Kidney Function Studies on Three Species of Marine Fish with Varying Degrees of Glomerular Development (Myoxocephalus octodecimspinosus, Myoxocephalus scorpius and Lophius piscatorius).

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These three fish, all common in the waters of Frenchman's Bay, were selected for study because they presented a unique opportunity to observe renal function as affected by variations in glomerulo-tubular balance. The renal tubules of all of them are undifferentiated and can be considered as homologous with the proximal segment of the mammalian nephron. The common longhorn sculpin, **M. octodecimspinosus,** has a completely glomerular kidney, the shorthorn or daddy sculpin, **M. scorpius,** has many aglomerular tubules and some with patent glomeruli, and the goosefish, **Lophius piscatorius,** is completely aglomerular. In the latter the blood supply to the kidney is purely venous. The glomerular circulation, when present, is always arterial and the tubular supply is mixed arterial and venous (see Marshall for review[1]).

Standard clearance procedures were employed to measure renal function. P-aminohippurate clearances were used for minimal renal plasma flow determinations and inulin clearances for the measurement of glomerular filtration rates (see Smith for review[8]). Freezing point depressions were determined with a "thermistor" (Western Electric Co.), sodium with a Perkins-Elmer flame photometer, and chlorides with Van Slyke and Hiller's modification of Sendroy's method[9]. Urine samples were usually collected by indwelling catheter using bladder washes, and blood was taken from the caudal artery.

Physiological evaluation of glomerular activity. Inulin is never eliminated by tubular secretory activity and hence does not appear in the urine of aglomerular fish. Forty shorthorn (daddy)) sculpins were examined to assess glomerular activity by determining the efficiency of inulin excretion. No correspondence was found between the size or age of the fish and the degree of glomerular activity. Six could be considered essentially aglomerular (wts. = 282, 304, 479, 710, 770 and 1060 grams) where only unmeasurable traces of inulin could be detected in the urine when simultaneous plasma concentrations were as high as 200 mgm. per cent. Most of the non-

diuretic shorthorn sculpins had glomerular filtration rates of less than 3 ml. per kgm. body weight per day when simultaneous renal plasma flows were 1500 - 3000 ml. per kgm. per day. Similarly handled longhorn sculpins had filtration rates averaging 30 ml. per kgm, per day with renal plasma flows similar to those in the shorthorn sculpins.

Recruitment of glomerular function in "laboratory diuresis". Glomerular and aglomerular fish alike exhibit increased urine flows when maintained under laboratory conditions, even when kept in live cars and with minimal handling. This diuresis is associated with elevated urinary osmotic pressures, a fall in the percentage of red blood cells and a marked elevation in glomerular filtration rates in the sculpin. Inulin clearances in the shorthorn sculpins rose from less than 3 ml. per kgm. per day to as high as 55, and longhorn sculpins were observed with filtration rates as high as 189 ml. per kgm. per day. These increases of 15-fold or more are such a constant feature of renal function in fish (see also Clarke[1-2]) that one is led to conclude that in the normal state marine fish are essentially aglomerular physiologically, even though morphologically their nephron units may be completely glomerular. The mechanism which operates to recruit glomerular activity under these circumstances is unknown at present.

Renal plasma flow measurements. As in the mammals and other classes of vertebrates p-aminohippurate excretion by the kidneys proceeds at a very rapid rate. Inasmuch as its clearances are self-depressed with plasma concentrations higher than 5 mgm. per cent, along with other criteria, its clearance at low plasma levels can be taken as an approximation of the minimal renal plasma flow. Renal plasma flows in all three fish fall in the range of 1 - 4 liters per kgm. body weight per day when the animals are maintained in the laboratory. There is a tendency for the plasma flows to rise with "laboratory diuresis" but not in proportion to the rise in glomerular filtration rate. Less than one per cent of the renal plasma flow is filtered at the glomerulus in the shorthorn sculpin even at the height of diuresis, and rarely more than 5 per cent is ever filtered in the longhorn sculpins. There seems to be little doubt that compared with the mammals, and even amphibians, the arterial glomerular circulation is relatively unimportant in all three of these fish. The venous renal-portal circulation sustains kidney function exclusively in the aglomerular goosefish, and presumably to a very large part in the shorthorn and longhorn sculpins.

P-aminohippurate Tm. It has previously been demonstrated that a maximal limit is imposed upon the amount of secreted materials which can be transported by aglomerular tubules (Shannon [5-6]). Studies relating the amount of PAH delivered to the renal tubules to the amount of PAH transported demonstrate that such a limitation also exists for the glomerular undifferentiated tubule of the longhorn sculpin. Maximal values for the rate of excretion are reached with plasma PAH concentrations of 5-10 mgm. per cent. Above these concentrations, while wide variations exist between individual fish, no further relationship between PAH transported and load is obtained.

Water excretion by the renal tubules. All the water and solute appearing in the urine of aglomerular fish is formed purely by tubular activity. In the partially aglomerular shorthorn sculpin most of the water in urine must be contributed by the tubules because urine volumes are usually much higher than simultaneous glomerular filtration rates. A chance observation led to the finding that water, under certain circumstances, was excreted by the renal tubules even in the completely glomerular longhorn sculpin. When these fish were kept in isotonic or slightly hypotonic sea water for several days they characteristically had urine flows which exceeded simultaneous glomerular filtration rates (inulin clearances) by as much as 2-fold. Inulin urine/plasma concentration ratios are never less than 1.0, even at the height of diuresis, when these fish are kept in normal sea water.

Extra-renal excretion. PAH and phenol red injected into the blood stream of glomerular and aglomerular fish was found to be highly concentrated in gall bladder fluid as well as in urine. Four hours after the injection of 4 mgm. into longhorn sculpins 1.95 mgm. was accounted for in urine, 0.56 mgm. was recovered in bile and residual intestinal fluids, and 0.34 mgs. remained in the blood stream. The role of the liver in elimination seems to be quite important in fish.

Influence of cold on renal function. Sculpins are cold-adapted animals and can remain vigorously active in water approaching freezing temperatures. Studies of 31 longhorn sculpins observed at both ordinary temperatures (17-19°C.) and about 10° lower disclosed that filtration rates were not significantly affected by the lower temperatures but the rate of PAH transport was: the latter being characterized by a temperature coefficient of roughly 2.0 for these fish.

Osmotic pressure relations in normal animals. Urine samples taken from freshly caught fish were always significantly hypotonic to simultaneously collected plasma in the glomerular and in the aglomerular fish. The essential data concerning freezing point depressions of biological fluids taken from freshly caught goosefish are listed in table 1. Urine/plasma, ratios averaged 0.84.

TABLE 1

Freezing point depressions of biological fluids taken from 3 goosefish (Lophius piscatorius) immediately after capture.

Animal	Plasma	Urine	Pericardial Fluid	Coelomic Fluid	Urine / Plasma
No. 1	0.655°C	0.570°C	0.580°C	-	0.87
No. 2	0.670	0.545	0.555	-	0.81
No. 3	0.670	0.570	0.610	0.620°C	0.85

These signficant pressure differences amounting to more than one atmosphere suggest that water is excreted per se, although the equally awkward alternative exists that water and solute pass into the lumen at one level of the tubule and solute alone is preferentially reabsorbed at another. The latter explanation is especially difficult to accept because of the completely undifferentiated nature of the renal tubule in this fish. Almost identical differences in the osmotic pressures of simultaneously collected urine and plasma were noted in freshly caught shorthorn and longhorn sculpins. It is interesting to note that pericardial and coelomic fluid is also hypotonic to plasma. These urine samples taken from non-diuretic fish are frequently essentially chloride-free and have concentrations of sodium much lower than in the plasma.

Osmotic pressure changes during "laboratory diuresis". Smith[7] has shown that marine teleosts swallow sea water which is absorbed in the gastro-intestinal tract. While blood traverses the gills Na, K and Cl are actively secreted and the plasma remains markedly hypotonic with respect to environmental sea water. Mg and SO_4 are selectively eliminated by the kidneys. After marine fish are handled to any extent the urine flow markedly increases. Grafflin and Ennis[3] have interpreted this diuresis as due to skin traumatization which subsequently results in water being lost osmotically to environment-

al sea water, which in turn leads to an aggravation of the drinking process accompanied by corresponding increases in solute concentration in plasma and urine. However, it seems unlikely that osmotic loss of water through the skin initiates the diuretic state inasmuch as 5 longhorn sculpins placed in isotonic or slightly hypotonic sea water all exhibited the customary increases in glomerular filtration rate and urine flow. Total solute concentrations of both plasma and urine increased with handling glomerular and aglomerular fish alike. The essential data on freezing point depressions observed during "laboratory diuresis" in a goosefish is given in table 2. The fish

TABLE 2

Progressive diuresis over a 60 hour period in the aglomerular goosefish. Note the steady increase in urine flow with corresponding changes in freezing point depressions and chloride concentrations. Similar changes are observed in plasma samples taken at the beginning and end of the series. Only in this diuretic state is the urine and plasma essentially isotonic. Normally urine is significantly hypotonic to plasma.

Sample	Time Hours	Urine Flow ml/kgm/day	Freezing point depression	Chloride m.eq.NaCl/L
Plasma No. 1	7		0.610°C	168
Urine No. 1	0-7	29.4	0.610	180
Urine No. 2	7-24	38.4	0.625	199
Urine No. 3	24-30	54.8	0.650	208
Urine No. 4	30-46	65.0	0.680	222
Urine No. 5	46-54	75.0	0.720	232
Urine No. 6	54-60	?	0.760	242
Plasma No. 2	60		0.775	199

was maintained in a live-car for 24 hours after capture, then catheterized, and the urine samples quantitatively collected as indicated. Blood samples were taken at the beginning and end of the series of urine collections. The fish was in a highly diuretic state at the start of the urine collections, and it died suddenly 60 hours after the start of the first urine collection. It was presumably failing in the sixth period. The urine volume measurement was technically faulty for this period, but it clearly was lower than in the previous collection. At the end of the series, pericardial fluid had a freezing point depression of 0.740°C. and chloride concentration of 215 m. eq. NaCl per liter. Values for freshly caught goosefish are .582°C. and 160 m. eq. respectively.

The simplest explanation of the cause of diuresis is that there is an initial depression of secretory activity by the gills which results in flooding the animal with solute. This, in turn, is probably associated with increased fluid uptake from the gut, a rise in blood volume (fall in hematocrit), and, in the sculpins, an increase in glomerular filtration rate. Solute output by the kidneys of both glomerular and aglomerular fish increases along with corresponding rises in plasma solute concentration.

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The experiments summarized in this paper will be published in detail elsewhere.

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