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ASPECTS OF OSMOTIC AND IONIC REGULATION IN THE STURGEON

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INTRODUCTION

The sturgeons and their close relatives the paddle fish, *Polyodon*, are the living representatives of an ancient and isolated group of fishes. They are believed to have descended from the fresh water palaeoniscids of the Devonian period independently of both the holosteans and the teleosts. Like the salmon and some other teleosts, many sturgeons are euryhaline, breeding in fresh water but spending most of their lives in the sea. Other sturgeons, such as *Acipenser fulvescens* of the Great Lakes and the paddle fishes of the Mississippi basin, are confined to fresh water. The ability to live in sea water must have been acquired independently by the sturgeons and by the teleosts but as far as it is known both groups of fishes maintain similar blood concentrations, equivalent to about 30% sea water, in both sea water and fresh water. The osmotic concentration of the blood of *A. stellatis* is only 339 m-osmole/kg water when in sea water compared with 292 m-osmole when in fresh water (Kalashnikov & Skadovskii, 1948), and Urist & van de Putte (1967) found that the composition of the plasma of the white sturgeon, *A. transmontanus* was similar in both sea water and fresh water. An extensive series of analyses of plasma and urine of *A. sturio* and *A. oxyrhynchus* from both fresh water and brackish water (*c.* 40% S.W.) by Magnin (1962) showed that the blood concentration increased by less than 10% in the more concentrated medium.

Progress has recently been made in the study of salt and water balance in teleosts, especially since the introduction of isotopic tracers, but little is known of the physiology of salt and water balance in the sturgeons, because their localized distribution and large size make them inconvenient fish to work on. Two species of sturgeon, the green sturgeon *A. medirostris* (Ayres) and the white sturgeon *A. transmontanus* (Richardson) are readily available on the Oregon coast and this paper reports the results of some preliminary studies on salt metabolism in the two species. The white sturgeon can live in completely fresh water but the green sturgeon penetrates only into the estuaries. Bajkov (1951), Cleaver (1951).

MATERIALS AND METHODS

Most of the sturgeon were caught by gill net in the estuary of the river Umpqua, above Reedsport, Oregon. The estuary is tidal but has a large volume and a narrow exit to the sea. The concentration varies widely but was about 60% sea water and the temperature was 20–21 °C at the time of capture in July 1970. A few of the sturgeon,

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including both the white sturgeon, were caught in trawls in the Coos estuary in unrecorded but probably similar conditions. The sturgeon were kept in large circular tanks made of fibre-glass containing approximately 900 l of water, in the open air alongside the station laboratory. The tanks were kept vigorously aerated and were supplied with a continuous flow of sea water or fresh water or a mixture of both. The usual rate of water supply was 20 l/min. With running sea water the tanks maintained a temperature close to 14·5 °C, but with running fresh water the temperature was about 20 °C. In full sunlight, in late afternoons, the temperature ran one or two degrees higher. The fresh water at Charleston contained 0·36 mM-Na/l and 3 mM-Ca/l. The fish varied in weight between 3·5 and 17 kg but most were about 7 kg. The great weight and power of the fishes made it necessary to develop special experimental techniques.

Blood was obtained by hypodermic syringe from the caudal vein, just behind the anal fin. In order to collect blood the fish were removed from the tanks with a net, wrapped in coarse sack cloth and laid on their backs on the ground. Their movements were less frequent and less violent when the head and eyes were covered by the sacking, which also protected both the fishes and the physiologists from serious injury. The sturgeon blood did not clot readily and no special precautions were taken to prevent clotting. When plasma was required for analysis, between 1 and 5 ml of blood was transferred to glass centrifuge tubes and centrifuged at once. When whole blood was required for the measurement of sodium activity, 1–2 ml of blood was drawn and weighed immediately but counted at leisure. Whole blood required for tritium measurements was distilled in a vacuum apparatus similar to that described by Rudy (1967). No consistent differences in blood compositions between male and female were observed (cf. Magnin, 1962). As the numbers of fish available were limited possible sex differences have been ignored.

Blood and urine constituents

Sodium and potassium and calcium were estimated by flame photometry using a Coleman Model 21 flame photometer. Chloride was estimated by comparison with standards using a Cotlove chloridemeter. Magnesium was estimated by the method of Sky-Peck (1964) and sulphate by the method of Berglund & Sorbo (1960). The water content of blood plasma was calculated from the weight loss after drying at 105 °C overnight. Cortisol was estimated by the competitive protein-binding radioassay method of Murphy (1967). Ammonia was determined by comparison with standards at 410 mμ after nesslerization.

Sodium and water fluxes

^{22}Na activity was measured using a Picker Nuclear scintillation system consisting of a sodium iodide detector, a pulse height analyser, ratemeter and recorder. Tritium was assayed using a Beckman LS-150 liquid scintillation counter. The water content of blood plasma was calculated from the weight loss after drying at 105 °C overnight.

The large size of the animals made it impracticable to measure influxes as the quantity of isotope required to label the medium would have been excessive. Sodium effluxes were usually measured by following the declining activity of ^{22}Na in the blood following the injection of 10–30 μC of ^{22}Na into the peritoneal cavity. In some of the

early experiments the sodium was injected directly into the caudal vein but this method was later abondoned in order to reduce the damage in that region and to avoid the possibility of contaminating the blood samples, and the isotope was injected into the peritoneal cavity instead. Following the injection into the caudal vein the rate of loss of sodium from the blood remained high for 3 or 4 h, even in fish adapted to fresh water, where the rate of loss to the medium was low. For example, in one specimen the rate of disappearance of ^{22}Na was still as high as 6·5 %/h 3 h after injection although the loss eventually declined to only 1·5 %/h. This disappearance from the blood was no doubt mainly due to exchange with sodium in the tissues rather than loss to the medium. In order to allow the sodium to equilibrate within the fish measurements of the activity in the blood were begun 5 h after intraperitoneal injection, in fishes maintained in sea water, and after 12 h or overnight in fresh-water-adapted fishes. The disadvantages of this method were that the blood sample drawn was only a small part of the total animal so that even a large injection of sodium tracer gave only a low count, while the repeated sampling and associated disturbance might have altered sodium balance of the fish. As an alternative whole-body counts were made on one small sturgeon (M_{14}) by removing it from the tank, wrapping it in polythene bags and positioning it over the counter. Blood samples for tritium assay were also collected from the caudal vein. Tritium was counted by liquid scintillation after vacuum distillation of the water from the blood. 1 mC of tritiated water was injected into the peritoneal cavity and blood samples were taken after 2 h and again after 6 h.

Owing to the large size of the fish and the relatively small size of the tanks it is necessary to consider whether the calculated fluxes could have been distorted by recycling of the isotopes. The declining activity in a finite pool in dynamic equilibrium with an infinite pool may be represented by the equation:

$$C_t = C_0 e^{-KT} \qquad \text{(X)}$$

where K is the rate constant of exchange, C_0 the initial activity in the pool and C_t the activity after time T.

The declining activity in a finite pool (1) capacity A, in dynamic equilibrium with another finite pool (2) capacity B, may be represented by the equation:

$$C_t = (C_0 - C_\infty)e^{-(K_1+K_2)T} + C_\infty \qquad \text{(Y)}$$

when K_1 is the rate constant of exchange of pool (1) and K_2 the rate contant of exchange of pool (2), and C_∞ the activity in pool (1) after infinite time. As the efflux from (1) is the same as the influx into (2), $K_1 A = K_2 B$. The error arising from using equation (X) instead of equation (Y) when the second pool is finite is negligible initially but increases as equilibrium is approached. For example, if $K = 0{\cdot}3$ h^{-1} then after 1 h C_t will have declined to 0·741 of the initial activity when the second pool is infinite, but to only 0·743 when the second pool is ten times as large as the first. After 2 h the values would be 0·549 and 0·562 respectively. The volume of water in the tanks was about 900 l, or about 100 times the water content of the average fish. Any back flux of tritiated water from the tank to the fish would therefore reduce the calculated efflux by much less than 1 % even if there were no change of water in the tank. The rate constant of water exchange in the tank was about 1·5 h^{-1} while the rate constant of water exchange between fish and tank was in the range 0·1–0·3 h^{-1} so the activity in the water, and the consequent back flux, would be reduced by a further factor of 10, making the overall

error negligible. As the sodium concentration of the blood of the fish is only one-third that of sea water, and the average sodium concentration of the whole fish is even less, the error in sodium efflux in sea water will also be negligible. With sodium effluxes in fresh water the position is not so favourable. The sodium content of the fresh water was 0·36 mM/l so the tank would contain *c.* 300 mM-Na, while a 7 kg fish might contain about the same quantity of sodium. In the absence of any circulation of water through the tank the back flux of sodium would reduce the apparent efflux of ^{22}Na significantly, but the rate constant of water exchange in the bath was about 1·5 h^{-1} and even if the rate constant of sodium efflux from the fish was as high as 0·03 h^{-1} at equilibrium the specific activity of the bath would be only 2% of that of the fish and the error in the calculated efflux, induced by ignoring the back flux, would be even smaller. No correction was therefore made for the back fluxes.

On a number of occasions, when fish were removed from tanks of sea water for the collection of blood samples, it was observed that they ejected a thin jet of urine. It was found that 5 or 10 ml of urine could usually be collected if pressure was applied along the ventral surface in front of the cloaca. Curiously, it was difficult to collect urine in this fashion from fish adapted to fresh water but fish from sea water produced urine on most occasions.

Some urine was collected by catheter. The size and strength of the fish made it impracticable to restrain them during the collection periods so attempts were made, with a limited success, to implant a catheter in the ureter, holding it in place with a suture around the base of the ureter inside the cloaca, together with further sutures in the anal fin. The urine was collected in polythene bags of double thickness tied to the base of the tail. Urine was collected on several occasions by this means both from fresh-water-adapted and sea-water-adapted fish but this procedure was rarely successful for more than a few hours at a time. When left for longer periods the bags were invariably dislodged by vigorous strokes of the tail or torn open between the flanks of fish and the side of the tank. The short periods of collection made reliable estimates of the rates of urine production impossible as the process of catheterization generally resulted in the loss of an unmeasured amount of retained urine which would have to be replaced before the urine could enter the bag.

Individual fishes belonging to the species *A. medirostris* were identified for reference as M_1 to M_{14} and specimens of *A. transmontanus* as T_1 and T_2. Fishes M_1 to M_5 and T_1 were obtained in the summer of 1969, the remaining fishes in 1970.

RESULTS

The dry weight of the plasma varied between 20 and 30 mg/ml and the water content was about 990 mg/ml. Results are expressed per litre of plasma, concentration per kilogram plasma water would be about 1% higher.

Composition of blood of Acipenser *in fresh water*

In the first experiments with sturgeon in fresh water, two green sturgeon (M_3 and M_5) were transferred directly to fresh water, M_3 from estuarine water and M_5 directly from sea water. Both fish died within 48 h of transfer. After 24 h the concentration of the blood plasma of both fishes was already very low (Table 1). Two other specimens

Table 1. *Composition of serum and urine in fresh water.* M_1–M_{10} Acipenser medirostris; T_1 A. Transmotanus. *Concentrations in mM/l*

Fish	M_1	M_2	M_3	M_5	M_8	M_9	M_{10}	T_1	
Weight	17 kg	6·65 kg	5·3 kg	7·2 kg	8·0 kg	7·6 kg	—	3·5 kg	
Period in fresh water	1 day	3 days	1 day	1 day	3 days	3 days	3 days	5 days	
	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Urine
Na	167	140	105	85	119	115	114	125	12·5
K	4·8	5·6	—	—	1·5	2·0	1·7	5·5	2·5
Ca	3·1	3·5	2·5	2·0	1·3	1·8	2·0	3·4	5·0
Mg	1·6	1·4	2·1	1·9	0·6	0·42	0·46	—	—
Cl	143·5	114	83·7	61	106	106	100	—	13
SO_4	—	—	—	—	2·76	2·8	2·84	—	—

of *A. medirostris*, M_1 and M_2, having previously been used in sea-water experiments, survived direct transfer from sea water to fresh water, although they were less active than they were in sea water or brackish water. One animal, M_1, survived for 5 days in fresh water, cutting down salt loss effectively as discussed below. After 24 h in fresh water the plasma concentration was still high (Table 1), but the concentration may have declined further before equilibrium was established. After 5 days in fresh water it was returned to sea water. In order to survive for 5 days the fish must have been at, or close to, equilibrium. The second fish, M_2, maintained a sodium content of 140 mM/l after 3 days in fresh water, similar to that of many teleosts (Table 1). A white sturgeon, T_1, also survived well in fresh water. After 5 days it was still vigorous and was then transferred back to sea water. The composition of the blood and urine after 5 days in fresh water is shown in Table 1. A sodium concentration of 125 mM/l would be rather low for a teleost. In view of the loss of fishes M_3 and M_5 in later experiments the fish were adapted more slowly to fresh water, usually over a period of 2 days, and the blood was analysed after 3 whole days in fresh water (M_8, M_9 and M_{10}, Table 1). Only one good urine sample was collected from these animals (Table 1). A small sample of urine collected from the second *A. transmontanus*, T_2, contained only 2·5 mM Cl/l.

Composition of blood and urine in sea water

Specimens of the green sturgeon, M_1, M_4 and M_5, transferred directly from estuarine water to sea water survived well. The composition of the blood, and of two urine samples, after 48 h in sea water is shown in Table 2. Fish M_6, M_7 and M_{12} were adapted more slowly to sea water by gradually increasing the salinity of the medium over a period of 2 days. After 3 days in full sea water the concentration of their blood plasma was rather lower than that of fish transferred directly to sea water (Table 2). The large excess of chloride over sodium found in all the urines analysed suggested that the urine might contain a high concentration of ammonium ions, but analysis showed that the concentrations of ammonia were quite insufficient to account for the cation deficit.

Sodium fluxes

The rate constants of efflux of sodium from fish adapted to sea water *c.* 15 °C ranged from 0·065 h^{-1} to 0·3 h^{-1}, similar in magnitude to those of teleosts (Table 3). Fish adapted to fresh water of *c.* 20 °C had much lower rate constants. As mentioned above

Table 2. *Composition of serum and urine in fish adapted to sea water. Concentrations in mM/l.*

Fish	M_1	M_3		M_4		M_6		M_7		M_{13}	
Weight	17·0 kg	7·2 kg		5·5 kg		6·0 kg		7·3 kg		9·6 kg	
Period in sea water	2 days	2 days		2 days		3 days		3 days		3 days	
	Plasma	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine
Na	218	222	40	182	15	165	21	138	10	134	5
K	—	—	1·2	—	4·2	2·5	0·6	2·0	4·2	1·7	1·0
Ca	4·9	4·5	3·8	5·0	1·3	2·0	1·0	2·2	8·6	1·8	1·6
Mg	—	—	—	—	—	1·98	24	1·02	22·1	1·82	22·6
Cl	186	197	167	183	70	169	61	146	162·5	139	78
SO_4	—	—	—	—	—	2·48	12	2·84	8·6	2·72	8·4
NH_4	—	—	—	—	—	—	2	—	15	—	—

Table 3. *Rate constants of sodium efflux from sturgeon adapted for three days to sea water or to fresh water*

Sea water 15 °C

Fish	M_1	M_4	M_5	M_6	M_7	M_{12}	M_{14}	T_3
K (h^{-1})	0·092	0·065	0·089	0·18	0·30	0·18	0·12	0·20

Fresh water 19 °C

Fish	M_1	M_2	M_8	M_9	M_{14}	T_1	T_2
K (h^{-1})	0·015	0·011	0·024	0·022	0·030	0·026	< 0·01

some fish failed to adapt to fresh water after direct transfer from sea water. M_3 died after about 30 h in fresh water but shortly before death the rate constant of sodium loss was still as high as 0·11 h^{-1} and the blood concentration at death was reduced to only 235 m-osmole/*l*. Survival was better when the sea water was replaced gradually as in the cases of fish M_8, M_9, M_{10}, T_2, although the number of fishes is too small to be significant, and some fish, e.g. M_1, survived direct transfer well and reduced the rate constant of efflux to 0·014 h^{-1} on both the fourth and fifth days. These rates of efflux are similar to those found previously in small teleosts which generally reduce the rate constant of efflux to 1 % or less in fresh water, e.g. salmon parr maintain a rate constant of efflux of 0·01–0·02 h^{-1} at 10 °C (Potts, Foster & Stather, 1970) and *Fundulus kansae* maintain a mean rate constant of 0·012 h^{-1} at 20 °C (Potts & Fleming, 1971).

Changes of sodium flux on transfer from sea water to fresh water and back again

Immediately following transfer from sea water to fresh water the rate of efflux of sodium from euryhaline teleosts usually continues at a high rate and the rate constant is generally closer to the sea-water rate than to the equilibrium rate in fresh water, but the rate of efflux usually declines rapidly during the first hour following transfer. If this reduction did not occur then the survival of the fish would be limited to a few hours as the rate of loss would far exceed the capacity of the uptake system. Sturgeons also cut down the rate of efflux when transferred to fresh water but the time required for adaptation seems to be considerably longer than in the teleosts so far examined, although our data are limited. The rate of efflux was monitored at frequent intervals by whole-body counts in the case of only one animal, M_{14}. In this experiment the sea

Table 4. *Change of rate constant of efflux (K) following transfer from sea water to fresh water (time 0 h) and from fresh water to sea water (time 50 h). M_{14}. For details see text*

	Sea water to fresh water						
Time (h)	0–2½	2½–5	5–8	8–20	20–29	29–43	43–50
K (h^{-1})	0·12	0·12	0·17	0·064	0·034	0·037	0·030

	Fresh water to sea water			
Time (h)	50–53	53–57	57–67	67–83
K (h^{-1})	0·053	0·052	0·067	0·079

Table 5. *Changes in blood concentration on transfer from sea water to fresh water (time 0 h) and fresh water to sea water (time 18.36 h). M_{18}*

Time (h)	0	5·33	8·35	18·36	27·87
Conc (mM Cl/*l*)	152	137	113	108	134

water was gradually replaced by fresh water. The water flow was set to reduce the salinity by about 30%/h so that the water was virtually fresh after 15 h. However, even after 20 h the rate of efflux was still as high as 6·4%/h and only declined to 3%/h after 2 days, by which time the blood chloride had declined to 134 mM/*l*. The fresh water was then replaced by sea water in a similar fashion and the rate of efflux rose gradually to 7·9%/h after 16 h, by which time the blood concentration had risen to 157 mM-Cl/*l* while the urine contained 206 mM/Cl/*l* and 50 mM-NH_4/*l*. The experiment was discontinued after 16 h in sea water because the declining activity of the ^{22}Na made counting difficult. The rate constants at various intervals following transfer are shown in Table 5. The temperature rose, during the replacement of the sea water with fresh water, from 15·5 to 19 °C and declined to 15 °C when the sea water was restored. In a second experiment a fish, M_{13}, was transferred directly from sea water to fresh water and the declining concentration of the blood was monitored. After 18 h the fish was returned to sea water. The changes which occurred in the rate constant of the efflux are shown in Table 5. Not all animals adapted successfully to the changes of medium. As mentioned above, sturgeons M_3 and M_5 died after being transferred to fresh water and efflux rate constants continued at a high level immediately before death averaging 0·05 h^{-1} in one fish and 0·011 h^{-1} in the second.

Water permeability

It has recently been shown that euryhaline teleosts are more permeable to water when adapted to fresh water than when adapted to sea water (Evans, 1969). This change in permeability is due in part to the lower concentration of calcium in fresh water compared with sea water and in part to the higher concentration of the hormone prolactin in the fresh-water-adapted fishes (Potts & Fleming, 1970). As the sturgeons are distant, and in some respects more primitive, relatives of the teleosts, the permeability of the sturgeons to tritiated water was examined. The results are given in Table 6. The sample is small but the results are consistent. The mean value of the rate constant of efflux of tritiated water from the four sea-water-adapted sturgeons is 0·19 h^{-1} but for the five fresh-water-adapted sturgeons the mean is 0·42 h^{-1}. The ranges do not over-

Table 6. *Rate constants of tritium efflux from sea-water-adapted and fresh-water-adapted sturgeons*

Sea water 15 °C

Fish	M_6	M_7	T_2	M_{13}
K (h^{-1})	0·29	0·150	0·153	0·169

Fresh water 19 °C

Fish	M_8	M_9	M_{10}	M_{11}	T_2
K (h^{-1})	0·57	0·334	0·297	0·467	0·430

Table 7. *Concentration of cortisol in blood plasma of* Acipenser medirostris, *µg/100 ml*

Medium	Cortisol concentration
Fresh water	1·4
Fresh water	1·7 } Mean 1·7
Fresh water	2·0
Sea water	1·4
Sea water	1·0 } Mean 1·2
Sea water	1·2

lap. In the only specimen measured in both conditions, T_2, the values of the rate constant were 0·153 and 0·430 h^{-1} respectively. However, part of the difference will be due to higher temperature of the fresh water. Evans (1969) found that the Q_{10} of the fluxes of tritiated water in teleosts was 1·9. Assuming the same value holds for sturgeons a temperature difference of 5 °C would reduce the water permeability by a factor of 0·725; the sea-water value of 0·19 h^{-1} at 15 °C should therefore be compared with a computed fresh-water value of 0·305 at 15 °C. Some part of the difference will be due to the lower calcium content of fresh water but the fresh water was very hard and the difference in the permeabilities is so great that it is likely that prolactin has a similar function in sturgeons to that in the teleosts. It will be difficult to hypophysectomize sturgeons but histological examination of the pituitary gland would throw some light on this problem.

Cortisol

In the mammals the steroid hormones of the adrenal glands are of major importance in the regulation of salt balance. Much less is known of the hormonal control of salt balance in the fishes but amongst the teleosts cortisol is quantitatively the major corticosteroid produced by the interrenal glands. Cortisol has been shown to influence the movement of sodium ions across the gill, in both fresh-water-adapted and sea-water-adapted eels (Chan, Rankin & Chester Jones, 1969; Mayer, Maetz, Chan, Foster & Chester Jones, 1967), and to affect water movement across the gut (Henderson, Chan, Sandor & Chester Jones, 1970). Little is known of the hormonal control of salt movement in the sturgeons so the opportunity was taken to collect blood samples from sea-water-adapted and fresh-water-adapted fish for cortisol assay. The results are shown in Table 7. Cortisol was found in both sea-water-adapted and fresh-water-adapted sturgeons, being rather more concentrated in fish from fresh water, although the number of determinations was too low to be sure that the difference is significant.

DISCUSSION

Although physiologists usually prefer to work with large animals rather than small, mature sturgeons are too large to be convenient. The difficulties of collection and maintainance kept the number of experimental animals low. When individuals vary considerably around the mean, average values based on less than ten or a dozen specimens are not very satisfactory but, nevertheless, some conclusions may be drawn from the data described above. The similarities between euryhaline sturgeon and euryhaline teleosts are numerous and striking. Both in fresh water and in sea water the composition of the blood is similar to that of teleost fishes. In fresh-water teleosts the sodium content usually lies between 140 and 160 mM/*l.* (Holmes & Donaldson, 1969). The blood concentration of sturgeons in fresh water is rather lower than in teleosts even in the more euryhaline *A. transmontanus*. Urist & van de Putte (1967) reported similar values with a mean sodium concentration of 123 mM-Na/l in gravid females and 129 mM-Na/l in non-gravid females. In the less euryhaline *A. medirostris* the sodium concentration after 3 days in fresh water had fallen below 120 mM-Na/l in three individuals although one individual (M_2) still maintained 140 mM-Na/l. These low values are not characteristic of all sturgeons. Magnin (1962) reported a mean sodium concentration of 145 mM-Na/l in a series of *A. sturio* and of 148 mM-Na/l in *A. fulvescens* in fresh water. In sea water the blood concentrations were generally rather higher than in fresh water, although two animals, M_7 and M_{12} (Table 2) maintained rather low blood concentrations. Similarly Magnin (1962) found a relatively high blood sodium, mean 172 mM-Na/l., in *A. oxyrhynchus* even in brackish water although Urist and van de Putte recorded a mean of 130 mM-Na/l in *A. transmontanus* in sea water. The blood sodium concentrations in marine teleosts usually range from 160 to 180 mM-Na/l (Homes & Donaldson, 1969). Our results seem to indicate that sturgeon normally regulate their blood concentration at a rather lower level than do teleosts but the larger specimens were very difficult to keep for long periods in captivity with the tanks available and some allowance should be made for the stress of captivity. The potassium concentrations recorded in the plasma vary between 1·5 and 6 mM-K/l but blood plasma potassium in all vertebrates is peculiarly susceptible to contamination by potassium loss from the erythrocytes and generally the lower values are to be preferred. Recorded values for *Anguilla anguilla* in fresh water vary from 1·49 to 9·0 mM-K/l (Holmes & Donaldson, 1969, Table XVII). Urist & van de Putte (1967) recorded 2·0–2·7 mM-K/l in *Acipenser transmontanus* while Magnin (1962) reported means of 2·7 and 4·3 mM-K/l in *A. oxyrhynchus* and *A. sturio* in fresh water and 2·8 and 4·6 mM-K/l respectively in brackish water. The concentration of calcium found in the blood, though higher in sea water, is well within the range recorded for teleosts in both media. Urist and van de Putte found 1·7 mM-Ca/l in *A. transmontanus* in sea water, 1·8 mM-Ca/l in females in fresh water but 4·6 mM-Ca/l in gravid females in fresh water. Again Magnin found rather higher values in *A. sturio* than in *A. oxyrhynchus*. Magnesium regulation appears to be less effective than calcium regulation, declining from about 2 mM-Mg/l in sea water to only 0·5 mM-Mg/l in fresh water in some individuals. Urist & van de Putte (1962) recorded 2·1 mM Mg/l in sea water, 2·0 in non-gravid females in fresh water but only 1·1 in gravid females. Idler & Tsuyuki (1958)

recorded values of 0·5 mM-Mg/l in the serum of *Onchorhyncus nerka* in fresh water. Chloride concentrations are similar to those of sodium, but are sometimes higher, sometimes lower. In most land vertebrates the sodium concentrations exceed the chloride concentrations, the deficit being made up by bicarbonates. In teleosts bicarbonate is less important and chloride concentrations sometimes exceed sodium concentrations, being balanced by the minor cations. Lahlou (1967) records a considerable excess of chloride over sodium in *Platichthys flesus* when adapted to sea water. Urist & van de Putte recorded 5–6 mM-HCO_3/l in both marine and fresh-water *Acipenser transmontanus*. The sulphate content of the plasma was similar in both sea water and fresh water. The value in sea water, about one-tenth that of the medium, was much as expected but the high value in fresh water was not. The sulphate should clear from the blood at about the same rate as magnesium but the ions may be of organic origin. Urist & van de Putte found only 0·5 mM-SO_4/l in both media; Robertson *et al.* (1961) reported that Pacific salmon *Oncorhyncus tschawytscha* when in fresh water had a higher sulphate concentration in the plasma than when in sea water.

The composition of the urine in sea-water-adapted fishes, with a relatively high concentration of magnesium and sulphate ions, a chloride concentration similar to but lower than that of the blood, and containing only a low concentration of sodium can be paralleled amongst the teleosts. An apparent deficit of cations is not balanced, as was expected, by ammonium ions, which were found in relatively low concentrations; Magnin (1962) recorded similar urine compositions from *A. sturio* from brackish water, although the highest chloride concentration he recorded was only 33 mM-Cl/l. The composition of the sample of urine from a fresh-water-adapted sturgeon was unexceptional, containing about 13 mM-Na and Cl/l. The eel in fresh water has been found to produce urine containing 13 mM-Na/l (Chester Jones, Henderson & Rankin, 1969), and *Fundulus kansae* 12·8 mM-Na/l (Stanley & Fleming, 1964). Magnin recorded a mean sodium content of 35 mM-Na/l and a mean chloride concentration of 5 mM-Cl/l in urine collection from *A. sturio* in fresh water.

The sodium fluxes found in sea-water-adapted sturgeon also compare closely with those of marine teleosts. Evans (1969) showed that in a variety of teleosts isotopic fluxes varied as $(\text{weight})^{0.89}$. A 1000-fold change in weight would therefore reduce the rate constant of the flux by a little over half. Rate constants of 0·3–0·4 h^{-1} have been reported in *Fundulus kansae* (Potts & Fleming, 1971) and in *Tilapia* (Potts *et al.* 1967) both weighing only a few grams. The sturgeon are therefore similar to teleosts when compared on a similar weight basis. However, in fresh water the rate constant of salt efflux was rather higher than that found in fresh-water teleosts and this may be correlated with the rather low blood concentrations observed in fresh water. The two white sturgeon were not obviously better in respect to salt conservation than the green sturgeon although the former are said to penetrate further into fresh water. In addition to remaining relatively permeable to salt, when in fresh water, the sturgeons contrast poorly with the teleosts (Potts & Fleming, 1971) and with the lampreys (unpublished observation) in the rate of decline of salt loss when transferred to fresh water. After 6 h the reduction of salinity had begun, the efflux was apparently higher than in sea water in M_{14} and during the next 12 h, when the medium contained only a few per cent of sea water, the efflux still averaged 5-6%/h. In comparison euryhaline teleosts cut down to 1%/h after one or 2 h in fresh water (Potts & Fleming, 1971).

It is apparent that the sturgeons must have an effective salt-uptake system in order to survive at all in brackish water. The white sturgeon is not more effective on the basis of the slender evidence described here. If these observations are typical of sturgeons then it would seem that sturgeon are ill adapted to an immediate transition from sea water to fresh water. If this is the case, then unlike the salmon and sea trout they are unlikely to penetrate small rivers and streams flowing directly into the sea, but should be confined to major river systems with large estuaries providing large volumes of brackish water in which the fish may either adapt gradually to lower salinities or else spawn before returning to the sea.

The permeability to tritiated water is markedly higher in fresh water than in sea water. Part of the difference may be attributed to the lower calcium content of the fresh water, but in view of the common ancestry of the sturgeons and the teleosts it is very likely that prolactin is involved with the increased permeability found in fresh water. Although no prolactin-like substance could be demonstrated in the lampreys (Aler, Bage & Fernholm, 1971) prolactin probably occurs in the selachians as hypophysectomy has recently been shown to decrease water permeability in *Scyliorhinus* while prolactin restored it (Payan & Maetz, 1971). If prolactin occurs in the latter group, as well as in the teleosts, it is likely to be found in the sturgeons as well. The rate constant of turnover of water, *c.* 0·4 h^{-1} at 19 °C, may be compared with a rate constant of *c.* 1·4 h^{-1} for 1·5 g *Fundulus kansae* at 20 °C. Once again, allowing that a three or 4000-fold difference in weight would more than halve the rate constant, the comparison is close. The osmotic permeability constant and the diffusion permeability constants of water are very similar in marine teleosts but the osmotic constant is several times larger in fresh-water teleosts (Motais *et al.* 1969). If the same holds true for sturgeons then an accurate estimate may be made of the drinking rate in marine sturgeon and a rough estimate of urine production in the fresh-water sturgeon. The mean flux of tritiated water in sea-water-adapted fish has a rate constant of 0·19 h^{-1}. If the sea water is assumed to have an osmotic concentration of 1000 m-osmole/l and the fish are assumed to have an osmotic concentration of 350 m-osmole/l and a water content of 70%, then the mole fraction difference in water concentration between the fish and the sea water will be (1·00–0·35)/55·4 or 0·0117 and the net or osmotic water flux will be $0{\cdot}0117 \times 0{\cdot}7 \times 0{\cdot}19$ or 0·00156 h^{-1}. That is 1·56 ml/h. The drinking rate would have to be slightly larger than this to allow for urine production. This would correspond to a sodium intake of about 700 μM-Na/kg fish. The sodium content of the sturgeon is unlikely to be less than 50 mM-Na/kg. As the rate of sodium turnover has a rate constant of *c.* 0·15 h^{-1} or perhaps 7 mM-Na/kg/h then it follows that drinking can contribute only a small part of the total sodium influx, most of which must take place directly through the body surface as in the teleosts. Whether the influx takes place by diffusion, implying a high permeability to sodium in sea water, or by exchange diffusion, is uncertain in teleosts but the continuance of the efflux at a high rate after transfer to fresh water suggest that exchange diffusion is unimportant in sturgeons.

The osmotic uptake in fresh water can be only approximately calculated. If it is assumed that the blood concentration in fresh water is 300 m-osmole/l and that the osmotic permeability coefficient is three times the diffusion permeability coefficient, as in the eel (Motais *et al.* 1969), then the osmotic inflow will be *c.* $0{\cdot}4 \times 3 \times 0{\cdot}7 \times 0{\cdot}3/55{\cdot}4$, equivalent to 4·5 ml/kg/h. The urine flow should be approximately the same. This is

rather lower than in most fresh-water teleosts but this is mainly the consequence of the large size. The sodium loss in the urine, *c.* 50–60 μM/kg/h, will be small compared with the total sodium loss of *c.* 800 μM/kg/h.

The cortisol content of sturgeon blood is low compared with some teleosts, but similar to that found in the European eel *Anguilla anguilla*. A recent study by Ball *et al.* (1971) has shown that the cortisol content of eel plasma is also similar in both fresh-water-adapted and sea-water-adapted fish but adaptation following transfer from either sea water to fresh water or from fresh water to sea water is accompanied by a transitory increase in the cortisol levels in the plasma to several times that of the normal level. Ball suggests that this pulse of cortisol triggers off the adaptive changes in the gills and gut. An assay of corticosteroids in *Acipenser oxyrhynchus* demonstrated even lower quantities of cortisol, only 18 μg/100 ml in the plasma of a single immature specimen but other steroids occurred in even lower concentrations (Sangalang, Weisbart & Idler, 1971).

In general, it is clear that the sturgeons are very similar to the teleosts in the main features of their osmotic regulation. This is true not only of the composition of the blood in sea water and fresh water but also in the high flux of sodium ions across the gill in sea water. In addition the regulation of water permeability is similar to that found in teleosts and it is probable that both cortisol and prolactin are involved in the maintenance of salt and water balance. However, there is evidence that at least in the two species examined the regulation of salt balance on change of medium is not as rapid as in some euryhaline teleosts. This requires confirmation but could be of considerable ecological significance.

SUMMARY

1. Analyses have been made of the blood and urine of the euryhaline sturgeon *Acipenser medirostris* and the rates of turnover of sodium and water in both sea water and fresh water have been measured.

2. The blood concentration is rather lower in fresh water than in sea water and the concentration of magnesium ions declines markedly.

3. The rate of turnover of sodium ions is high in sea water and similar to that of marine teleosts. The rate of turnover of sodium is much lower in fresh water but adaptation to fresh water is slow and the animals are more permeable to sodium than are teleosts.

4. The rate of turnover of tritiated water is more rapid in fresh water than in sea water but in each medium it is similar to that of teleosts of a similar size.

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