PROTEOGLYCANS IN THE FILTRATION BARRIER OF THE ATLANTIC HAGFISH MYXINE GLUTINOSA

Lüder M. Fels[1], Brigitte Decker[2], Hilmar Stolte[1]
[1]Laboratory of Experimental Nephrology, Division of Nephrology, Department of Internal Medicine, [2]Laboratory of Cell Biology and Electron Microscopy, Medical School Hannover, Germany

Proteins of the extracellular matrix are important constituents of the glomerular filtration barrier. The composition and metabolism of extracellular matrix proteins like proteoglycans is of interest under aspects of (i) comparative physiology and (ii) pathophysiology. The first because it has been assumed that they play a key role in regulating the assembly and porosity of the basement membrane meshwork (Farquhar M.G., In: Hay E.D., ed. Cell biology of extracellular matrix, Plenum Press, New York, London, chapt. 11, p. 408, 1991). The latter because extracellular matrix can play an important role in nephrotoxic processes and in many renal diseases (Martinez-Hernandez A. et al., Lab. Invest. 6: 656, 1983). Proteoglycans have been demonstrated histochemically in the glomerular basement membrane of several vertebrates (Decker B., Reale E., Bas. Appl. Histochem. 35: 15, 1991) and characterised as heparansulfate-proteoglycans (Timpl R., Eur. J. Biochem. 180: 487, 1989). However, the biochemical composition of the filtration barrier of Myxine glutinosa has not yet been extensively studied, yet. In the study presented here, it was attempted to demonstrate proteoglycans histochemically with the cationic dye cuprolinic blue in glomeruli of the hagfish.

Preparation of glomeruli of the hagfish was performed essentially as described elsewhere (Fels et al., Renal Physiol. Biochem. 16: 276, 1993). Glomeruli were perfused in situ with Ringers solution to remove the blood. The fixative contained 2.5 % glutaraldehyde, 25 mM Na-acetate, 300 mM $MgCl_2$, 0.2% (w/v) cuprolinic blue, pH 5.6 (cuprolinic blue was a gift by Dr. T. Koob). A perfusion fixation of the glomeruli of 15 minutes was followed by an immersion fixation over night. Some tissue blocks were rinsed with 1% sodium tungstate in water for 30 minutes in order to better visualize the glycosaminoglycan-cuprolinic blue precipitate. Samples were dehydrated in alcohol, cleared in toluene, and embedded in Araldit CY 212. For an identification of the glycosaminoglycan composition of the proteoglycans, glomeruli were perfused free of blood, mechanically torn into smaller pieces, first incubated over night with heparitinase I (0.01 U/200 µl, in 50 mM Tris acetate, pH 8) or enzyme buffer as control, subsequently incubated with cuprolinic blue and immersion fixed.

Histochemical staining with cuprolinic blue followed by incubation with sodium tungstate revealed a predominant binding of the cationic dye in the electron dense glomerular basement membrane and along the epithelial surface coat (fig. 1a). The glomerular basement membrane and the network of electron dense structures was less intensively stainned without this sodium tungstate incubation. However, cuprolinic blue-glycosaminoglycan precipitates could still be observed (fig. 1b). The collagen fibrils in the mesangium showed staining with electron dense filament like structures. Following digestion with heparitinase I the network of cuprolinic blue-glycosaminoglycan precipitates was no longer visible in the glomerular basement membrane (fig. 1c).

The identification of proteoglycans in the glomerular basement membrane confirm earlier results on other vertebrates. The results with heparitinase I show that the glomerular basement membrane of Myxine glutinosa contains heparansulfate-proteoglycan and in this respect is comparable to that of higher vertebrates.

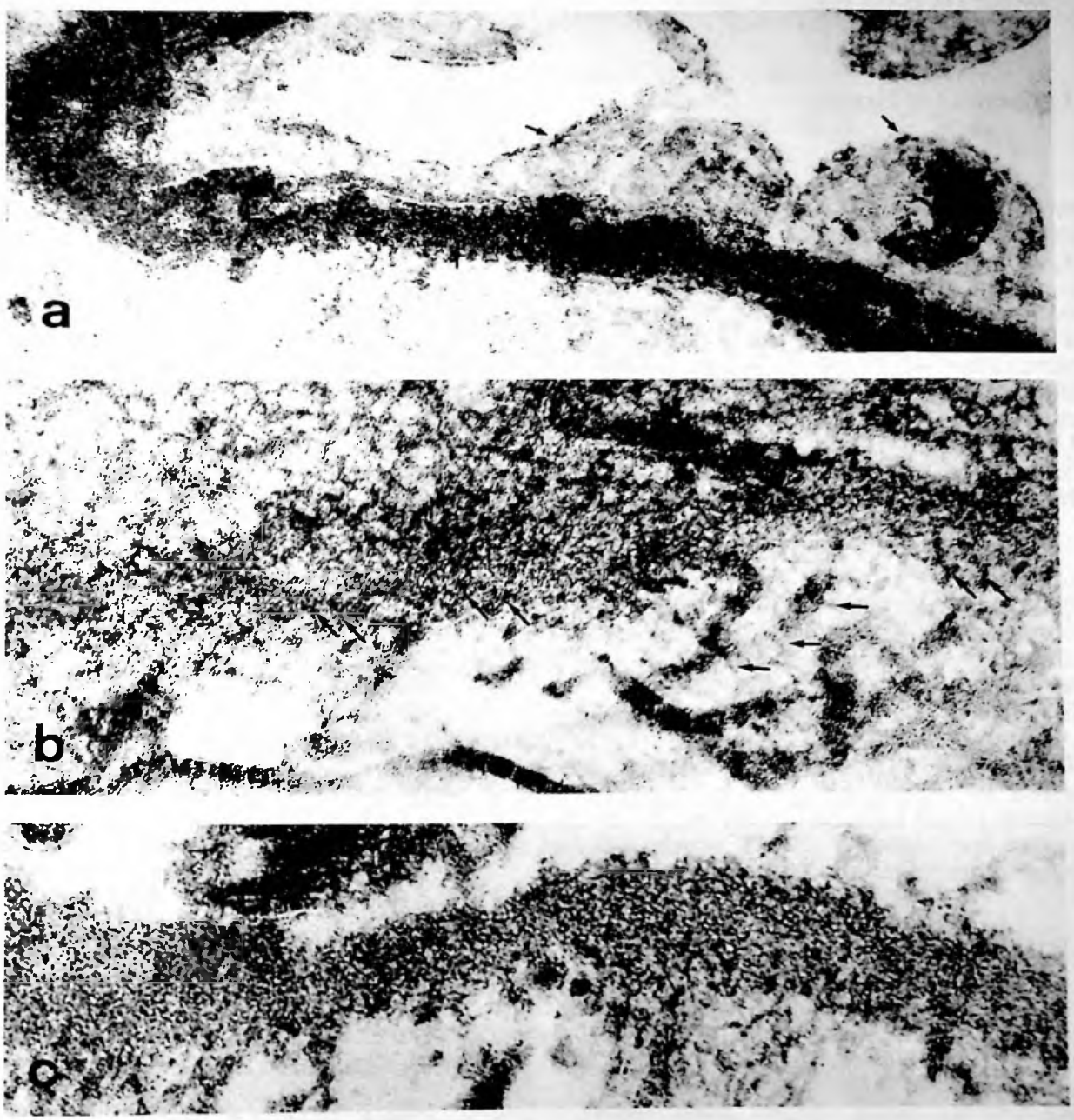

fig. 1 (a) Glomerular basement membrane of Myxine glutinosa following perfusion with cuprolinic blue and incubation with sodium tungstate shows a network like distribution of electron dense structures and a discontinously stained surface coat (arrows). x 80000
(b) Without treatment with sodium tungstate the glomerular basement membrane appears less electron dense. The arrows point at the network of filaments of cuprolinic blue-glycosaminoglycan precipitates that is faintly stained. Further filaments can be detected along the mesangial collagen fibrils (arrows). x 120000
(c) Following treatment with heparitinase I there is almost no positive staining with cuprolinic blue. x 120000

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