IN VIVO-EXPERIMENTS ON THE RENAL EXCRETION OF NATIVE PROTEINS IN THE UNANESTHETIZED WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS.

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The morphology of the glomerular filtration barrier in the winter flounder is comparable to the mammalian glomeruli. The functional mechanism which prevents the filtration of anionic antifreeze peptides is basically a charge to charge repulsion by the anionic glomerular capillary wall (Petzel and DeVries, Bull. MDIBL 21: 35-37, 1981). The aim of this study was to investigate whether and to what extent serum proteins are filtered and present in the urine.

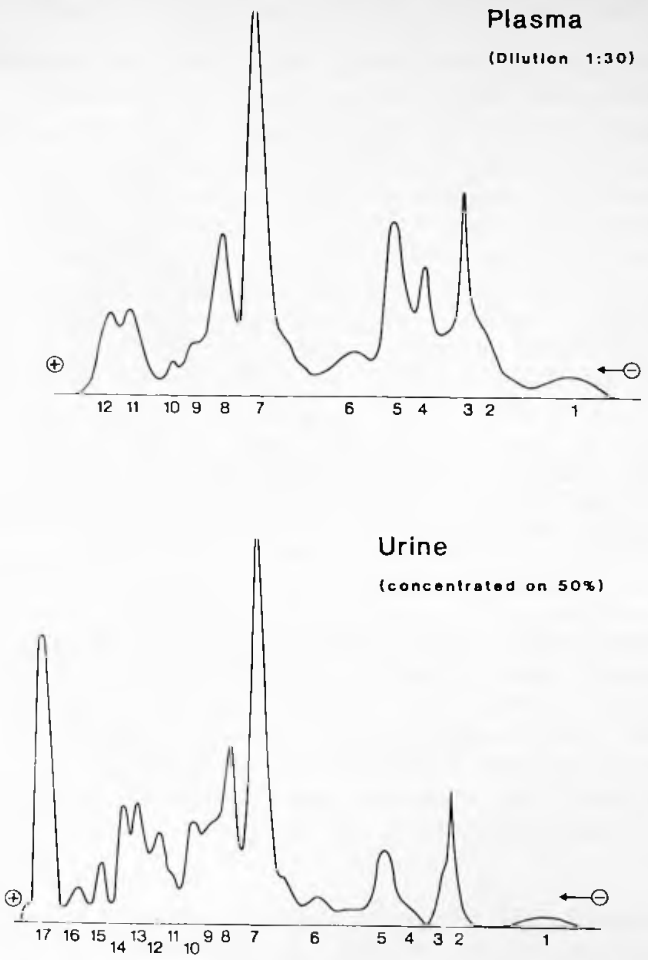
Materials and Methods

Immature winter flounders (Pseudopleuronectes americanus) were captured in Frenchman Bay and maintained without feeding for at least one week prior to use for experiments. Catheterization of the urinary bladder and the cardinal vein with polyethylene tubing was carried out under anesthezia with MS 222 (0.006 g/100 ml). The animals were placed in shallow chambers which were supplied with aerated seawater (15°C). Kidney function of 16 unanesthetized, quietly resting animals was studied the next day after catheterization: blood samples (0.3 ml) were drawn by cardinal vein catheter and urine was collected under paraffin oil in tared plastic tubes. Total protein was measured by the Lowry method and protein patterns were obtained by microelectrophoresis on linear polyacrylamide gel gradients as previously described (C.A.Baldamus et al, Contr. Nephrol. 1: 37-49, 1975). The migration of proteins in these gels depends primarily on the size and molecular weight of the proteins.

Results and Discussion

The total plasma protein concentration was 3.21 ± 0.07 g/100 ml in 16 winter flounders ($\bar{x}$ ± SEM). This value is lower than in mammals but in the range of other teleost fish (M. Florkin and B.T.Scheer, Chem. Zool., VIII, London 1974). At least twelve different proteins were identified in the plasma by separation with the microelectrophoresis (Fig. 1). Addition of standard proteins to the samples showed that the protein bands number 2 and 3 are similar in molecular weight to ferritin (MG 450.000), peak 8 corresponds to bovine serum albumin (MG 69.000). Thus the protein bands 1-7 may be called high molecular weight proteins (HMW) and distinguished from the other proteins with lower molecular weight (LMW). Quantification of the different protein groups by integrating the electrophoretic bands revealed that HMW proteins dominate in the plasma (Fig. 3).

The renal excretion rate of protein is low (0.21 ± 0.04 mg/h/kg body weight) because only small amounts of protein (0.104 + 0.01 g/100 ml) are present in the urine. Therefore, urine samples had to be concentrated in the vacuum to 50% of the original volume before the electrophoretic analyses could be performed. Figure 1 shows that HMW serum proteins were also present in the urine but in smaller concentration than band 7. Interestingly, additional urinary proteins with a molecular weight less than albumin were detected. Thus, LMW proteins make up 56.8 ± 4.04 % in the urine (Fig. 2).



Conclusions

In the winter flounder, similarities to mammals exist not only in the glomerular ultrastructure but also in the permeability for macromolecules. Like in mammals, HMW serum proteins are largely restricted by the glomerular filtration barrier in the winter flounder. Further experiments are especially necessary to clarify the nature and origin of the urinary LMW-proteins, which are mainly handled by the tubular apparatus.

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Figure 1. Representative pherograms from polyacrylamide gel electrophoresis of plasma and urine. Start from the right. Numbers indicate different protein bands.

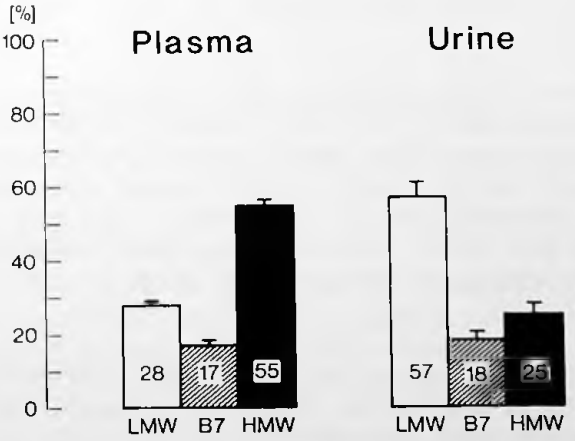

Figure 2. Fractions of HMW, band 7 and LMW proteins as percentage of the total area below the curves. $\bar{x} \pm$ SEM, n=16 animals.