RENAL EXCRETION OF PROTEIN AND NITROGEN ENDPRODUCTS IN THE HAGFISH, *Myxine glutinosa*

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The cyclostome *Myxine glutinosa* possesses one of the most primitive renal systems (mesonephros), which gives the opportunity to study the development of single mechanisms in the kidneys of vertebrates.

In earlier reports experiments on the handling of protein and electrolytes and the single nephron filtration rate have been described (Bull. MDIBL 11:11, 1971; Bull. MDIBL 13:120, 1973). The study has been extended including the renal excretion of glucose, urea and ammonia.

Experimental Procedure

Freshly caught hagfish (30-46 cm long) from Frenchman Bay or Oslofjord (300-350 feet depth) were maintained in a container with running seawater. The fish were anesthetized either with MS 222 (600 mg/L) or with propylenphenoxetol (30 drops in 500 ml seawater), the gills were perfused and the kidneys exposed as described before (Bull. MDIBL 11:11, 1971).

Plasma samples were drawn from the large cardinal vein or from the dorsal aorta. Urine was collected by catheterization with PE tubing of the last part of the archinephric duct (E-AND) near the cloaca.

Inulin and glucose were measured enzymatically with the micromethod described by Zwiebel (Pflügers Arch. 307:127, 1969). Total protein in the plasma was determined by the Lowry method. A qualitative analysis of proteins in plasma and urine was performed by micro-disc-electrophoresis or gradient electrophoresis according to Neuhoff (Micromethods in Molecular Biology, Springer Verlag Heidelberg, Berlin, 1973). The protein bands were stained with amido black and scanned with a double-beam microdensitometer. For urea and ammonia determination an enzymatic micromethod with the Biochemica Test Boehringer (Mannheim/Germany) was used.

Results

Results are summarized in Table 1. As has been shown by others (see also Eisenbach et al., Bull. MDIBL 11:11, 1971) there was no difference neither in regard to the osmolality in plasma and urine nor to the concentration of sodium. However in this study the osmolality in plasma and urine are significantly raised as compared to seawater (1037 ± 31, $n = 13$, $p < 0.0025$). The urinary concentration of glucose was lower than in plasma ($p < 0.01$), as revealed by the U/P ratio. In comparison to plasma the concentration of ammonia was very low. Here the mean value includes six measurements out of nine where no ammonia could be detected. The mean concentration of urea in the E-AND urine was higher than in plasma, but only with a significance of $p < 0.05$ due to the large scatter.

The urinary total protein was measured after electrophoresis by integrating the area of all peaks as scanned with the microdensitometer (for results see table). The values are in fairly good agreement with the ones measured with the Lowry method by Eisenbach et al. (Bull. MDIBL 11:11, 1971).

Five fractions of plasma proteins can be separated by gradient electrophoresis (Figure 1). Fraction 2 and 3 consist of high molecular weight proteins and constitute more than 50% of the total protein.

TABLE 1

Mean values (±S.D.) of measurements of osmolality, glucose, NH_4^+, urea and total protein in plasma and urine and calculated urine/plasma ratios in *Myxine glutinosa*

	Osmolality (mOsmol/kg)	Glucose (mg/100 ml)	NH_4^+ (mM)	Urea (mM)	Total Protein (mg/ml)
Plasma	1139 ± 111	38.1 ± 9.4	0.646 ± 0.244	0.785 ± 0.467	33.69 ± 4.03
	n = 20	n = 10	n = 9	n = 9	n = 14
Urine	1153 ± 150	23.6 ± 13.5	0.020 ± 0.036	1.280 ± 0.62	0.388* ± 0.130
	n = 12	n = 7	n = 9	n = 9	n = 11
Urine/Plasma	1.02 ± 0.16	0.672 ± 0.336	0.031 ± 0.05	1.923 ± 1.085	0.012 ± 0.004
	n = 12	n = 6	n = 9	n = 9	n = 11

*Determined from the integral of all peaks after electrophoresis. The method was calibrated with bovine albumin.

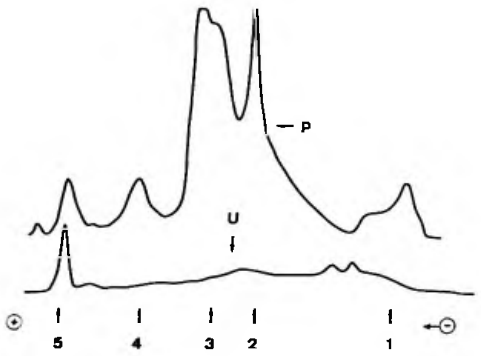

Figure 1. Gradient electrophoresis of proteins in plasma (P) and urine (U) of *Myxine glutinosa* (Dilution: Plasma 1:100, Urine 1:1).

Fraction 2 has the same electrophoretic mobility as ferritin. The low molecular weight fraction 5 has the same electrophoretic mobility as human albumin. This fraction can easily be identified in the urine while the remaining urinary protein is not well separated. Concerning the lack of fluid uptake in the archinephric duct as revealed by the U/P ratio for inulin in glomerulus perfusion experiments of 1.00 ± 0.02, n = 32 (Bull. MDIBL 13:120, 1973) the calculated sieving coefficient for this fraction 5 is 0.024 ± 0.014, n = 13 (ratio $C_{E\text{-}AND}$ over C_p). Pre-albumins are not detected in urine as well as in plasma.

The above experiments suggest (1) uptake of glucose, (2) secretion of urea, (3) minor contribution of NH_4^+ ions for the renal regulation of acid-base balance and (4) selectivity for excretion of plasma proteins in the mesonephros of *Myxine glutinosa*.

Supported by NSF (General Research Support Grant No. 1-Sol R12 05764) and DFG (SFB 146, B 4).