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SODIUM- AND POTASSIUM-ACTIVATED ADENOSINE TRIPHOSPHATASE IN KIDNEYS OF *FUNDULUS HETEROCLITUS* ADAPTED TO FRESH AND SALT WATER¶

It is well known that glomerular teleosts excrete a profuse dilute urine in fresh water and a scanty urine isotonic to plasma in seawater.[1] The striking changes in renal function that ensue when euryhaline fish are transferred from seawater to freshwater or the reverse have been studied in five species: the European eel, *Anguilla anguilla*,[2] the Japanese eel, *Anguilla japonica*,[3] rainbow trout, *Salmo gairdneri*,[4] European flounder, *Platichthys flesus*,[5] and the plains killifish, *Fundulus kansae*.[6] In all, the mechanism of adjustment involves changes in glomerular filtration and tubular reabsorption of sodium. Depending on the species, glomerular filtration rate is three to twenty times higher in fresh water than in salt water, while urine excreted in fresh water contains very little sodium. The quantity of sodium reabsorbed by the kidneys in freshwater must therefore exceed by a considerable amount that transported by the renal tubules in seawater. Sodium transport by the kidney thus changes in a direction opposite to that of sodium transport by the gill when the salinity of the medium is changed.

Sodium- and potassium-activated adenosine triphosphatase has been implicated in the transport of sodium across epithelial membranes[7] and we have shown previously that the specific activity of this enzyme in the gills of *Fundulus heteroclitus* falls after prolonged adaptation to freshwater.[8] Our present experiments indicate that the activity of sodium- and potassium-activiated adenosine triphosphatase rises in the kidneys of Fundulus transferred to freshwater, an adaptive change consistent with the increase in transport work by the kidneys known to occur under these circumstances.

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Two groups of male *Fundulus heteroclitus*, caught in the vicinity of New Haven, Conn., were employed in these experiments. The 1967 series were captured between April 9 and 16 of that year and transferred to either fresh or salt water at about 20°C. after preliminary treatment with dilute formalin and inspection for parasites. Fresh water tanks were supplied with dechlorinated running city water. Salt water tanks were on a recirculating filtered system. All fish were kept on an 8 hour daylength and fed daily. The 1968 series were captured on October 18, 1967 and similarly treated and inspected. They were maintained in standing water aquaria through December and later transferred to the running freshwater or recirculating seawater systems. Some trouble from skin infections was experienced in the standing water aquaria but when medication was required (acriflavine or malachite green dips) the same treatment was given to all fish in all groups.

At autopsy each fish was weighed and a blood sample taken for serum sodium and potassium. The testes were weighed as a measure of the sexual maturity of the fish. Gills and kidneys (mesonephros without the leucopoietic head kidney) were taken for enzyme study, as described below.

Table 1 summarizes the autopsy data. The relative size of the testes (GSI*) shows that our samples cover the major phases of the reproductive cycle: the 1967 groups killed in May were in full sexual maturity; later samples from this population showed that the fish passed gradually into sexual regression. The 1968 series, on the other hand, were undergoing maturation in March.

The kidneys from two or three fish were pooled and homogenized in 2 ml. of an ice-cold solution, pH 6.8, containing 0.25 mole of sucrose, 5 mMole of sodium ethylenediaminetetraacetate, 1 g. of sodium deoxycholate, and 30 mMole of histidine buffer per liter. Gill homogenates were prepared by homogenizing all the gills including gill arches from a single fish in 2 ml. of the homogenizing solution. Homogenization was carried out with a Teflon pestle at 1725 rev/min. The homogenate was filtered through a double layer of gauze and assayed within two hours. In some cases, a crude microsomal suspension was prepared from gill homogenates as described in reference 8. Samples (0.1 ml.) of the whole homogenate, containing 1 to 2 mg. of protein per ml. in the case of kidneys, and 5-7 mg. of protein per ml. of gill homogenate, were assayed for adenosine triphosphatase as outlined by Epstein, Katz, and Pickford,[8] except that incubation was carried out in a shaking water bath at 37°C. for 15 minutes instead of 5 minutes. The activity of Na^+- and K^+-activated adenosine triphosphatase was derived from the difference between the amount of inorganic

* Gonosomatic Index (Testis Wt./Body Wt. × 100).

TABLE 1. SUMMARY OF CONDITION OF FISH AT AUTOPSY

Date	Acclimation (weeks)	Number		GSI*		Serum Na^+ (mEq/liter)		Serum K^+ (mEq/liter)	
		SW	FW	SW	FW	SW	FW	SW	FW
1967 series:									
5/15†	4	3	3	4.44	3.91	182.6	168.5	9.6	7.5
5/31	6	8	7	3.95	4.55	174.6	163.1	7.1	2.8
7/31	11.5	5	5	2.67	1.42	179.4	169.6	4.5	3.9
9/7	16	10	9	2.36	2.05	180.0	165.4	4.3	5.2
9/27	22	5	4	1.87	1.21	175.7	172.8	6.2	3.6
1968 series:									
3/11	20.5	6	6	2.58	2.42	175.1	168.1	11.4	5.5
3/20	22	6	6	3.07	2.60	176.4	165.7	8.4	3.6
3/28	23	6	6	4.00	3.79	170.1	171.0	5.5	4.1
Mean ± SE		—	—	—	—	176.7 ±1.4	168.0 ±1.1	7.1 ±0.9	4.5 ±0.5

* GSI = Gonosomatic Index (Testis Wt./Body Wt. × 100).
† "Untrained" fish, i.e., not accustomed to daily handling.

phosphate (Pi) released from adenosine triphosphate in the medium containing K^+ and that released in medium containing no K^+. It is expressed as micromoles of Pi released per hour per milligram of protein. Breakdown of adenosine triphosphatase in the medium containing no K^+ is referred to as "residual adenosine triphosphatase." The concentration of protein in the tissue suspensions was measured by the technique of Lowry.[9]

All determinations of adenosine triphosphatase were performed in triplicate. Measurements of enzyme activity carried out on the same day agreed well with each other but there was greater scatter when the values from fish sacrificed several weeks or months apart were compared. Part of this difference may be due to minor changes in technique from one experimental group to another. The most valid comparisons are those between the tissues of groups of fish in fresh and salt water killed and processed in the same way on the same day.

The specific activity of sodium-potassium-activated adenosine triphosphatase in the kidneys of Fundulus adapted to freshwater was about 50 per cent higher than in the kidneys of fish kept in salt water (Table 2). The significance of this difference is underlined by the fact that there was no change in the residual, or Mg^{++}-activated adenosine triphosphatase. In gill homogenates, sodium- and potassium-activated adenosine triphosphatase changed in the opposite direction, being approximately 40 per cent lower in freshwater fish than in saltwater animals. These results are consistent with those reported earlier in *Fundulus heteroclitus*[8] as well as with the

TABLE 2. CHANGES IN ATPASE IN THE GILL AND KIDNEY OF *Fundulus heteroclitus* AFTER ADAPTATION TO FRESHWATER*

Date	Gills						Kidneys					
	Sodium-potassium ATPase			Residual ATPase			Sodium-potassium ATPase			Residual ATPase		
	SW	FW	% change	SW	FW	% change	SW	FW	% change	SW	FW	% change
1967 series:												
5/15	10.9	9.0	—17.4	25.1	20.1	—15.9	25.4	40.1	+ 57.9	18.0	18.4	+ 2.2
5/31	14.5	12.2	—15.9	25.0	25.8	+ 3.2	28.7	48.9	+ 70.4	20.2	35.8	+77.2
7/31	12.0	6.7	—44.2	10.3	6.6	—35.9	7.8	12.3	+ 57.7	15.0	9.8	—34.9
9/5	6.7	3.7	—44.8	12.0	10.4	—13.3	21.8	34.8	+ 59.6	21.1	25.1	+19.0
9/7	12.8	5.5	—57.0	16.4	11.9	—27.5	17.3	26.1	+ 50.9	20.7‡	29.8	+44.0
9/27	5.4	4.8	—11.1	15.6	12.2	—21.8	6.3	13.3	+111.1	20.4	16.2	—25.5
1968 series:												
3/11	†7.3	†4.0	—45.2	†20.7	†10.0	—51.7	16.4	24.2	+ 47.6	19.5	16.2	—16.9
3/20	†8.2	†4.2	—38.8	†19.3	†17.7	— 8.3	15.7	20.2	+ 28.7	11.4	14.4	+26.3
3/28	†2.6	†0.7	—73.1	†14	†11	—21.4	15.5	18.4	+ 18.7	40.3	32.5	—19.4
Mean			—38.6			—21.4			+ 55.8			+ 8.0
SE			± 6.8			± 5.4			± 8.8			±12.3
P			<0.001			<0.001			<0.001			NS

* Values of adenosine triphosphatase represent the average of 5 or 6 fish in each group expressed as μM Pi/mg. protein/hr. Each value for kidney ATPase is the mean of two separate pools of kidney tissue, each containing 2 or 3 fish. Incubation time 15 minutes except for the last (3/28/68) samples, which were incubated for 45 minutes.

† Crude microsomal fraction.

‡ One very low value (4.2) omitted.

data on gill adenosine triphosphatase in eels during adaptation to seawater published recently by Kamiya and Utida.[10] The residual ATPase of gills appeared to fall slightly after transfer to freshwater, though this was not the case in other experiments.[8,10]

F. heteroclitus is a remarkably good osmoregulator. The data here reported on serum sodium in fresh and salt-water adapted groups confirm more extensive studies[11] and illustrate the effectiveness of the adaptive changes in sodium transport by gill and kidney. On the average, serum sodium in the present experiments was only 4.9 per cent lower in freshwater. Serum potassium declines to a greater extent; in these experiments the decline averaged 36.6 per cent (Table 1). Neither serum sodium nor serum potassium showed any significant correlation with the state of sexual maturity.

The work of the kidneys in transporting sodium in both freshwater and seawater can be estimated roughly from the measurements of glomerular filtration and urinary sodium made by Fleming and Stanley in *Fundulus kansae*,[6] a species closely related to *Fundulus heteroclitus*. In freshwater, inulin clearance is about 0.5 ml/kg/min., and although one-third to one-half of this volume is excreted as urine, 95 per cent of the filtered sodium is reabsorbed. After seven days acclimation to seawater, glomerular filtration is 3-8 per cent and urine flow 2-5 per cent of their previous values in freshwater. Similar though less marked changes are seen in the eel[3] and the European flounder.[5] It is likely that in the present experiments renal tubular reabsorption of sodium was several times higher in Fundulus adapted to freshwater than in fish accustomed to seawater. The increase in sodium-potassium-activated adenosine triphosphatase probably reflects this change in active ion transport and may help to mediate it. Similar changes in the activity of this enzyme have been shown to accompany increases in filtration rate and tubular reabsorption induced in rats by unilateral nephrectomy, protein feeding and glucocorticoids.[12]

A role for sodium-potassium-activated adenosine triphosphatase in the transport of sodium across epithelial membranes is suggested by the changes that take place in the concentration of the enzyme in gill and kidney when euryhaline fish are adapted to salt and to fresh water. In seawater, sodium transport by the gill is high and by the kidney low. In freshwater, active outward transport of sodium by the gill ceases, while the kidney must reabsorb large quantities of sodium from the greater volume of glomerular filtrate. In each of these circumstances, the specific activity of sodium-potassium-activated adenosine triphosphatase changes in the same direction as does the work of sodium transport, and therefore in opposite directions in gill and kidney.

SUMMARY

The activity of adenosine triphosphatase stimulated by sodium and potassium is higher in the kidneys of the euryhaline killifish, *Fundulus heteroclitus*, after adaptation to freshwater than when the fish is adapted to seawater. Enzyme activity changes in a direction parallel to the work of the kidneys in transporting sodium, and opposite to simultaneous changes in the adenosine triphosphatase activity and ion transport of the gills.

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