# RENAL PROTEIN EXCRETION IN THE AGLOMERULAR TOADFISH, OPSANUS TAU: CHARACTERIZATION OF PROTEINS IN PLASMA AND URINE BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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## Introduction

The urinary excretion of numerous high and low molecular weight proteins has been demonstrated in the winter flounder, Pseudopleuronectes americanus (Elger et al., Bull. MDIBL 24:76-77, 1984). Despite glomerular restriction, remarkable amounts of plasma proteins were determined in ureteral urine. In addition to these proteins which are comparable in size to bovine serum albumin (MG 69,000) and larger, some low molecular weight proteins were detected only in the urine.

It is widely assumed that urinary protein excretion is solely defined by glomerular leakage and tubular reabsorption. In order to determine a possible tubular origin and/or postglomerular passage of plasma proteins into the urine, we investigated in the present study ureteral urine of the aglomerular toadfish, Opsanus tau.

## Materials and Methods

Toadfish weighing 300-540 g were obtained from Woods Hole and allowed to recover from capture and transportation for at least one week before they were used for an experiment. Catheterization of the urinary bladder, collection of ureteral urine, determination of total protein and electrophoretic protein separation were performed as in our previous experiments with the winter flounder (Elger et al., ibid.). At the end of the experiments a caudal blood vessel was punctured under light anaesthesia of the animals with MS 222 (0.25 g/l seawater). The blood was mixed with one drop of heparinate (Liquemin, Roche), centrifuged to obtain the plasma and stored at -20 $^{o}$C like the urine samples for the analyses.

## Results

Urine flow rate of six non-anaesthetized toadfish was on an average 0.043 $\pm$ 0.014 $ml \cdot h^{-1} \cdot kg^{-1}$ ($x \pm$ S.D.). Total protein concentrations of plasma and urine were 3.01 $\pm$ 0.66 g% and 0.014 $\pm$ 0.005 g%, respectively. Urinary protein excretion rate was 5.95 $\pm$ 2.76 $ug \cdot h^{-1} \cdot kg^{-1}$.

Separation of plasma proteins by micro-electrophoresis on linear polyacrylamide gel gradients yielded protein patterns as shown in figure 1. Since the migration of proteins in these gels depends primarily on the size of the proteins it is possible to derive information cn the molecular weight of the proteins by the addition of marker proteins e.g. bovine serum albumin (MG 69 000, $\Delta$) and ferritin (MG 450 000, $\blacktriangle$) to the samples. Band 8 showed the same electrophoretic mobility like bovine serum albumin and therefore, the fractions 1-7 may be termed high molecular weight (HMW) proteins and fractions 9-11 low molecular weight (LMW) proteins. The bands were numbered according to the plasma protein pattern of the winter flounder (Bull. MDIBL (1984) vol. 24, p. 77, fig. 1) because both species revealed extensive conformity except for the protein bands 1,2 and 12.

Urine samples of all toadfish investigated contained proteins corresponding to the plasma protein fractions 7,8,9 and 11 (fig. 2). Further HMW-protein bands were not detected but in contrast to the plasma protein pattern a considerable amount of protein was present as a shoulder of band 7 (see arrow in fig. 2).

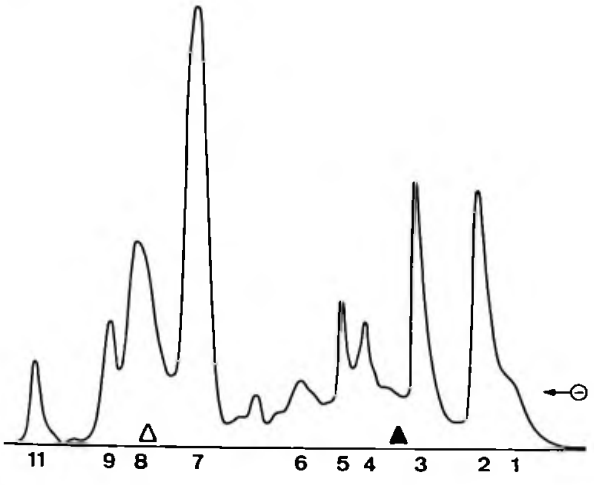


Figure 1. Densitometer curve showing a representative plasma protein pattern (toadfish no. 8444) after polyacrylamide gel electrophoresis and staining with amidoblack. Sample dilution was 1:30 and start from the right.

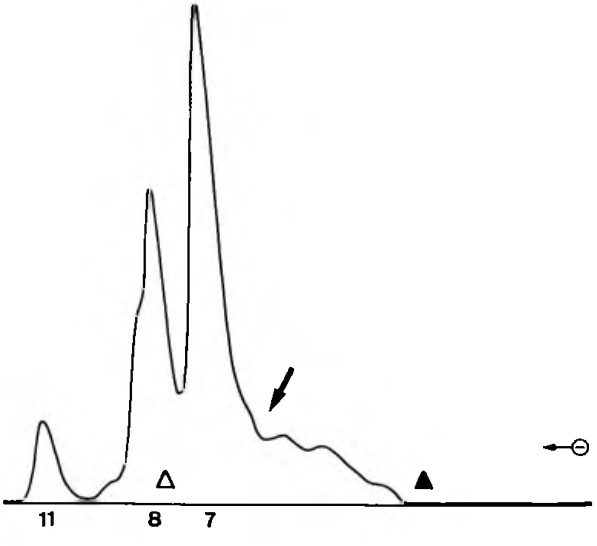


Figure 2. Urinary protein pattern of the same animal. Urine samples were concentrated in the vacuum to 50% of the initial volume.

## Discussion

The amounts of urine produced by the aglomerular kidneys of O. tau were low because these animals usually do not develop "laboratory diuresis" like the goosefish, Lophius americanus (Forster, Fortschr. Zool. 23: 232-247, 1975).

Protein content was measurable in undiluted urine samples of all six toadfish by the method of Lowry et al. (J. Biol. Chem. 193:265-275, 1951) which is more sensitive than the methods used by Bieter (J. Pharm. Exp. Therap. 8:407-412, 1931) who found no protein in the urine of Opsanus tau. It may be concluded from a comparison of the plasma and urinary protein patterns that the plasma protein bands 7,8,9 and 11 were also present in the urine. Since the other HMW plasma proteins (some of them possess a molecular weight higher than that of ferritin) were not detectable in the urine it may be assumed that a size selective tubular transfer of intact plasma proteins into the urine takes place by paracellular and/or transcellular pathways.

Further experiments are required to characterize the proteins which were present in the urine as a shoulder of band 7. Since this region of the urinary protein pattern has a different appearance in the plasma it is possible that these proteins represent either modified plasma proteins (LMW-protein aggregates and/or subunites of HMW-proteins) or proteins of tubular origin (e.g. glycoproteins).

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