1971 #6

RENAL HANDLING OF PROTEIN IN THE HAGFISH, *Myxine glutinosa*

G.M. Eisenbach, M. Weise, R. Weise, K. Hanke, H. Stolte and J.W. Boylan, Medizinische Hochschule, Hannover and State University of New York, Buffalo

Although some preliminary description of the plasma proteins of cyclostomes has been published by Manwell (The Biology of Myxine ed. by A. Brodal and R. Fänge, Universitetsverlaget, Oslo, 1963, 516), there are no direct experimental data which define the excretory urinary pattern of protein in the nephron of the hagfish, *Myxine glutinosa*. Our interest in protein excretion by the mammalian kidney prompted us to examine this process in the hagfish which possesses one of the most primitive of filtering kidneys. We have included some observations on the handling of the electrolytes, sodium and potassium in this nephron.

EXPERIMENTAL PROCEDURE:

Experiments were carried out on 14 hagfish weighing 80-140 g. They were caught in Frenchman Bay in a water depth of 300-350 feet and maintained in a suitable container in running sea water at 12-15°C. Anesthesia was performed with 100 mg/kg BW Nembutal in 1 ml Ringer's solution adapted to sea water osmolality. In addition, some animals were first put in 2 L sea water containing 600 mg/L ME 222. After 30-45 minutes the gills were perfused via an oral tube with 300-400 ml/kg BW/minute oxygenated sea water thermostated at 10-12°C. The kidneys were exposed by an incision in the second third of the body length. Two types of experiment were performed (1) Fluid samples were taken from Bowman's capsular space using micropuncture techniques. (2) Glass tubes were inserted in the archinephric duct in such a way as to allow sampling of the fluid filtered from 2-3 glomeruli. In all animals urine was collected from the archinephric duct near the cloaca. Plasma samples were drawn by puncturing the large cardinal vein or the dorsal aorta. In some cases arterial blood pressure was measured by water manometry in the dorsal aorta; the fish being maintained in a perfectly horizontal position. Anatomical studies were performed after casting the arterial, venous and archinephritic duct system with Microfil.

Fluid samples were examined for osmolality, Na^+, K^+ concentrations, total protein and fractional protein concentrations. In the gill perfusion water, osmolality, Na^+, and K^+ concentrations were measured. For protein fractionation, micro-disc-electrophoresis on 20 per cent polyacrylamide gel was used. The gels were stained with 1 per cent Coomassie blue and the protein bands were scanned by a microdensitometer.

RESULTS

Anatomical description of the renal system (see also Figs. 1 and 2).

The renal system (Mesonephros) of the hagfish, *Myxine glutinosa* has been described by Fänge (The Biology of Myxine ed. by A. Brodal and R. Fänge, Universitetsverlaget, Oslo, 1963, 516). It consists of two archinephric ducts (ureter) draining 30-40 *glomeruli* (Gl). The archinephric ducts (AD) situated lateral to the cardinal veins (V) begin at 3-6 percent of the whole body length just caudal to the abdominal opening of the gills and end in the cloaca. Glomeruli are present on the first two thirds of the whole length of the archinephric ducts. The distance between adjacent

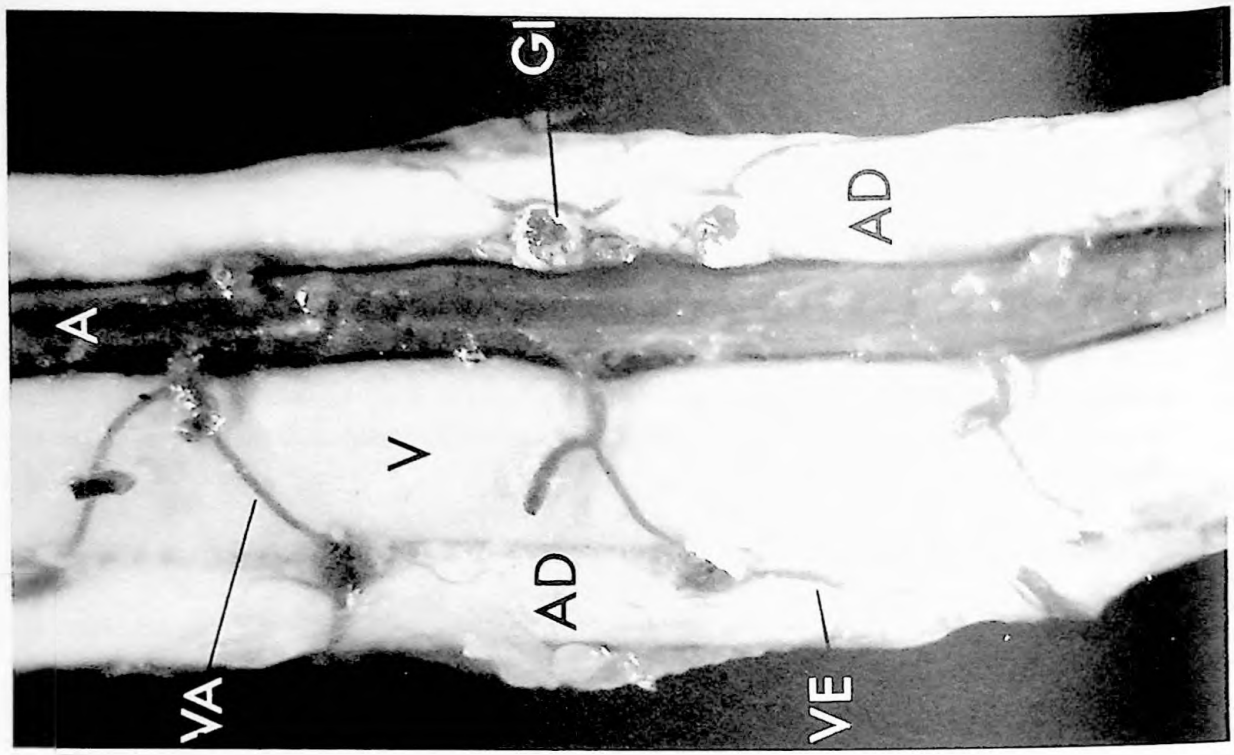

Figure 1: Microfil injection of the archinephoric ducts, cardinal vein, and aorta (x12)
A: Aorta, V: Cardinal Vein, AD: Archinephoric duct, VA: Vas afferens, VE: Vas efferens.
GL: Glomerulus

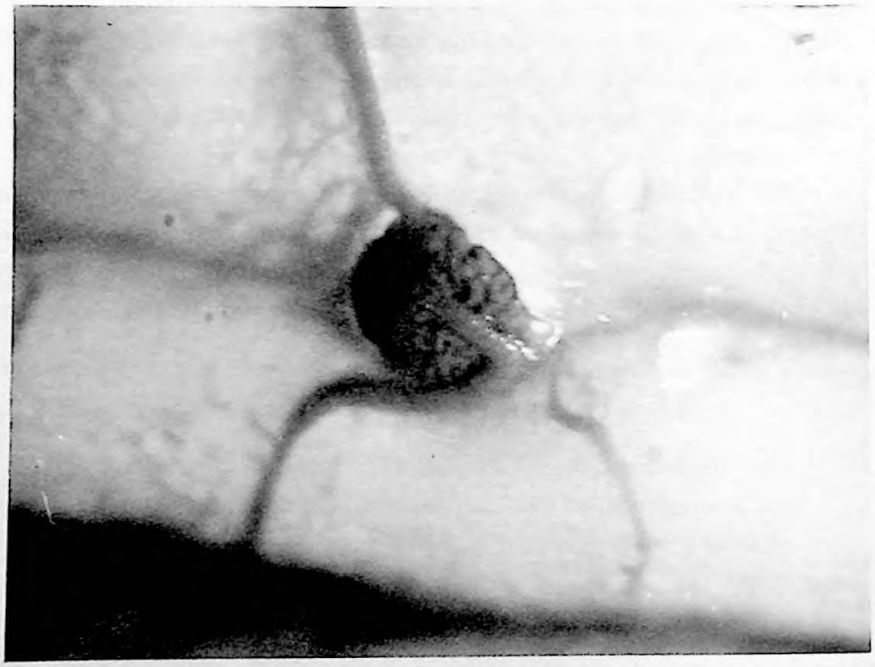

Figure 2: Microfil injection of the archinephoric ducts, cardinal vein and aorta (x48)

glomeruli is 3-4 mm.

The glomeruli have a diameter of 200-600 μ depending on the amount of fluid in Bowman's capsular space. Bowman's capsular space surrounds the glomerular capillaries in such a way that the capillaries are only visible from the dorsal aspect. The space is drained by a thin-walled tubule of small diameter barely visible *in vivo*. This tubule (neck segment) courses parallel to the archinephric duct in upstream or downstream direction and enters the duct on its lateral side forming a little diverticulum. The glomerular blood supply consists of a *vas afferens* (VA) branching off from one of the segmental arteries arising from the dorsal aorta (A). All vasa afferentia are situated on the dorsal side of the great vessels. The glomerular tuft is drained by a vas efferens (VE) branching in most cases in two larger and one smaller postglomerular arteriole. The larger ones branch in the postglomerular network which invests the archinephric duct. This network is drained by a vein which is in close contact to the base of the neck on the archinephric duct. There is no portal circulation. The small branch of the vas efferens mentioned above runs between Bowman's capsular space and archinephric duct and enters the collecting vein in close proximity to the base of the neck, thus forming a venous loop surrounding the neck. This particular structure suggests analogy to the feed-back mechanism thought to play a role in the control of glomerular filtration in higher organisms. The collecting veins open to the cardinal veins. The cardinal veins are connected by bulblike anastomoses.

HISTOLOGY

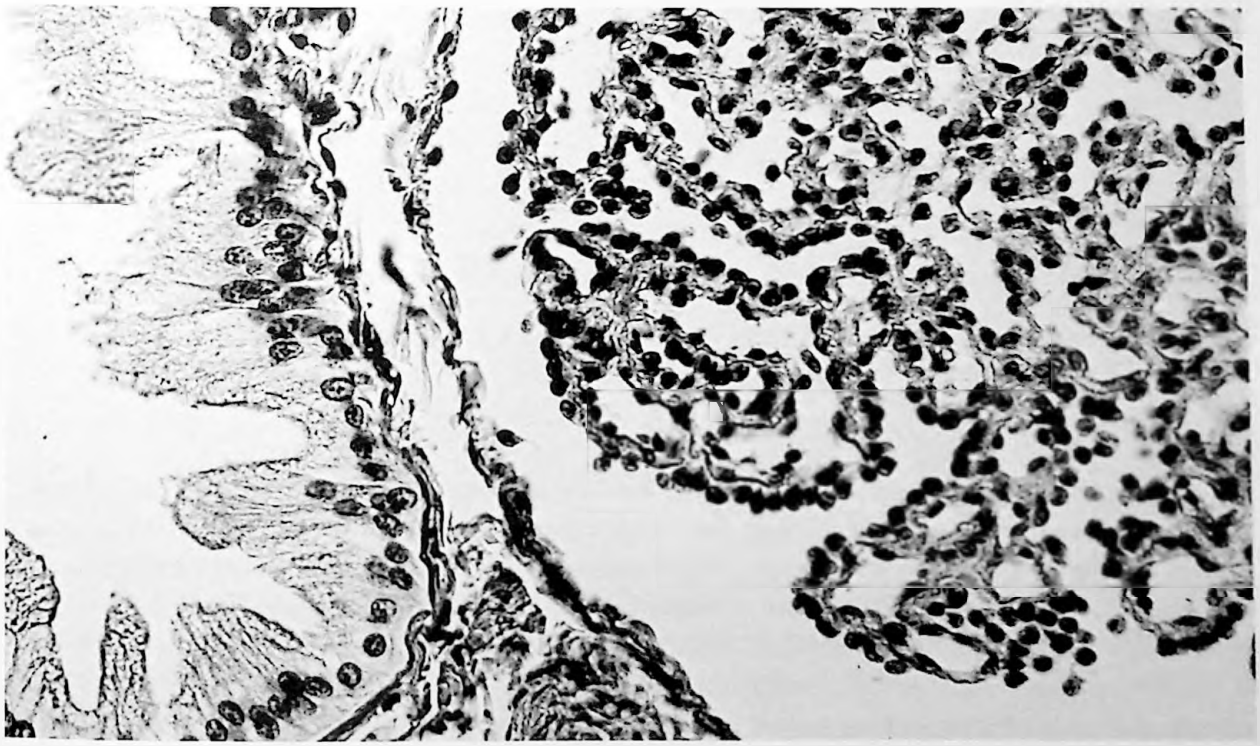

Figure 3: Cross section of Hagfish glomerulus and archinephric duct

In the glomerulus the nuclei of epithelial cells are prominent and numerous. Endothelial cells can be seen. The archinephric duct is lined by cylindric epithelium with converging and diverging cells, which exhibit a brushborder at the lumen.

OSMOLALITY AND ELECTROLYTES

As demonstrated by Munz and McFarland (Comp. Biochem. Physiol. 13:381-400, 1964) and by Morris (J. Exp. Biol. 42:359-371, 1965) there is almost no change in osmolality or sodium concentration in the ultrafiltrate passing through the hagfish nephron and this is confirmed by the present findings (Table). We find, however, that potassium concentration has already increased in

TABLE

	Osmolality *(mosm/1)*	Sodium *(mEq/1)*	Potassium *(mEq/1)*	Protein + *(g %)*	Albuminfraction *(%)*
SEA WATER	944.9 ± 48.7 (7)	454.9 ± 45.6 (8)	9.96 ± 2.85 (8)		
PLASMA	1109.6 ± 118.6 (8)	497.2 ± 67.1 (10)	9.05 ± 1.35 (10)	2.92 ± 0.21 (9)	18.0 ± 5.0 (4)
GLOMERULUS	908.7 ± 89.2 (6)	475.7 ± 120.6 (7)	7.26 ± 3.2 (8)		32.2 ± 4.8 (5)
ARCHINEPHRIC DUCT	1159.1 ± 183.4 (8)	544.2 ± 84.4 (9)	15.69 ± 2.8 (9)		21.0 ± 1.7 (3)
FINAL URINE	956.7 ± 161.7 (13)	482.2 ± 63.3 (17)	13.45 ± 4.07 (17)	0.0625 ± 0.026 (4)	25.75 ± 3.5 (8)

+ as calculated for the protein with similar electrophoretic migration characteristics like human albumin.

the archinephric duct with a tubular fluid to plasma ratio of 1.73± 0.31 S.E., implying that an important site of potassium secretion is located in the neck segment.

PROTEIN

Total protein was measured by the Lowry method in plasma and final urine. As can be seen in the Table the protein content of final urine with a mean of 0.0625 ± 0.026 g per cent in comparison to plasma with 2.92 ± 0.21 g per cent establishes the very low permeability of this filtering membrane for proteins. This is further documented by micro-disc electrophoresis of samples taken by micro-puncture from the glomerulus, the archinerphric duct and the final urine (see Figure 4). In the plasma, three main protein bands comprising about 50 per cent of the total amount of protein were found. It is of interest that several proteins with a m.w. much lower than albumin are present, which are not found in mammalian serum. In micropuncture samples of glomerular filtrate these three plasma protein bands were found, but as can be seen in Figure 4, in a much lower concentration.

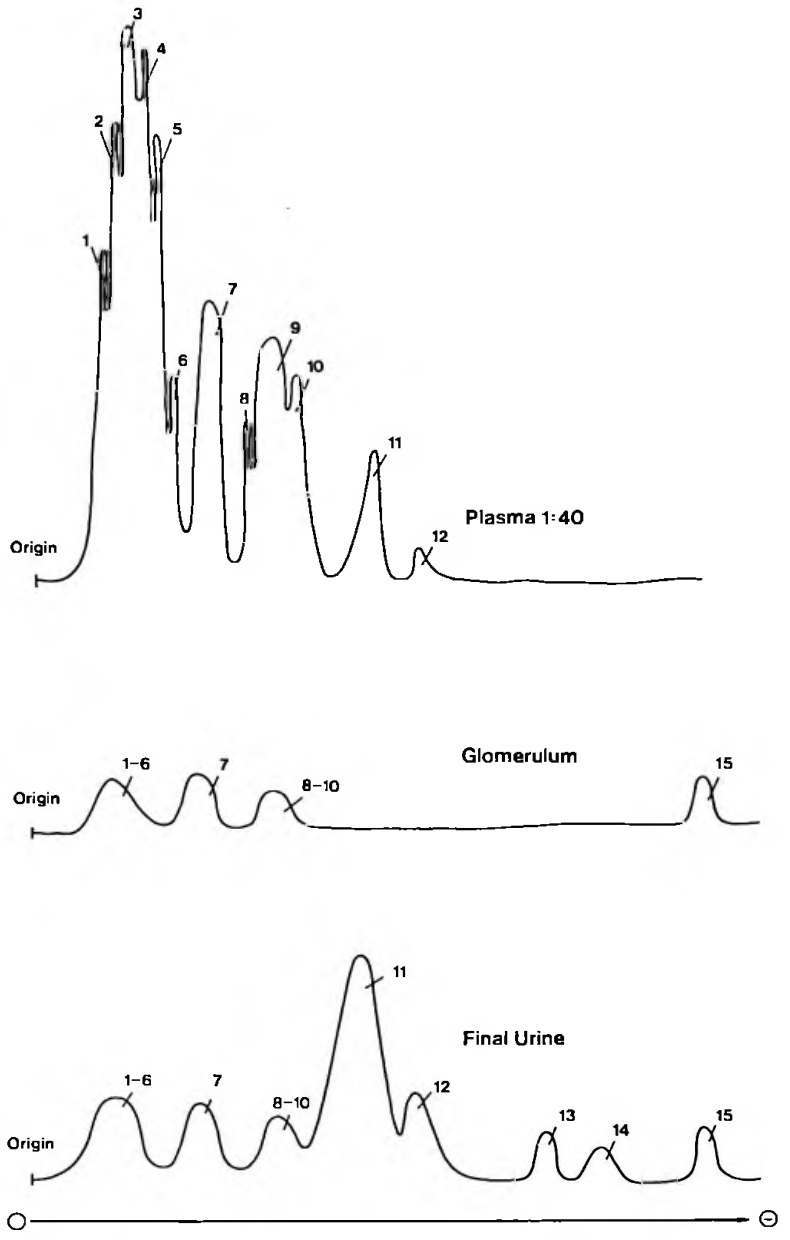

Figure 4: Scans of micro-disc-electropherograms

In samples of the *archinephric duct*, the same proteins were found. In urine samples (end of archinephric duct), the protein pattern was similar to that of the archinephric duct fluid. But in addition to the low m.w. protein, urine also contained proteins of higher m.w. (13-15), probably of ureteral origin.

Supported by National Institutes of Health Grant No. AM-14424-02 to John W. Boylan.

We are grateful to Dr. E. Reale and Dr. O. Muller for preparing the histological sections.