the relative volume changes. Independent of cell volume changes, raw data were also corrected for fluorescence drift by calculating the ratio of fluorescence of the sample and control wells for each time point. Diagrams were plotted as relative volume in percent ($100 \times V_t/V_0$), as a function of time, where $V_t$ is the cell volume at time t; $V_0$ is the initial relative cell volume (set to 1). The data were recorded as triplicates which had an error of <2%. The statistical significance was analysed by the ANOVA test and the significance was calculated by the areas under the curves. Post hoc multiple comparison tests were applied to compare mean values. These calculations indicated that differences of 2% relative volume changes were significant. The effects of hyaluronan and chondroitin sulphate on fibroblasts and HEK cells were also visualised by fluorescent microscopy of calcein labelled cells.

## RESULTS

### CELL VOLUME CHANGES INDUCED BY EXTRACELLULAR HYALURONAN

As cell volume changes might be induced by the osmotic pressures exhibited by the glycosaminoglycan solutions, we determined the osmolarities for each solution in a preliminary experiment. The culture medium had an osmotic pressure of 318 mOsm and the increase by the glycosaminoglycans stayed within the range of experimental error up to concentrations of 2 mg/ml. Theoretically, a hyaluronan solution of 2 mg/ml should have an osmotic pressure of 5.6 mOsm raising the osmotic pressure of the medium by 1.8%.

The influence glycosaminoglycans exerted on the cell volume was studied with hyaluronan-producing fibroblasts, which retain an extensive hyaluronan coat on the cell surface by binding to the CD44 receptor, and with hyaluronan-deficient human embryonic kidney cells (HEK). The HEK cell line was also devoid of the CD44 receptor as determined by a sensitive immunostaining method [Mitchell et al., 1996]. Relative volume changes of human fibroblasts and HEK cells were recorded by the fluorescent calcein-AM method in the presence of increasing concentrations of high molecular weight hyaluronan over a period of 30 h. Figure 1A shows that extracellular hyaluronan induced a concentration- and time-dependent volume response. For lower concentrations up to 0.5 mg/ml, it steadily increased the fibroblast volume in a concentration-dependent manner to a maximum for about 14 h. Then it dropped again. At a concentration of 1 mg/ml the time-dependent changes were marginal. Merely a volume decrease was observed at 2 mg/ml after about 18 h. HEK cells responded to extracellular hyaluronan only by a volume decrease in a concentration-dependent manner (Fig. 1B).

The calcein-loaded fibroblasts and HEK cells were also observed under fluorescence microscopy after the addition of culture medium containing 1 mg/ml of hyaluronan or chondroitin sulphate over a period of 10 min (Fig. 2). Both hyaluronan and chondroitin sulphate induced blebbing 4 min after addition. This blebbing occurred with hyaluronan preparations from rooster comb as well as with hyaluronan from *Streptococcus equi* and appeared more prominent at higher concentrations. In contrast, HEK cell did not show this blebbing (data not shown).

These results suggested that multiple factors determined cell volume as a function of concentration, time and nature of cell line. The difference in response patterns between hyaluronan coated fibroblasts and hyaluronan-deficient HEK cells indicated that the hyaluronan coat may have profound effects on the regulation of the cell volume regulation.

### FIBROBLASTS VOLUME CHANGES BY HYALURONIDASE TREATMENT

In order to verify that the above observations indeed have a physiological significance, fibroblasts were treated with hyaluronidases for them to digest hyaluronan from the cell surface. Figure 3 shows that hyaluronidases caused a reduction in cell volume within 4 h. Thus, the endogenous hyaluronan cell surface coat appeared to increase the cell volume.

### CELL VOLUME CHANGES BY EXTRACELLULAR CHONDROITIN SULPHATE

Chondroitin sulphate also caused volume changes in fibroblasts (Fig. 4A) and HEK cells (Fig. 4B) in concentration- and time-dependent manner that resembled the hyaluronan effect. This result suggested that hyaluronan and chondroitin sulphate had similar cellular functions.

### CELL VOLUME CHANGES BY EXTRACELLULAR HEPARIN

Heparin at different concentrations markedly increased the cell volume of fibroblasts (Fig. 5A) and even more pronouncedly of HEK cells (Fig. 5B). This result suggested that the hyaluronan coat may attenuate the swelling effect of heparin.
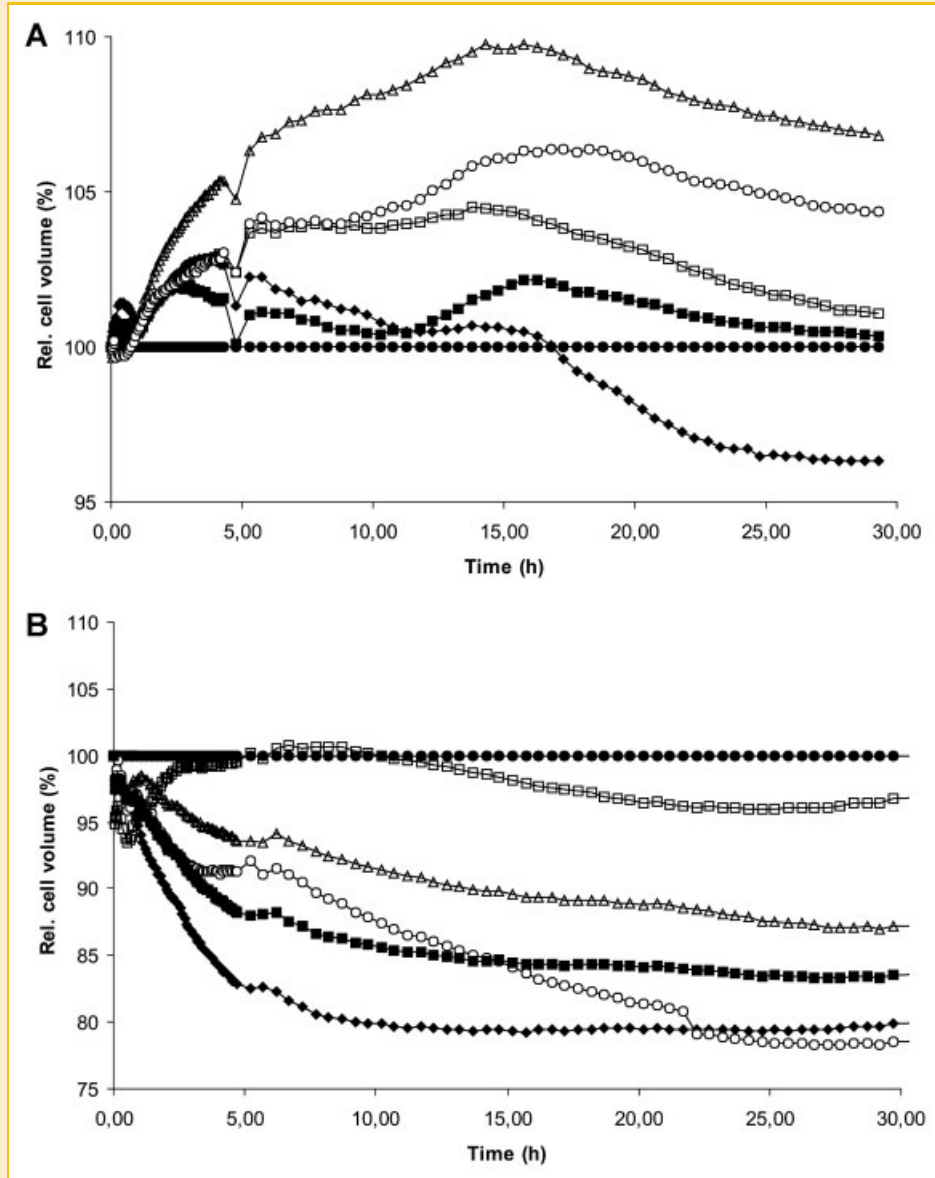
Fig. 1. Volume changes by extracellular hyaluronan. Human fibroblasts (A) and HEK cells (B) were grown in 96-well microtiter plates to confluency. The media were changed to serum-free Quantum containing 10 µg/ml calcein-AM and incubated for 1 h to load the cells with the volume-sensitive dye. The cells were washed and incubated with Quantum containing 2 mg/ml (◆), 1 mg/ml (■), 0.5 mg/ml (△), 0.25 mg/ml (□), 0.125 mg/ml (○) hyaluronan and control medium without hyaluronan (●).

## CELL VOLUME CHANGES BY HYALURONAN EXPORT INHIBITION

We have previously shown that hyaluronan export from fibroblasts through MRP5 can be inhibited by zaprinast, a cGMP analogue that blocks both, phosphodiesterase 5 and hyaluronan exporter MRP5 [Deiters and Prehm, 2008]. Export from epithelial cells through CFTR can be inhibited by GlyH101 which clogs the channel from outside and thus does not interfere with the intracellular metabolism [Muanprasat et al., 2004; Schulz et al., 2010]. We inhibited hyaluronan export by zaprinast from mouse fibroblasts, and by GlyH101 from MRP5-deficient mouse fibroblast and a human breast cancer cells that both express the CFTR channel. As a control for a cell line which does not produce hyaluronan, we used HEK cells. Figure 6 shows that export inhibition of hyaluronan-producing cells caused cell shrinkage, whereas HEK went through a transient shrinkage at 25 µM zaprinast and then swelled like the ones in the 50 µM treatment. This experiment indicated that accumulation of intracellular hyaluronan led to cell shrinkage.

## DISCUSSION

Glycosaminoglycans have several physicochemical effects in solution. First, they attract water. Second, they have an osmotic effect. This effect was responsible for the swelling pressure of corneal stroma [Friedman et al., 1972] or cartilage [Urban et al., 1979]. In our experiments these effects appeared marginal, because
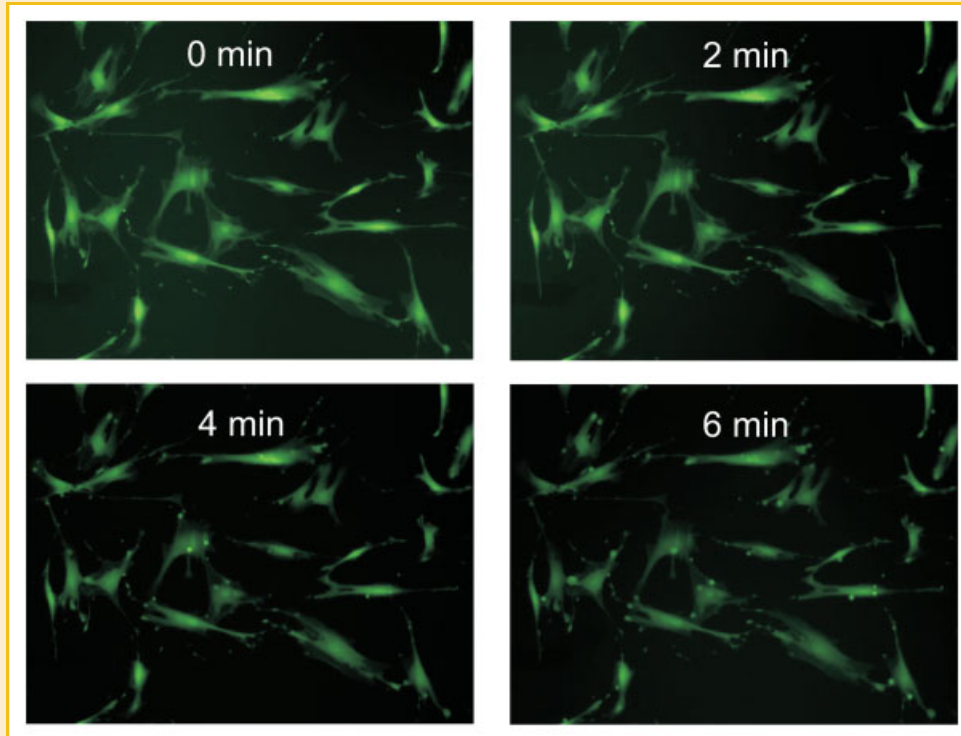
Fig. 2. Cell blebbing by hyaluronan in the culture medium. Fibroblasts were incubated serum–free Quantum containing 10 μg/ml calcein–AM and incubated for 1 h to load the cells with the volume–sensitive dye. The cells were washed and incubated with Quantum containing 1 mg/ml of hyaluronan at time point 0 over a period of 10 min. Fluorescent images were taken at the times indicated.
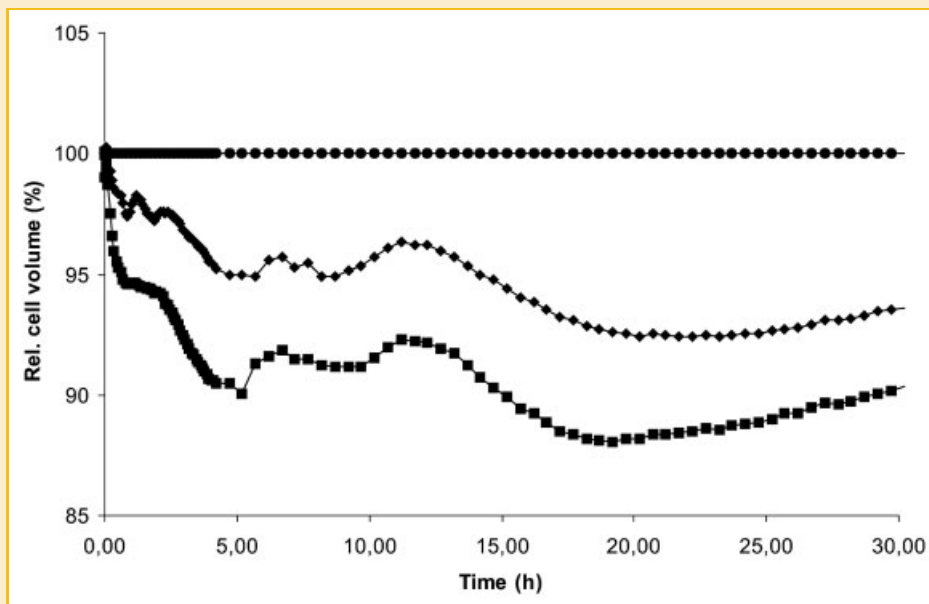


Fig. 3. Fibroblast volume changes through hyaluronidases. Human fibroblasts were grown in 96–well microtiter plates to confluency. The media were changed to serum–free Quantum containing 10 μg/ml calcein–AM and incubated for 1 h to load the cells with the volume–sensitive dye. The cells were washed and incubated with Quantum containing 2 mg/ml hyaluronidase from bovine testis (■) or from Streptomyces (◆) and control medium without hyaluronidases (●).
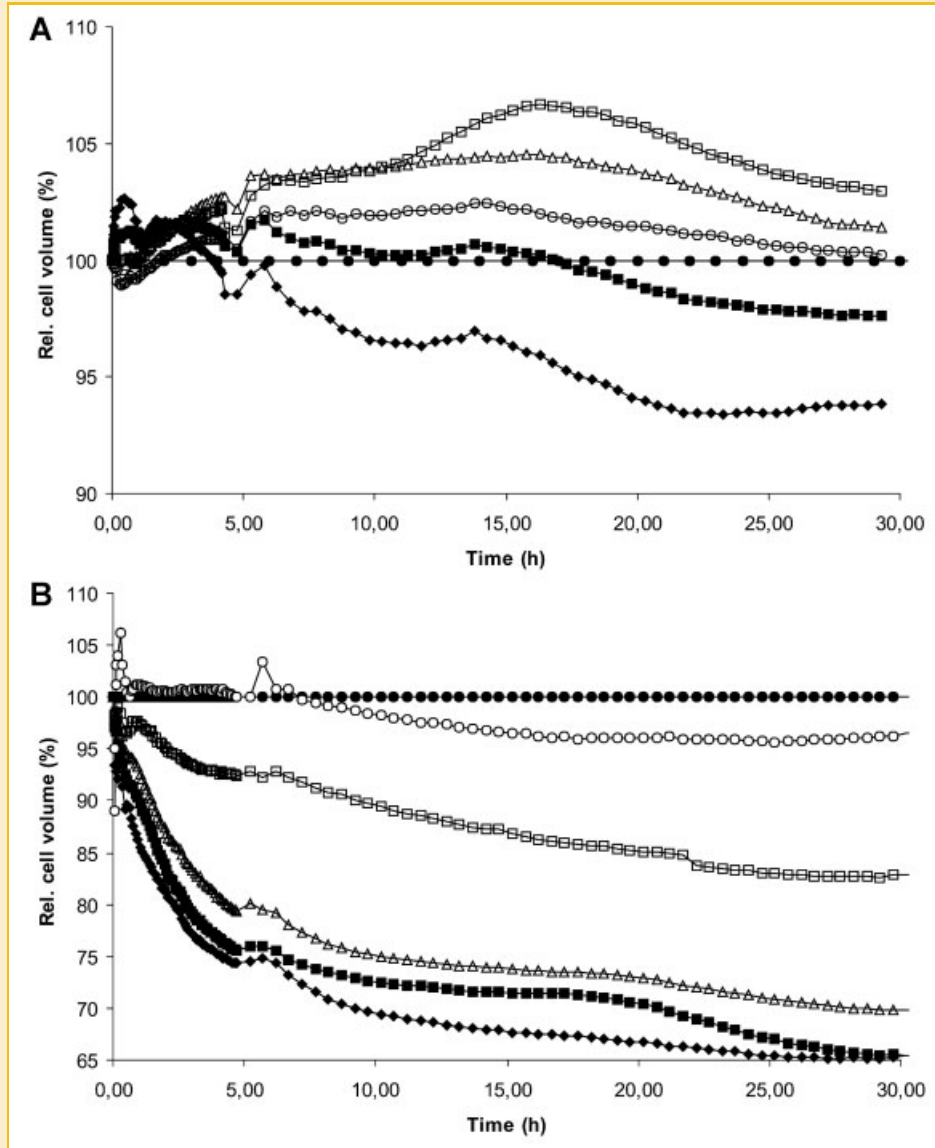
Fig. 4. Cell volume changes through extracellular chondroitin sulphate. Human fibroblasts (A) and HEK cells (B) were grown to confluency in 96-well microtiter plates. The media were changed to serum-free Quantum containing 10 μg/ml calcein-AM and incubated for 1 h to load the cells with the volume-sensitive dye. The cells were washed and incubated with Quantum containing 2 mg/ml (◆), 1 mg/ml (■), 0.5 mg/ml (△), 0.25 mg/ml (□), 0.125 mg/ml (○) chondroitin sulphate and control medium without chondroitin sulphate (●).

the glycosaminoglycans did not significantly enhance the osmotic pressure of the culture medium. Third, the Donnan effect influences the membrane potential and drives counterions into the neighbouring compartment. In our recent publication [Hagenfeld et al., 2010], we found that a solution of 2 mg/ml of hyaluronan caused a pH shift of 0.9 units over a dialysis membrane which converts into an eightfold concentration difference. Such an increase in concentration of counterions into the neighbouring compartment should also apply for other ions. Fourth, large glycosaminoglycans and in particular hyaluronan produce molecular crowding at higher concentrations for example exclusion of other macromolecules from their territory due to insufficient water for solvation. The phenomenon of steric exclusion is strongly dependent on molecular weight and concentrations. It is particularly large for high molecular

weight hyaluronan that has a critical concentration of about 0.1 mg/ml above which steric exclusion sets in [Laurent and Gergely, 1955; Laurent, 1964]. The water attraction leads to concentration of other macromolecules in the residual water and potentiates the osmotic pressure. So far, molecular crowding has been reported to play an important role in osmosensing and cell volume regulation in bacteria [Al-Habori, 2001; Poolman et al., 2004]. Fifth, the Donnan osmotic effect in combination with molecular crowding leads to fixed charge densities in the inhomogeneous micro-areas where the concentration of glycosaminoglycan is higher. The glycosaminoglycans then behave like ion exchangers and impede-free diffusion of counterions [Wu, 1926; Maroudas, 1968]. The influence of fixed charge densities and the Donnan effect on pressure increase, tissue swelling and oedema has only recently been acknowledged [Elkin
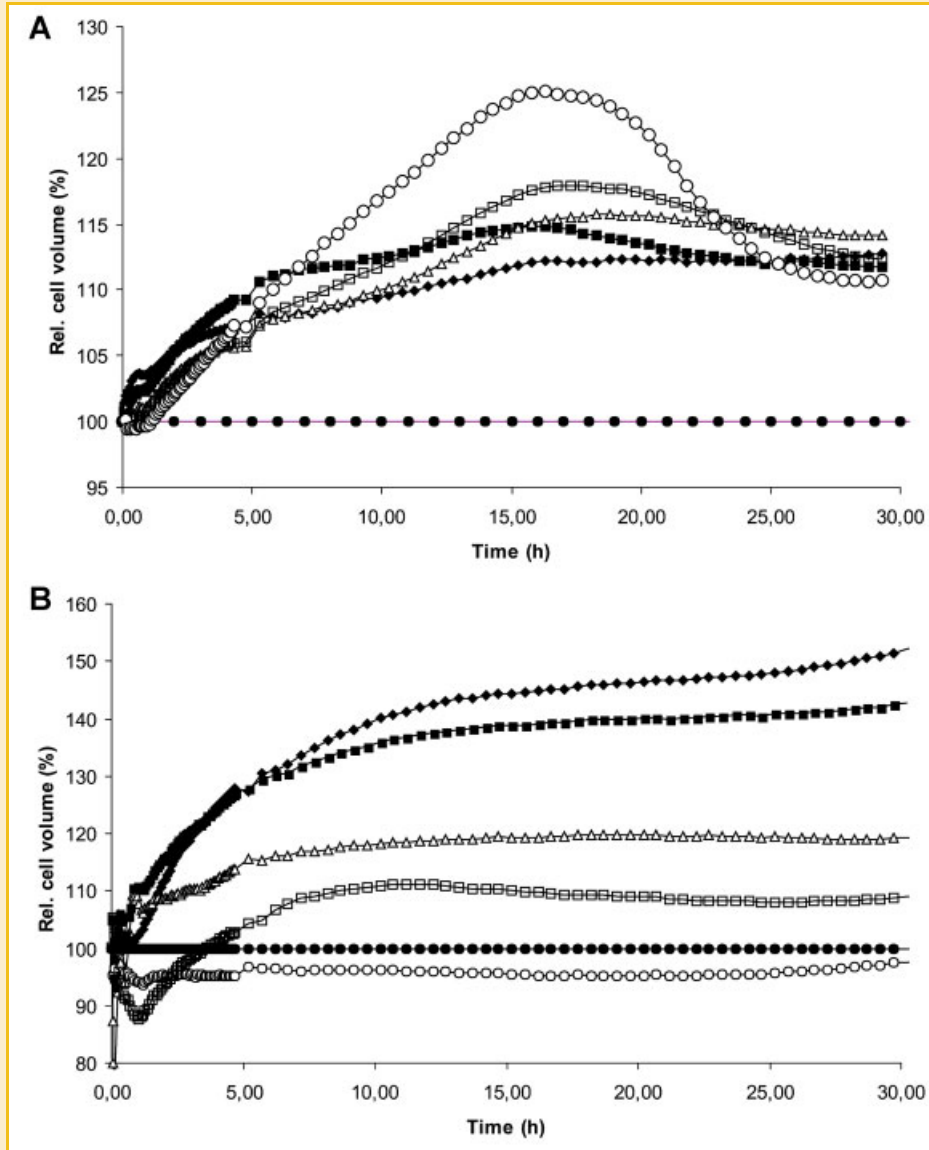
Fig. 5. Cell volume changes through extracellular heparin. Human fibroblasts (A) and HEK cells (B) were grown in 96-well microtiter plates to confluency. The media were changed to serum-free Quantum containing 10 μg/ml calcein-AM and incubated for 1 h to load the cells with the volume-sensitive dye. The cells were washed and incubated with Quantum containing 2 mg/ml (◆), 1 mg/ml (■), 0.5 mg/ml (△), 0.25 mg/ml (□), 0.125 mg/ml (○) heparin and control medium without heparin (●).

et al., 2010]. The final outcome of the interaction of cells and extracellular matrix is difficult to predict, as cellular ion pumps and channel gating will attempt to maintain homeostasis.

Cell surface receptors for glycosaminoglycans provide a concentrated glycocalix which is particularly large for hyaluronan retained by CD44. The chemical properties of hyaluronan are exceptional among glycosaminoglycans, because it has the largest molecular weight ($>10^6$ Da) and lowest charge density. Chondroitin sulphate has a lower molecular weight ($<5 \times 10^4$ Da) and has a higher charge density due to sulphation. It can also self-aggregate [Scott et al., 1995] and binds to CD44 [Kawashima et al., 2000]. Heparin, too, has a lower molecular weight and possesses the highest charge density. All these properties must be taken into account, if an understanding of the determinants of cell volume is the aim.

We must also consider, whether the employed glycosaminogly-can concentrations of 0.125–2 mg/ml are physiologically relevant in vivo. Human adult skin has hyaluronan concentrations of about 0.5 mg/g dry weight and 0.1 mg/g wet weight [Oh et al., 2011]. The distribution of glycosaminoglycans in skin is as follows: 56% consists of hyaluronan, 15.6% is dermatan sulphate and 9.1% makes up the remaining chondroitin sulphate [Hardingham and Phelps, 1970], a fact that is roughly reflected by the production of skin fibroblasts in cell culture [Kapoor et al., 1983]. In cell culture, human skin fibroblasts produce about 10 μg/ml of hyaluronan within 24 h of which 14% remain associated with cells creating a more than 10 μm thick hyaluronan coat. The amount of intracellular hyaluronan shows a near to twofold increase, when hyaluronan export is inhibited [Schulz et al., 2010]. Epithelial cells prodzuce
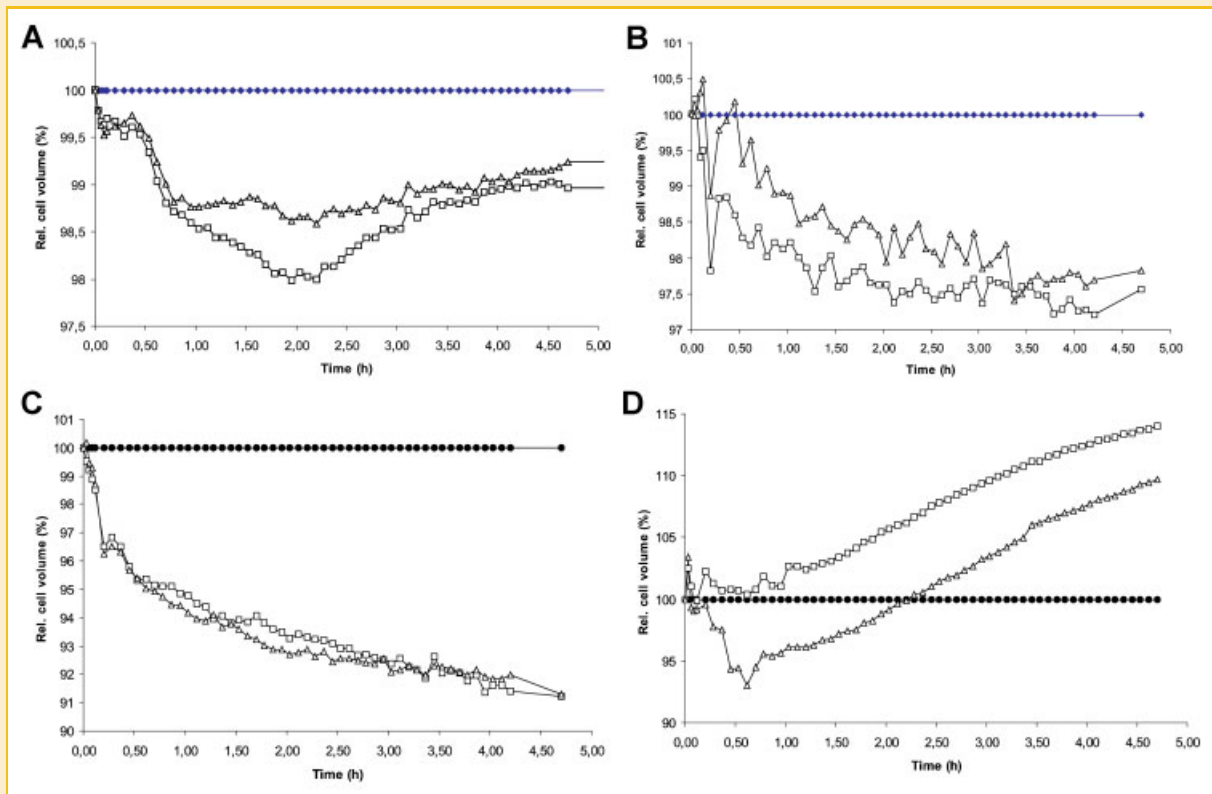
Fig. 6. Cell volume changes through inhibition of hyaluronan export. Human fibroblasts expressing MRP5 (A), MRP5-deficient mouse fibroblasts expressing CFTR (B), human breast cell carcinoma cells expressing CFTR (C) and HEK cells that did not produce hyaluronan (D) were grown in 96-well microtiter plates to confluency. The media were changed to serum-free Quantum containing 10 µg/ml calcein-AM and incubated for 1 h to load the cells with the volume-sensitive dye. The cells were washed and incubated with Quantum containing the hyaluronan export inhibitors zaprinast (A,D) and GlyH101 (B,C) at 25 µM ($\triangle$), or 50 µM ($\square$). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

about tenfold less hyaluronan. The amount of heparin in skin was determined to be 400–800 µg/g dry weight [Straus et al., 1984].

The kidney medulla, which is involved in water balance, contains about 1 mg of glycosaminoglycans per gram of dry tissue with a distribution of 54.6% hyaluronan, 10.1% chondroitin sulphate, 13.9% heparan sulphate and 13% dermatan sulphate [Vasan et al., 1983]. With a water content of about 77% [Jansen, 1961], this converts to about 180 mg of hyaluronan, 33 mg of chondroitin sulphate, 46 mg of heparan sulphate and 43 mg of dermatan sulphate per gram of wet weight. These data indicate that the concentrations of glycosaminoglycans used in this study are in the range of physiological conditions.

Our results showed that the glycosaminoglycans caused cell immediate blebbing and sustained swelling of fibroblasts which was more prominent at lower concentrations. Cell blebbing and regulatory volume increase are survival mechanisms under stress conditions due to massive chloride influx followed by influx of water [Svoboda et al., 2009; Hoffmann et al., 2009]. Enhanced cell blebbing should reduce the cell volume and may explain that lower concentrations of glycosaminoglycans yielded higher volume increase. It is also possible that molecular crowding for example the attraction of water for self hydration, became more prominent at higher glycosaminoglycan concentrations. The driving force for chloride influx is likely to be the Donnan effect exerted by extracellular glycosaminoglycans which was also responsible for the depolarisation of plasma membranes [Hagenfeld et al., 2010].

The kinetic response curves for fibroblasts also showed an interesting feature, as they peaked at about 18 h. This may indicate that the cells underwent cell division during this time period, because quiescent cells had been stimulated with new growth medium at time point 0. Removal of the hyaluronan coat from fibroblasts or inhibition of hyaluronan export led to cell shrinkage. The latter result indicated that the hyaluronan coat of fibroblasts maintained the cells in a swollen state. This might have been even be boosted by additional glycosaminoglycans in the culture medium. In contrast, HEK cells swelled only in the presence of heparin and shrunk in the presence of hyaluronan and chondroitin sulphate.

Heparin stood out in its effect on HEK cells, as it caused swelling in contrast to hyaluronan and chondroitin sulphate. Heparin is also exceptional amongst the glycosaminoglycans as it has an extremely high affinity for $Ca^{2+}$ with a $K_D = 31$ µM, compared to 310 µM for chondroitin sulphate and 1,408 µM for hyaluronan [Hunter et al., 1988]. With 4 negative charges per heparin disaccharide, a solution of 2 mg/ml has the capacity of binding roughly 4.7 mM of $Ca^{2+}$, whereas the normal $Ca^{2+}$ concentration in culture medium is only about 2 mM. Thus, heparin behaves like EDTA which is known to increase the cell volume in astrocytes [Olson et al., 1990]. The mechanism might be depletion of intracellular $Ca^{2+}$, closure

of $Ca^{2+}$-activated $K^+$ channels and increase of intracellular osmotically active $K^+$ concentration.

Inhibition of hyaluronan export led to cell shrinkage in all hyaluronan producing cell lines. This effect could also be explained by the prevalence of the Donnan effect that expelled salt outside to decrease the intracellular osmotic pressure. Under physiological conditions, hyaluronan export is inhibited by intracellular cGMP [Schulz et al., 2007]. At high intracellular cGMP concentrations, hyaluronan remains in the cytosol where it can inhibit its own chain elongation through its synthase [Lüke and Prehm, 1999].Furthermore, the amount of extracellular hyaluronan decreases and again reduces the level of hydration of the extracellular matrix, because the existing hyaluronan is turned over by CD44-mediated endocytosis, intracellular degradation or dissipation with a half life of 1–2 days [Fraser et al., 1997; Rugheimer et al., 2009]. Thus, intracellular hyaluronan accumulation and extracellular hyaluronan loss both lead to tissue constriction and dehydration.

This scenario stands in marked contrast to the pathological situation of edema formation. There are ample, hitherto unrecognised correlations between intracellular cGMP levels and hyaluronan production resulting in edema formation. Pulmonary inflammation is accompanied by increased hyaluronan production [Teder et al., 1995] and post-transplant lung edema can be treated by bromo-cGMP [Sandera et al., 2000]. In the post-ischaemic heart, the cardioprotective NO signalling and cGMP levels are depressed [Itoh et al., 2006] which might very well be the reason for the observed hyaluronan overproduction in myocardial tissue after infarction [Waldenstrom et al., 1991]. Similarly, diabetic nephropathy is characterised by decreased NO synthase activity and cGMP levels [Khamaisi et al., 2006]. Diminished competition with hyaluronan export could lead to the observed hyaluronan accumulation in the ischaemic inflamed renal cortex of diabetic rats [Melin et al., 2006]. These correlations indicated that tissue hydration or edema formation may be regulated by intracellular cGMP levels controlling hyaluronan export by MRP5.

It has long been known that accumulation of hyaluronan in the extracellular matrix led to tissue hydration and swelling [Hansell et al., 2000]. In conclusion, our results showed that the interstitial hyaluronan also caused swelling of the producing fibroblasts.


## ACKNOWLEDGMENTS

## REFERENCES

Al-Habori M. 2001. Macromolecular crowding and its role as intracellular signalling of cell volume regulation. Int J Biochem Cell Biol 33:844–864.

Capó-Aponte JE, Iserovich P, Reinach PS. 2005. Characterization of regulatory volume behavior by fluorescence quenching in human corneal epithelial cells. J Membr Biol 207:11–22.

Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP. 2000. Molecular mechanism of cGMP-mediated smooth muscle relaxation. J Cell Physiol 184:409–420.

Deiters B, Prehm P. 2008. Inhibition of hyaluronan export reduces collagen degradation in IL-1 treated cartilage. Arthritis Res Ther 10:R8.

Elkin BS, Shaik MA, Morrison B III. 2010. Fixed negative charge and the Donnan effect: A description of the driving forces associated with brain tissue swelling and oedema. Philos Transact A Math Phys Eng Sci 368:585–603.

Fraser JA, Huang CL. 2004. A quantitative analysis of cell volume and resting potential determination and regulation in excitable cells. J Physiol 559:459–478.

Fraser JRE, Laurent TC, Laurent UBG. 1997. Hyaluronan: Its nature, distribution, functions and turnover. J Intern Med 242:27–33.

Friedman MH, Kearns JP, Michenfleder CJ, Green K. 1972. Contribution of the Donnan osmotic pressure to the swelling pressure of corneal stroma. Am J Physiol 222:1565–1570.

Hagenfeld D, Schulz T, Ehling P, Budde T, Schumacher U, Prehm P. 2010. Depolarisation of the membrane potential by hyaluronan. J Cell Biochem 111:858–864.

Hansell P, Goransson V, Odlind C, Gerdin B, Hallgren R. 2000. Hyaluronan content in the kidney in different states of body hydration. Kidney Int 58:2061–2068.

Hardingham TE, Phelps CF. 1970. The glycosaminoglycans of neonatal rat skin. Biochem J 117:813–818.

Hoffmann EK, Lambert IH, Pedersen SF. 2009. Physiology of cell volume regulation in vertebrates. Physiol Rev 89:193–277.

Hunter GK, Wong KS, Kim JJ. 1988. Binding of calcium to glycosaminoglycans: An equilibrium dialysis study. Arch Biochem Biophys 260:161–167.

Itoh T, Haruna M, Abe K. 2006. Differential regulation of the nitric oxide-cGMP pathway deteriorates postischaemic heart injury in stroke-prone hypertensive rats. Exp Physiol 92:147–159.

Jansen H. 1961. Der Kalium-, Natrium- und Wassergehalt der inneren Organe zu bestimmten Zeiten nach dem Tode. Virchows Arch A Pathol Anat Histopathol 334:510–515.

Jojovic M, Delpech B, Prehm P, Schumacher U. 2002. Expression of hyaluronate and hyaluronate synthase in human primary tumours and their metastases in scid mice. Cancer Lett 188:181–189.

Kapoor R, Bourier S, Prehm P. 1983. Glycosaminoglycan synthesis in skin fibroblasts from patients with osteogenesis imperfecta. FEBS Lett 152:183–186.

Kawashima H, Hirose M, Hirose J, Nagakubo D, Plaas AH, Miyasaka M. 2000. Binding of a large chondroitin sulfate/dermatan sulfate proteoglycan, versican, to L-selectin, P-selectin, and CD44. J Biol Chem 275:35448–35456.

Khamaisi M, Keynan S, Bursztyn M, Dahan R, Reinhartz E, Ovadia H, Raz I. 2006. Role of renal nitric oxide synthase in diabetic kidney disease during the chronic phase of diabetes. Nephron Physiol 102:72–80.

Knepper MA, Saidel GM, Hascall VC, Dwyer T. 2003. Concentration of solutes in the renal inner medulla: Interstitial hyaluronan as a mechano-osmotic transducer. Am J Physiol Renal Physiol 284:F433–F446.

Laurent TC. 1964. The interaction between polysaccharides and other macromolecules. The exclusion of molecules from hyaluronic acid gels and solutions. Biochem J 93:106–112.

Laurent TC, Gergely L. 1955. Light scattering studies on hyaluronic acid. J Biol Chem 212:325–333.

Laurent TC, Ogston AG. 1963. The interaction between polysaccharides and other macromolecules. 4. The osmotic pressure of mixtures of serum albumin and hyaluronic acid. Biochem J 89:249–253.

Lüke HJ, Prehm P. 1999. Synthesis and shedding of hyaluronan from plasma membranes of human fibroblasts and metastatic and non-metastatic melanoma cells. Biochem J 343:71–75.

Maroudas A. 1968. Physicochemical properties of cartilage in the light of ion exchange theory. Biophys J 8:575–595.

Maroudas A. 1975. Biophysical chemistry of cartilaginous tissues with special reference to solute and fluid transport. Biorheology 12:233–248.

Maroudas A, Bannon C. 1981. Measurement of swelling pressure in cartilage and comparison with the osmotic pressure of constituent proteoglycans. Biorheology 18:619–632.

Maroudas A, Venn M. 1977. Chemical composition and swelling of normal and osteoarthrotic femoral head cartilage. II. Swelling. Ann Rheum Dis 36:399–406.

Maroudas A, Ziv I, Weisman N, Venn M. 1985. Studies of hydration and swelling pressure in normal and osteoarthritic cartilage. Biorheology 22: 159–169.

Melin J, Hellberg O, Funa K, Hallgren R, Larsson E, Fellstrom BC. 2006. Ischemia-induced renal expression of hyaluronan and CD44 in diabetic rats. Nephron Exp Nephrol 103:e86–e94.

Metry G, Hall C, Wikstrom B, Kallskog V, Hansell P, Danielson B. 2001. Fluid balance in patients with chronic renal failure assessed with N-terminal proatrial natriuretic peptide, atrial natriuretic peptide and ultrasonography. Acta Physiol Scand 171:117–122.

Mitchell BS, Whitehouse A, Prehm P, Delpech B, Schumacher U. 1996. CD44 exon variant 6 epitope and hyaluronate synthase are expressed on HT29 human colorectal carcinoma cells in a SCID mouse model of metastasis formation. Clin Exp Metastasis 14:107–114.

Muanprasat C, Sonawane ND, Salinas D, Taddei A, Galietta LJ, Verkman AS. 2004. Discovery of glycine hydrazide pore-occluding CFTR inhibitors: Mechanism, structure-activity analysis, and in vivo efficacy. J Gen Physiol 124:125–137.

Ogston AG. 1966. Measurement of osmotic properties of hyaluronic acid by equilibrium sedimentation. Fed Proc 25:1118–1119.

Oh JH, Kim YK, Jung JY, Shin JE, Kim KH, Cho KH, Eun HC, Chung JH. 2011. Intrinsic aging- and photoaging-dependent level changes of glycosaminoglycans and their correlation with water content in human skin. J Dermatol Sci 62:192–201.

Olson JE, Fleischhacker D, Murray WB, Holtzman D. 1990. Control of astrocyte volume by intracellular and extracellular $Ca^{2+}$. Glia 3:405–412.

Peitzsch RM, Reed WF. 1992. High osmotic stress behavior of hyaluronate and heparin. Biopolymers 32:219–238.

Poolman B, Spitzer JJ, Wood JM. 2004. Bacterial osmosensing: Roles of membrane structure and electrostatics in lipid–protein and protein–protein interactions. Biochim Biophys Acta 1666:88–104.

Prehm P. 1984. Hyaluronate is synthesized at plasma membranes. Biochem J 220:597–600.

Reed RK, Rodt SA. 1991. Increased negativity of interstitial fluid pressure during the onset stage of inflammatory edema in rat skin. Am J Physiol 260:H1985–H1991.

Rugheimer L, Olerud J, Johnsson C, Takahashi T, Shimizu K, Hansell P. 2009. Hyaluronan synthases and hyaluronidases in the kidney during changes in hydration status. Matrix Biol 28:390–395.

Sandera P, Hillinger S, Stammberger U, Schoedon G, Zalunardo M, Weder W, Schmid RA. 2000. 8-Br-cyclic GMP given during reperfusion improves post-transplant lung edema and free radical injury. J Heart Lung Transplant 19:173–178.

Schulz T, Schumacher U, Prehm P. 2007. Hyaluronan export by the ABC-transporter MRP5 and its modulation by intracellular cGMP. J Biol Chem 282:20999–21004.

Schulz T, Schumacher U, Prante C, Sextro W, Prehm P. 2010. Cystic fibrosis transmembrane conductance regulator can export hyaluronan. Pathobiology 77:200–209.

Scott JE, Heatley F, Wood B. 1995. Comparison of secondary structures in water of chondroitin-4-sulfate and dermatan sulfate—Implications in the formation of tertiary structures. Biochemistry 34:15467–15474.

Shiedlin A, Bigelow R, Christopher W, Arbabi S, Yang L, Maier RV, Wainwright N, Childs A, Miller RJ. 2004. Evaluation of hyaluronan from different sources: Streptococcus zooepidemicus, rooster comb, bovine vitreous, and human umbilical cord. Biomacromolecules 5:2122–2127.

Straus AH, Sant'anna OA, Nader HB, Dietrich CP. 1984. An inverse relationship between heparin content and antibody response in genetically selected mice. Sex effect and evidence of a polygenic control for skin heparin concentration. Biochem J 220:625–630.

Svoboda N, Pruetting S, Grissmer S, Kerschbaum HH. 2009. cAMP-dependent chloride conductance evokes ammonia-induced blebbing in the microglial cell line, BV-2. Cell Physiol Biochem 24:53–64.

Teder P, Nettelbladt O, Heldin P. 1995. Characterization of the mechanism involved in bleomycin- induced increased hyaluronan production in rat lung. Am J Respir Cell Mol Biol 12:181–189.

Urban JP, Maroudas A. 1981. Swelling of the intervertebral disc in vitro. Connect Tissue Res 9:1–10.

Urban JP, Maroudas A, Bayliss MT, Dillon J. 1979. Swelling pressures of proteoglycans at the concentrations found in cartilaginous tissues. Biorheology 16:447–464.

Vasan NS, Saporito RA, Jr, Saraswathi S, Tesoriero JV, Manley S. 1983. Alterations of renal cortex and medullary glycosaminoglycans in aging dog kidney. Biochim Biophys Acta 760:197–205.

Waldenstrom A, Martinussen HJ, Gerdin B, Hallgren R. 1991. Accumulation of hyaluronan and tissue edema in experimental myocardial infarction. J Clin Invest 88:1622–1628.

Wu H. 1926. Note on donnan equilibirium and osmotic pressure relationship between the cells and the serum. J Biol Chem 70:203–205.