ELECTROLYTE CHANGES ASSOCIATED WITH TRANSFER OF THE STEELHEAD TROUT (SALMO GAIRDNERI) INTO SEAWATER

by

MARY HOPE VICKERS
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Department of Zoology

The University of British Columbia,
Vancouver 8, Canada.

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ABSTRACT

The purpose of the investigation was to elucidate the mechanisms enabling the survival of euryhaline fish in salt water. Steelhead trout (Salmo gairdneri) were transferred to 60% seawater and serial measurements made of the serum and muscle sodium and potassium content and of the tissue water during a ten day period after transfer.

The initial 24 hours in seawater were characterized by a dehydration of the tissues and a great increase in the body electrolytes. This was followed by a regulatory phase which represented the mobilization of active transport mechanisms in the muscles and gill tissues, enabling the excretion of the dominant extracellular cation, sodium. The regulation of potassium, the main intracellular cation, was assigned to the kidney. The regulation of tissue potassium and water appeared to be dependant on the regulation of sodium.

After 110 hours in seawater the regulatory processes had returned the animal to a new equilibrium which was characterized by: 1) serum cations only 6% higher than fresh water controls, 2) muscle potassium 15% higher than fresh water controls, and 3) a lower tissue water content than the fresh water controls.

The control of this osmoregulatory adaptation to a hypertonic environment is discussed and possible hormonal action considered.

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INTRODUCTION

The capacity of teleost fish, whether they be freshwater or marine species, to withstand the varying osmotic demands of their environments is a remarkable physiological phenomenon. Fresh water fish must maintain concentrations of electrolytes in their body fluids which have an osmolarity significantly greater than their environment. Marine teleosts, on the other hand, maintain their body fluids against a hypertonic environment which tends to dehydrate the tissues and flood the body fluids with electrolytes. It is clear, therefore, that any fresh water fish which is capable of adjusting its regulatory mechanisms to withstand the osmotic hazards of seawater is well worthy of investigation. Such a euryhaline fish is the Steelhead trout (Salmo gairdneri).

The principal osmoregulatory mechanisms in fresh water fish are the efficient reabsorption of ions by the kidney tubules, the production of a copious hypotonic urine, and possibly the active uptake of ions through the gill epithelium. In order to conserve water, marine teleosts drink salt water and in effect distill it, since the gills appear to be able to actively excrete salts, possibly via the "chloride secreting cells". The kidneys of marine teleosts would seem to play a minor regulatory role by producing the minimum amount of urine necessary to remove toxic waste products from the body. This urine, although hypotonic to the body fluids, is more concentrated than that of fresh water fish, indicating a possible anti-diuretic action. These adaptations have been comprehensively reviewed by Krogh (1939) and Black (1957).

In freshwater fish, where salt retention is imperative, one would expect a priori that a salt retaining mechanism would be present. In mammals the adrenal cortex produces compounds which function in salt retention, and the most active of these, aldosterone, has recently been demonstrated in the peripheral blood of spawning salmon (Phillips et al, 1959) while the anterior interrenal has been identified as its site of synthesis in Fundulus (Phillips and Mulrow, 1959). This

would seem to be further indication of the homology thought to exist between the interrenal tissue of teleosts and the mammalian adrenal cortex. However, there is no evidence extant to indicate whether this aldosterone is physiologically active in the fish and if so what its action may be. The observation that the interrenal of Fundulus is quiescent in salt water (Pickford et al, 1957) supports the view that sodium retaining steroids (viz. aldosterone) produced by the interrenal promote survival in fresh water. The quiescence of the interrenal in salt water is considered to be a result of the decreased demand for salt retaining steroids (Chester Jones, 1956). The validity of this interpretation is open to question since Holmes (1959) has shown that the corticosteroids, DCA and hydrocortisone, promote loss of sodium through the gills in a salt loaded fish, and Sexton (1955) has demonstrated that DCA depressed the uptake of sodium against a diffusion gradient in the goldfish gill. Other steroids, which in the mammal effect predominantly carbohydrate metabolism, have been identified in the serum and interrenal tissue of salmonid fish. An increase in serum 17-hydroxycorticosteroids and an increase in interrenal volume have been observed in smolting salmon (Fontaine and Hatey, 1954; Olivereau, 1960). It is significant to note that during the smolting period salmonid fishes are most able to withstand transfer to seawater (Houston, 1959; Koch, 1959). In addition, structures other than the interrenal tissue have been postulated as possible organs of osmoregulation. Fontaine and Hatey (1959) claimed the identification of corticosteroids in the corpuscles of stannius, although this observation is at variance with the findings of Ford (1959) and Phillips (pers. comm.). Enami (1959) has described the urohypophysis which may well have an osmoregulatory role.

It is postulated that a neurohumour associated with the posterior pituitary is important in the maintenance of osmotic balance in teleosts upon transfer to sea water (e.g. Chester Jones, 1956). This postulate is based on the work of Arvy and Gabe (1954) who demonstrated an increase in neurosecretory material in the supra-optic

nucleus less than one half hour after transfer to seawater. Recent work by Fridberg and Olsson (1959) have confirmed these observations in Gasterosteus. As in the higher vertebrates, compounds having ADH, pressor, and oxytocic activity have been identified in the teleost neurohypophysis (Herring, 1915; Heller, 1941). The fact that pituitaries from marine teleosts are more active than those of fresh water fish in promoting water retention in the frog indicates the presence of a greater amount of oxytocin in the marine fish (Weise, 1959). Holmes (1959) showed that in vivo injections of oxytocin decreased the in vitro respiratory rate in the kidney in Salmo clarki clarki, while a large dose of vasopressin enhanced kidney respiration. Furthermore, Holmes (1959) has demonstrated that vasopressin diminished the renal excretion of sodium in sodium loaded trout.

It would appear that the posterior pituitary hormones are active in sea water teleosts, although their mode and site of action is by no means well understood.

When a fish is transferred from fresh to salt water, it must be able to pump out the incoming ions if it is to survive. If measurements are made of the changes observed in ionic content of the blood and tissues after a euryhaline fish is transferred to saltwater, then an insight into the osmoregulatory mechanism may be made. The purpose of this investigation was to examine these changes in electrolyte composition and tissue water. Steelhead trout, therefore, were transferred from freshwater to seawater and serial measurements were made of the changes in serum and muscle sodium and potassium levels and in tissue water content. In this way an adequate description of the normal osmoregulatory changes under these conditions could be obtained.

MATERIALS AND METHODS

1. Care and Maintenance of Fish

Hatchery raised yearling steelhead trout (Salmo gairdneri) of similar genetic stock were obtained from Cultus Lake Hatchery, British Columbia, by the courtesy of the B.C. Game Department. In Vancouver the fish were stored in outdoor cement tanks supplied with running dechlorinated water, the temperature of which remained between 4.5 and 5.0° C. during the experimental period. Stock fish were fed weekly (Clark's fingerling fish food), whereas the experimental fish were not fed for a week prior to, nor during time in seawater. All fish were allowed to equilibrate for at least a week after transportation before being used experimentally.

2. Experimental Treatment

Flame photometric (Zeiss FP 5) estimations of sodium and potassium for muscle and serum samples were made on each fish. These values were expressed in milliequivalents (meq.) of ion per kilogram of serum and muscle respectively. The water content of all muscle samples was calculated from fresh and dry weights of the samples.

Upon removal from the tank, the body weight and forklength were recorded. Paper towels were wrapped around the cloaca to prevent contamination of the blood, the caudal fin severed one inch posterior to the anus and the efferent blood collected in a centrifuge tube. Muscle samples were then taken. This process took about 8 minutes per fish to complete.

All glassware used in analyses was pyrex and all chemicals used were CP reagent grade.

a. Treatment and Analysis of Blood

The collected blood was allowed to clot in a refrigerator for 30-45 minutes. It was then centrifuged for 15 minutes at 1500 RPM, and the serum was pippetted off and stored in stoppered tubes.

Analyses were made on 0.2 or 0.5 ml. serum, which was digested with a drop

of HCl and diluted to 25 ml. (1/125 dilution) or to 50 ml. (1/100 dilution) respectively. Initially, duplicate dilutions were made from a series of samples in order to test the accuracy of dilution. Subsequently, only one dilution per fish was analyzed.

Sodium and potassium values were read on the same dilutions (1/100). Sodium was read against a 10 mgm.% sodium standard and no interference standard was necessary. Potassium values were read against a 0.5 mgm.% interference standard. Standard curves were obtained for sodium and potassium flame photometer readings and unknown samples were calculated from these standard curves. Precision for sodium was $\pm$ 2 meq./L and for potassium was $\pm$ .5 meq./L.

b. Treatment and Analysis of Muscle

Duplicate muscle samples (about 1 gram) were removed from the dorsal muscle mass on either side of the vertebral column just anterior to the dorsal fin. After the adhering skin and fat was removed, the sample was placed in a tared aluminum pan and weighed. Samples were dried to constant weight at 109° C., reweighed, and the per cent water content calculated.

Two methods of freeing the ions in the muscle were used. The first was an ashing technique. Weighed dry muscle samples (about 0.2 gram) were ashed in platinum crucibles at 1000° C. for six hours. The white crystalline material remaining was then dissolved in concentrated hydrochloric acid and diluted to 100 ml. in a volumetric flask.

The ashing technique is tedious and therefore the majority of the muscle samples were digested, using a modification of the technique described by Gordon (1959). Each muscle sample was placed in a test tube and to this was added 1.0-1.5 ml. 10N nitric acid, 0.2 ml. 30% H_2O_2, and a drop of caprylic alcohol. This was then heated for one hour at 60° C. The resulting solution was neutralized by the addition of 1 ml. 15N ammonia hydroxide, and made up to 100 ml. with distilled water.

An interference standard for potassium determination was found to be

unnecessary. The concentration of potassium was read against a 10 mgm.% standard curve and each value recorded was a mean of duplicate samples. Sodium values were read against a 0.768 mgm.% interference standard and similar duplicate samples were analysed. The accuracy of each mean value was calculated from the average deviation from the mean of twenty duplicate samples. The accuracy for the potassium was $\pm$ 2.5 meq./kg. wet weight, and for sodium $\pm$ 0.5 meq./kg. wet weight.

3. Fish Transferred to Salt Water

Experimental fish were transferred directly from fresh water to 60%[1] seawater (Na 287 meq./L, K 5.95 meq./L). The seawater tanks were aerated and the temperature was constant at $10 \pm 0.5^{\circ}$ C. Fish were removed for analysis at various intervals after to sea water during a ten day period.

4. Statistical Analysis

The statistical treatment of the data collected in this investigation follows the instructions given by Snedecor (1956) for t tests, regression analyses, and covariance analysis.

[1] Salinity was measured on an electrical conductivity bridge, using a salinity of 31.88 was considered 100% seawater.

RESULTS

1. Comparison of Muscle Techniques

It was necessary first of all to determine whether the sodium and potassium values obtained from the two different types of muscle analysis (ashing and digestion) were comparable, or whether the type of treatment introduced a systematic error which would render the two treatments not strictly comparable. Sodium and potassium values obtained in the two treatments were compared using a t test (Snedecor, 1956). In order to avoid differences due to the varying water content of the tissues, the ionic concentrations were expressed as meq./kg. dry weight. The results of this comparison are presented in Table 1.

Both the sodium and potassium values obtained by ashing were consistently higher than those obtained by digestion, although only the potassium values were significantly different ($p = <.01$). Both the ashing technique and the digestion technique have the same level of accuracy, as shown in Table 2.

All muscle cation concentrations reported in this investigation were obtained using the digestion technique.

2. Values of Sodium and Potassium in Fresh Water Fish

A series of fresh water steelhead were sampled to establish the normal concentrations of serum and muscle sodium and potassium in this hypotonic environment. The results were analyzed in order to estimate the influence of size and sex on the variables measured.

No significant correlation was demonstrated between body weight in the range of 60-200 grams and the variables measured. Nor could any significant differences in blood and muscle values be demonstrated between male (immature and mature) and female fish. However the serum sodium concentration of the female fish fell in the upper limits of the normal range and that of the males in the lower part (Table 1, Appendix).

Table 1. A Comparison of the Ashing Technique and the Acid Digestion Technique of Muscle Analysis.

Technique	Ionic Concentration in meq./kg. dry weight	
	Na	K
Ashing	43.5 ± 1.95 (N = 11)	526 ± 11.2 (N = 11)
Acid Digestion	39.8 ± 1.55 (N = 16)	486 ± 10.7 (N = 16)
	t = 1.18 P<.5, >.1	t = 2.60* P<.02,>.01

Table 2. A Comparison of the Precision of the Ashing Technique and the Digestion Technique of Muscle Analysis.

Technique	Average Deviation From the Mean			
	Wet Weight[1]		Dry Weight[2]	
	Na	K	Na	K
Ashing	0.57	2.62	1.63	5.70
Digestion	0.51	2.50	1.56	5.92

[1] Expressed in meq./kg. wet weight

[2] Expressed in meq./kg. dry weight

Table 3. Salmo gairdneri Fresh Water Values of Serum and Muscle Electrolytes
January, 1960

	Na			K			Na/K		
	Mean	± SE	N	Mean	± SE	N	Mean	± SE	N
Serum									
meq./L	150.0	± 146	(13)	3.24	± .14	(13)	47.8	± 3.4	(13)
Muscle									
meq./kg. wet muscle	8.88	± .32	(16)	109	2.30	(16)	12.5	± .5	(16)
meq./kg. dry muscle	39.8	± 1.5	(16)	486	± 10.7	(16)			
% water content	77.8	± .15	(24)						

Table 4. The Effect of Exercise and Temperature on the Ionic Concentrations* in the Serum and Muscle of Salmo gairdneri.

Treatment	Serum		Muscle		
	Na	K	% H_2O	Na	K
Exercise	151 ± 3 (3)	1.68 ± .3 (3)	77.7 ± 3.4 (3)	8.97 ± .32 (3)	106 ± 2 (3)
Controls	150 ± 1 (13)	3.24 ± .1 (13)	77.8 ± .2 (13)	8.88 ± .32 (13)	107 ± 2 (13)
P value	>.5	<.01	>.5	>.5	>.5
6 hours at 2° C	135 ± 6 (3)	2.7 ± .1 (3)			
Controls at 6° C	134 ± 2 (10)	3.46 ± .2 (9)			
P value	>.5	P<.08,>.02			

* Ionic concentrations expressed in meq./L serum, or meq./kg. wet weight

± One standard error of the mean, (S.E.). Sample size is indicated in parentheses.

It appears that for the purposes of these experiments neither sex nor body weight exerted a significant effect on serum and muscle electrolytes.

An attempt was made to evaluate the effects of two factors, exercise and temperature, which could possibly bias the experimental results. Fish exercised for 30 minutes showed a serum potassium concentration significantly lower than the unexercised controls, although none of the other variables measured was changed. A similar decrease in serum potassium was recorded for fish kept at 2° C. for six hours (when compared with controls kept at 6° C.), although again none of the other variables was changed (Table 3).

3. Transfer of Fish to Seawater

The response of Salmo gairdneri after transfer to 60% seawater is shown in Figures 1 and 2 (and in Tables 9, 10, and 11 of the Appendix). This response may be divided into three distinct phases: (1) a stage of adjustment; (2) a stage of active regulation; and (3) a stage of equilibrium.

The initial adjustment phase, which continued for the first 24 hours, was characterized by an increase in the total cation concentration of both blood and muscle[1] and by a dehydration of the tissues. During this period the serum and muscle sodium rose 24% and 125% respectively, while the tissue water and potassium both fell 4% below the control values. All these changes, with the exception of muscle potassium, were significantly different from fresh water values ($P < .01$).

This phase of adjustment was followed by a regulatory phase during which time (24–110 hours) the ionic balance of the fish was returned to equilibrium. This regulation first became apparent in the muscles after 24 hours in seawater. It

[1] Tissue concentrations referred to in the text will be expressed in terms of dry tissue weight, since values expressed in terms of wet weight would be influenced by the degree of hydration of the tissues.

Figure 1. Serial Changes in Sodium Content of Serum and Muscle After Transfer to 60% Seawater.

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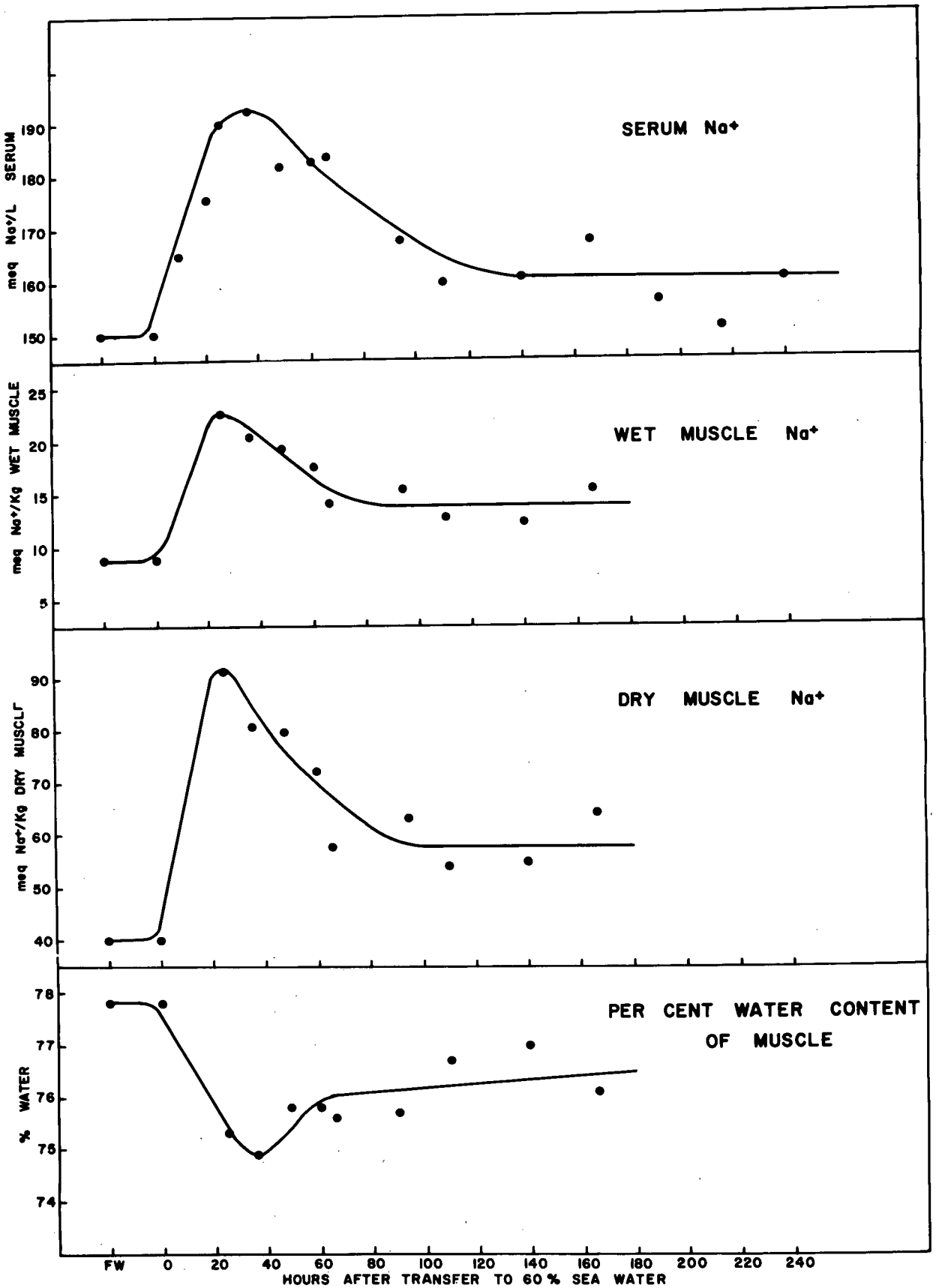
SERUM Na+
meq Na+/L SERUM
190
180
170
160
150
WET MUSCLE Na+
meq Na+/Kg WET MUSCLE
25
20
15
10
5
DRY MUSCLE Na+
meq Na+/Kg DRY MUSCLE
90
80
70
60
50
40
PER CENT WATER CONTENT OF MUSCLE
% WATER
78
77
76
75
74
FW
0
20
40
60
80
100
120
140
160
180
200
220
240
HOURS AFTER TRANSFER TO 60% SEA WATER

Figure 2. Serial Changes in Potassium Content of Serum and Muscle After Transfer to 60% Seawater.

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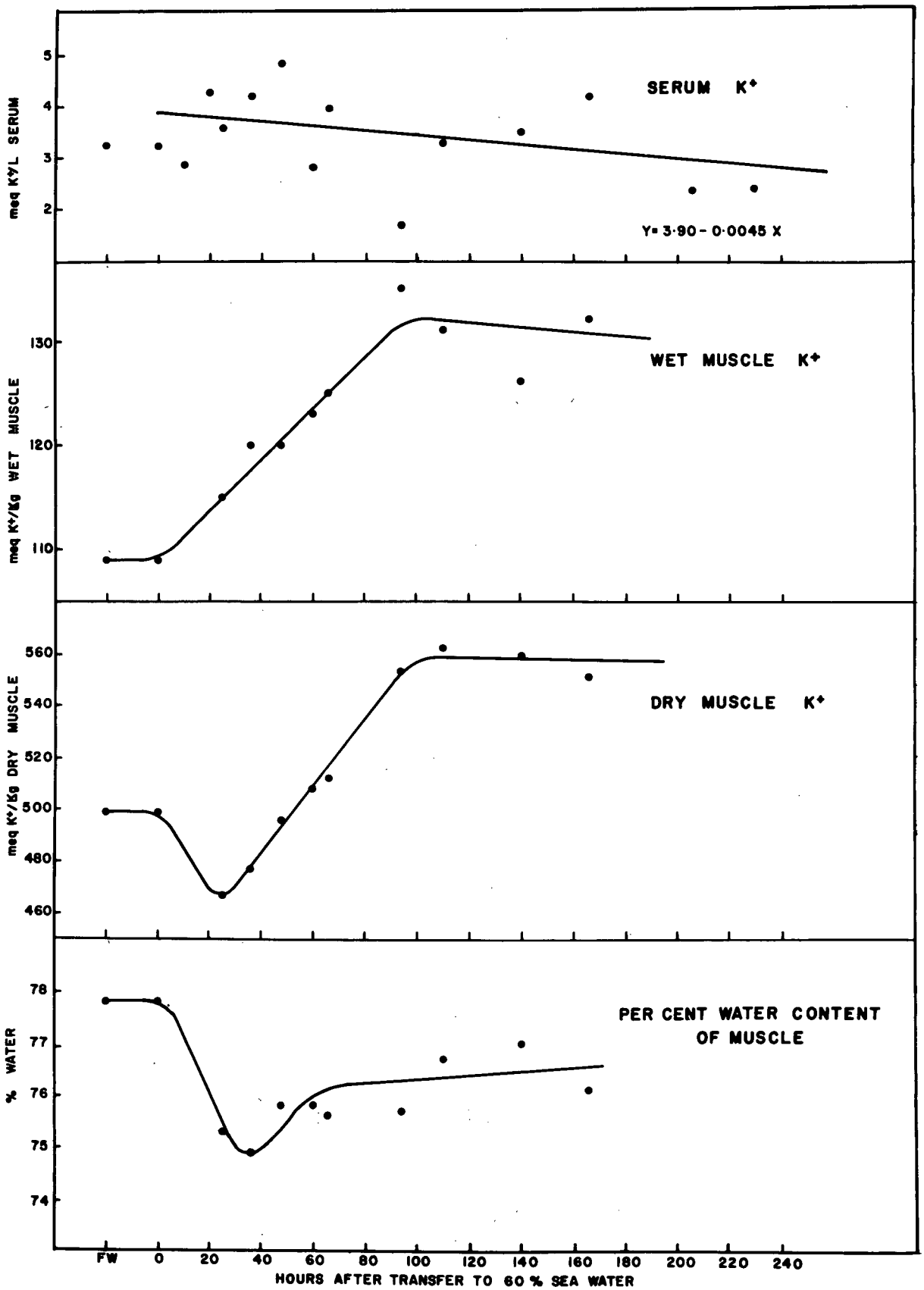
SERUM K+
Y= 3·90 - 0·0045 X
meq K+/L SERUM
5
4
3
2
WET MUSCLE K+
meq K+/kg WET MUSCLE
130
120
110
DRY MUSCLE K+
meq K+/kg DRY MUSCLE
560
540
520
500
480
460
PER CENT WATER CONTENT OF MUSCLE
% WATER
78
77
76
75
74
FW
0
20
40
60
80
100
120
140
160
180
200
220
240
HOURS AFTER TRANSFER TO 60 % SEA WATER

consisted of the active extrusion of sodium accompanied by an active accumulation of potassium in the muscles. After 36 hours in salt water the serum sodium value and the water content of the tissues started to return to normal, and appeared to reach an equilibrium after 110 hours in salt water. Presumably the fish had returned to a steady osmotic state and a new combination or balance of regulatory factors enabled them to maintain this constant milieu interieur in the hypertonic environment.

a. Changes in Serum Electrolytes

The initial increase in serum sodium represents the inward diffusion of ions with the osmotic gradient. The regulatory processes initiated after about 36 hours in salt water enabled the animal to excrete sodium at the rate of 0.41 meq./liter of serum/hour.

The changes observed in serum potassium were very difficult to interpret as only five values were significantly different from the fresh water controls. Since potassium occurs in such a low concentration in the serum when compared to sodium, any small errors in dilution could significantly affect the measurement of potassium but not influence the measurement of sodium. For this reason a linear regression of serum potassium against time in salt water was constructed. The formula so obtained ($Y = 3.90 - .0045X$) expresses the relationship between these two variables. The slope of -.0045 did not differ significantly from a slope of zero, indicating that there was probably no significant change in serum potassium during the experimental period.

The ratio of sodium to potassium was calculated for the serum to determine whether the cations varied independently during the treatment, or whether they remained proportional to each other throughout the experimental period. Serum sodium/potassium values showed no clear cut trend and hence are very difficult to interpret. One can say first that the value of 66, maintained after 216 hours in salt water is significantly higher than the fresh water sodium/potassium ratio ($P < .01$),

and second that although the proportion of sodium/potassium did vary considerably, it was maintained between the limits of 77 and 38.

b. Changes in Muscle Cations

During the period 24–66 hours after transfer to salt water, there was a linear decline in the dry muscle sodium concentration, according to the regression $Y = 108 - 0.69 X$. This rate of sodium loss of 0.69 meq./kg. dry weight/hour was highly significant with a correlation coefficient (r) of 0.92. Also during 24–96 hours after transfer to salt water there was a rise in muscle potassium according to the formula $Y = 434 + 1.24 X$. This rate of increase in muscle potassium of 1.2 meq./kg./hour was also highly significant ($r = 0.89$). Comparison of the rate of loss of sodium with the rate of gain of potassium showed that these rates are not significantly different (Figure 3).

Expression of the changes in muscle sodium and potassium in terms of fresh weight indicated trends similar to those described for the dry weight values although the changes were less dramatic.

The ratio of potassium to sodium in the muscle tissue shows a sigmoid relation when plotted against time in seawater (Figure 4). Upon transfer to seawater an increase in extracellular sodium caused the ratio to fall from a value of 12 to 5 in 24 hours, thereafter rising to an equilibrium level at 110 hours. The new equilibrium value of 10 is lower than the fresh water control although the difference is not significant, ($>.50$ $P<.10$).

c. Changes in Muscle Water Content

During the initial 36 hours in salt water the tissue water content fell from 77.8 % to 75.0 %. Thereafter the tissues slowly regained water until a new equilibrium of 76.5 % water content was established in the tissues after approximately 110 hours in seawater.

Figure 3. A Comparison of the Rates of Sodium Loss and Potassium Accumulation in the Muscle During Active Regulatory Phase.

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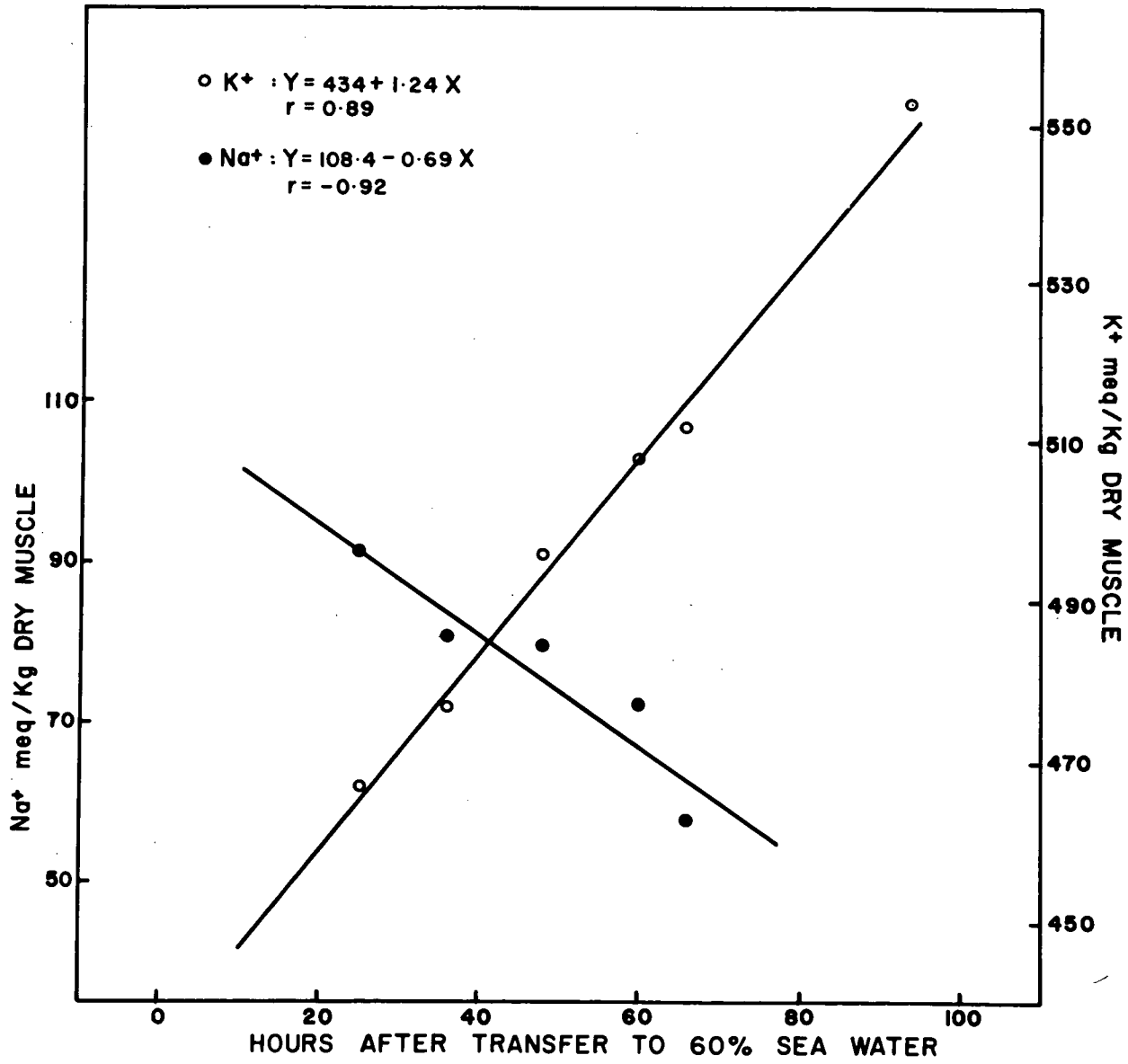
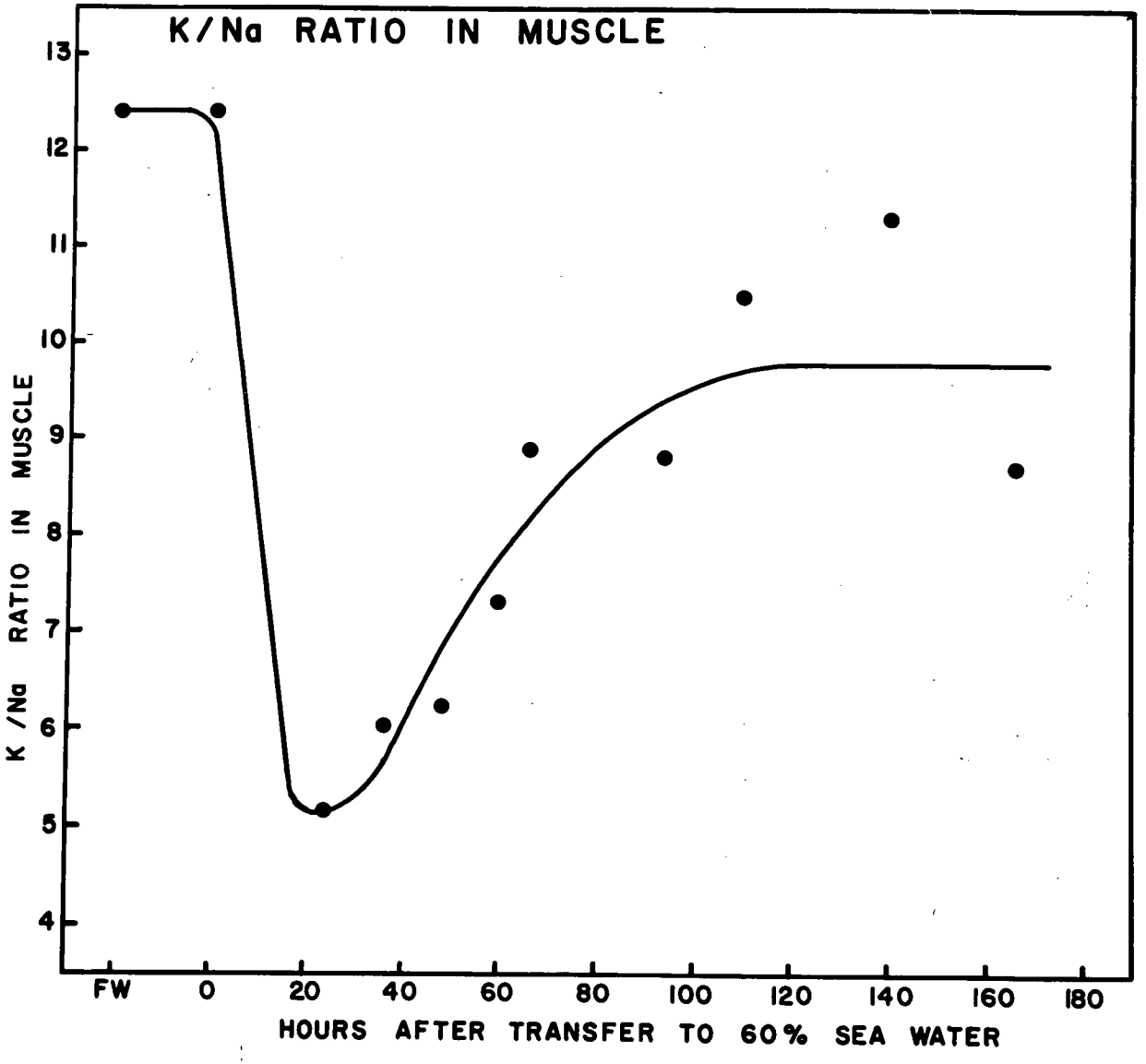

○ K+ : Y = 434 + 1·24 X
r = 0·89
● Na+ : Y = 108·4 − 0·69 X
r = −0·92
Na+ meq/Kg DRY MUSCLE
K+ meq/Kg DRY MUSCLE
110
90
70
50
550
530
510
490
470
450
0
20
40
60
80
100
HOURS AFTER TRANSFER TO 60% SEA WATER

Figure 4. K/Na Ratio in Muscle Tissue During Transfer to Seawater.

... to follow page 16


K/Na RATIO IN MUSCLE
K /Na RATIO IN MUSCLE
13
12
11
10
9
8
7
6
5
4
FW
0
20
40
60
80
100
120
140
160
180
HOURS AFTER TRANSFER TO 60% SEA WATER

d. Comparison of the Equilibrium Values With Fresh Water Fish

Comparison of these equilibrium values with those of the fresh water controls (Table 5) emphasizes the fact that a new equilibrium had indeed been established. This equilibrium was characterized by the following: (1) serum cation values only 6% higher than the fresh water controls; (2) a dry muscle cation concentration 15% higher than the controls; and (3) a lower water content of the tissues. It is interesting to note that the regulatory process(es) returned the blood ions to a level only slightly higher than the fresh water controls, while the cation content of the wet tissues was 21% higher than that in the controls.

Table 5. A Comparison of the Cation Levels of Serum and Muscle Between Fresh Water Controls and Fish Equilibrated to Salt Water.

Substance	Variable	Fresh Water	Salt Water Equilibrium	% Change	Probability
Blood	Na^+ meq./L	150	160.4	+ 6%	$<.02>.01^*$
	K^+ meq./L	3.24	3.29	- 1.5	$>.50$
	Total Cations	153	163	+ 6.53	
	Na/K	48	51	+ 7.3	$>.50$
Muscle	% H_2O	77.8	76.7	- 1.4	$<.01^{**}$
dry	Na^+ meq./kg.	40	54	+ 35	$<.01^{**}$
	K^+ meq./kg.	486	562	+ 16.0	$<.01^{**}$
	Total Cations	538	614	+ 14.1	
	Na/K	12.5	10.4	- 16	$<.10>.05$
wet	Na^+ meq./kg.	8.88	12.6	+ 42.0	$<.01^{**}$
	K^+ meq./kg.	108.6	131.0	+ 20.0	$<.01^{**}$
	Total Cations	117.5	143	+ 21.7	
Extracellular space	grams/kgm. wet tissue	61	93	+ 30	

DISCUSSION

1. Comparison With Values Reported in the Literature

The values obtained in the normal fresh water fish compare well with those described in the literature for Salmo gairdneri and other closely related salmonid fishes (Table 6). The observation that the serum sodium levels were higher in the female fish is in line with the well established fact that female sex hormones are more active than androgens in promoting sodium retention (e.g. Gennes and Bricaire, 1951). It is worthwhile stressing the fact that in a study of electrolyte metabolism, the age, sex and physiological condition of the animals must be taken into account, since the variability observed by different workers is no doubt related to one or more of these factors.

The general pattern of a rise in serum electrolytes during the first 36 hours after transfer to sea water and the gradual return to a new equilibrium which is reached after about 100 hours in this hypertonic medium, agrees with results of Koch (1959), Gordon (1959) and Houston (1960), as shown in Table 7.

The observation that the muscle potassium content of the fish equilibrated to seawater remains at a level 15% higher than the fresh water controls is at variance with the findings of Gordon (op. cit.) who reported that the muscle potassium after 240 hours in seawater had returned to the fresh water value.

2. Regulation of Serum Cations

The steelhead trout survives well when transferred to 60% seawater. If we examine its condition when a steady state has been reached, that is 110 hours after transfer, then we see that the fish has progressed in some degree towards the characteristics of a marine fish (Prosser, 1950). Thus, the concentration of sodium in the blood is somewhat elevated above that found in the fresh water fish, while the potassium concentration remains virtually unchanged. In the tissues, as represented by muscle, the water content has decreased in the seawater phase compared with that in the fresh water state. At the same time both the amount of sodium and potassium

Table 6. A Comparison of Sodium and Potassium Concentrations in Serum and Muscle of Salmonid Fish Sampled in Fresh Water.

Species	Date Sampled	Treatment	Serum meq./L Na	Serum meq./L K	$\%H_2O$	Muscle meq./kg. wet muscle Na	Muscle meq./kg. wet muscle K	Author
Salmo gairdneri	January	Fresh Water	150	3.24	77.8	8.8	109	This investigation
Salmo gairdneri	January		162.5	6.04				Houston (1959)
Salmo trutta	Summer	Fresh Water	155	5.0				Phillips and Brockway (1958)
Salmo trutta	Sept-April	Fresh Water 10°C	147	2.4		9.0 spring	138.2	Gordon (1959)
Salmo trutta	Sept-April Breeding Season	Fresh Water 20°C	148	4.2		14.4	143	Gordon (1959)
Salmo trutta			144	8.93		17.2	122	Spalding (1956)
Oncorhyncus	Juveniles catadromous migrants					10.3	123	McLeod (1958)

Table 7. A Comparison of Sodium and Potassium Concentrations in Serum and Muscle of Salmonid Fish Equilibrated to Sea Water.

Salmo gairdneri	January	110 hours in 60% Sea-water 10°C	160	2.8	76	14	125	This investigation
Salmo trutta	Sept-April	240 hours in 50% Sea-Water 20°C	162	3.8		9.7	141	Gordon (1959)
	Breeding Season	240 hours in 50% Sea-water 20°C				18		
Salmo salar	Downstream Migrants	110 hours in full Sea-Water 6.3°C	170					Koch and Evans (1959)

found in wet samples of muscle is greater when the trout is in a hypertonic medium.

We know that on transfer to seawater the fish takes seawater into its gut (Smith, 1930). The gut mucosa, although capable of some discrimination, nevertheless allows sodium chloride to pass into the body fluids in a concentration of 287 meq./L. This ingestion of seawater is responsible for the rise in the serum sodium during the first twenty four hours in seawater. Nevertheless, the initiation of additional active transport mechanisms enable the animal to rid itself of the excess ions, and eventually at equilibrium to maintain an internal concentration of 160 meq./L against an external medium containing some 287 meq./L. The work of Smith (1930), Keys (1932), Krogh (1939) and Holmes (1959), have demonstrated that the gills are the site of this active excretion of sodium.

In true marine fish, stenohaline to seawater, the potassium content of the blood does not differ significantly from that in fresh water stenohaline species (Prosser, 1950). So it is in the case of this euryhaline trout wherein the potassium concentration after 110 hours in seawater did not differ greatly from the fresh water control figures. Since Krogh (1939) demonstrated that the gill membranes of the goldfish were unable to transport potassium ion against a concentration gradient, while sodium and chloride ions were transported with ease, we are justified in assigning the regulation of potassium to the kidney. In mammalian physiology, the current concept of renal regulation of potassium involves three principles; filtration through the glomerulus, complete obligatory re-absorption in the proximal tubules and resecretion by distal tubules, in accordance with the needs of the animal. It is postulated that there exists in the distal tubule some exchange mechanism whereby a sodium ion is rotated into the tubule cell for every potassium ion secreted (Mudge, 1958). The hormonal control of renal function is by no means well understood, but it has been observed that mineralocorticoids increase urinary potassium, and at the same time decrease the concentrations of this cation in the body fluids and tissues.

It is postulated that these hormones facilitate the active secretion of potassium in the distal convoluted tubule.

This active excretion of potassium in the fish equilibrated to seawater is consistent with the enhanced oxygen consumption observed in the kidney of Salmo clarki clarki similarly adapted to 60% seawater, (Holmes and Stott, 1960). It appears then, that renal excretion of potassium ions accounts for the maintenance of a relatively constant potassium concentration in the blood, despite the varying ionic content of the serum.

3. Changes in Muscle Cations

In order to interpret the changes observed in the muscle tissue, one must first consider the constituent parts of this tissue. According to Manery (1954) any analysis of muscle electrolytes must take into account the two compartments into which all tissues are divided; viz the extracellular space and the intracellular space. It has long been established that Na^+ and Cl^- are predominantly extracellular in their distribution, and that K^+ and PO_4^{---} are found inside the cells. Studies with radioactive sodium have contributed to our understanding of this electrolyte inequality, for it has been demonstrated that radioactive Na^+ is permeable to the cell membrane and will replace unlabelled Na^+ present within the cell. The present concept of the cell is one of a dynamic equilibrium; Na which is present in a high concentration in the extracellular fluid diffuses into the cell, but is just as rapidly removed by an active process, with the net result that the cells contain virtually no sodium. K^+, on the other hand, tends to diffuse out of the cell with the concentration gradient but is pumped back into the cellular compartment at a rate which maintains a high intracellular K concentration. The actual mechanism of transport across the cell membrane is obscure, but the fact that it is inhibited by certain metabolic poisons and the recent isolation of ATPase from

the cell membrane shows that it is indeed dependent on metabolic processes.

Keeping in mind that muscle tissue is composed of 1) an intracellular space wherein 99% of the K^+ is located; and 2) the extracellular space which is essentially a plasma ultrafiltrate containing 99% of the total muscle Na^+, we can consider the movements of ions in the muscle after transfer to seawater.

The initial rise in serum sodium resulted in a more concentrated extracellular fluid (ECF) and possibly the diffusion of some sodium into the cells. This increase in osmotic pressure of the ECF tended to dehydrate the cells and some K^+ appeared to leave the cells, presumably with the osmotic water. Active regulation of the tissue electrolytes commenced twenty-five hours after transfer, the sodium being removed at a rate of 0.69 meq./Kg. dry muscle/hour and the K being deposited in the muscle at a rate of 1.24 meq/kg./hour, (Figure 3). The removal of Na may be interpreted as a combination of the initiation of the Na^+ excretory mechanisms in the gills or the kidney, thus decreasing the Na concentration of the ECF; and possibly of the action of a pump which removed any excess sodium from the muscle cells. The accumulation of K can only be explained by the presence of an active transport mechanism which carries the K^+ into the cell. Whether this pump is also responsible for the removal of Na from the cell is questionable for we have no conclusive proof that Na^+ actually accumulates in the cells or whether the initial increase in muscle sodium merely represents a more concentrated extracellular fluid.

It is impossible to describe accurately the mode of action of the K pump, or to determine whether it is linked with the transport of Na out of the cell, without a knowledge of the distribution of the two cations between the intracellular and extracellular compartments. Let us consider first of all the distribution of K in the muscle. Since the serum K, and therefore the interstitial fluid, did not change appreciably during transfer to seawater, we may be confident that any increase in the total muscle K represented an increase in the intracellular concentration of this cation.

The distribution of sodium on the other hand cannot be definitely determined from the data available. Considering the observation that yeast and frog muscle cells have been shown to accumulate Na when placed in media having a high Na concentration (Steinbach, 1954), it is reasonable to assume that a 30% increase in the extracellular Na concentration would result in a net increase in the intracellular Na concentration during the initial adjustive phase. Secondly a consideration of the muscle cation concentrations further clarifies the situation. At equilibrium the muscle tissue contains 23 and 76% more K and Na respectively than the fresh water animals. We would assume "a priori" that such a large increase in intracellular K must be accompanied by a proportionate increase in cellular sodium in order to maintain the electrolyte balance basic to all biological processes. That the intracellular sodium concentration is higher in the seawater animal is further supported by a consideration of the extracellular volume in the two media.

The simple formula:

$$ECS_{Na} = \frac{Na_T}{Na_s} \times \frac{H_2O}{r\,Na}\,s \qquad \text{(Manery, 1954)}$$

where ECS_{na} = the volume of the extracellular space, based on the assumption that all the muscle sodium is located extracellularly

Na_s = serum sodium concentration in meq./L

Na_t = total sodium concentration in muscle in meq./kg. wet tissue

rNa = .95 (Donnan factor)

H_2O_s = gms. H_2O/kg. serum 100 (assuming a specific gravity of 1) gives an approximate estimation of the extracellular volume

Considering the fish which have been in sea water for 110 hours as representative of an animal which is in equilibrium with its environment and substituting the values for serum and muscle sodium obtained at this time in the above formula, then

$$ECS = \frac{14}{160} \times \frac{1000}{.95} = 92 \text{ g/kg. wet muscle}$$

Similarly for the fresh water fish

$$ECS = \frac{8.8}{150} \times \frac{1000}{.95} = 62 \text{ g/kg. wet muscle}$$

Therefore, it would appear that the extracellular space of the equilibrated seawater fish is approximately 30% greater than the fresh water value. If, however, the assumption that all the Na^+ is distributed in the extracellular compartment is untrue, then a real increase in the intracellular sodium must in part account for the increase in the total muscle sodium of the equilibrated fish.

In view of this evidence it seems most likely that Na diffuses into the intracellular compartment during the first twenty-five hours in seawater. The onset of regulation is marked by the ability of the cells to pump out some of the excess sodium, and transport K^+ into the cells. Covariance analysis showed that the rates at which these two ions moved in and out of the muscle do not differ significantly from one another ($P < .25, > .10$), and we may therefore postulate that the movement of these ions in opposite directions is controlled by the same transport mechanism.

4. Possible Regulatory Mechanisms

Since twenty-four hours elapsed between the transfer of Steelhead trout to seawater and the initiation of regulatory processes, we may infer that this slow return to osmotic equilibrium was possibly under hormonal control. We are then justified in considering certain endocrine organs, albeit of uncertain osmoregulatory importance, and their possible participation in the adaptation of this euryhaline teleost to salt water. The established action of the hormones produced by the interrenal tissue, the thyroid, and the neurohypophysis will be discussed in light of the data obtained in this investigation.

It is difficult to determine whether the hormones of the interrenal are active in the adaptation of this animal to sea water. The decrease in sodium content of the serum and muscles and increase in the muscle potassium concentration recorded during the regulatory phase, resemble the changes observed after adrenalectomy or during adrenal insufficiency in higher vertebrates (Chester Jones, 1957). However, if we characterize the regulatory mechanisms as initiating the excretion of sodium from the muscle cells and also from the gill epithelium, we must include the adreno-corticoids as possible regulators, for in vivo treatment with DOC promotes extrarenal excretion of sodium in the salt loaded Salmo gairdneri (Holmes, 1959). On the other hand, injections of the same hormone (DOC) caused an increase in the sodium and decrease in the potassium content of the muscles of Salmo trutta (Spalding, 1956), changes which are opposite to those described in this investigation. Although smolting fish which have an active interrenal gland, are more able to withstand transfer to seawater than non-smolting fish, (Koch, 1959; Houston, 1959; Olivereau, 1960), it has yet to be demonstrated that adreno-corticoids increase the salinity tolerance of any euryhaline fish.

The thyroid appears to play an important part in enabling the survival of euryhaline fish in salt water. Hickman (1959) has correlated the higher metabolic rate of the euryhaline starry flounder (Platyichthyes stellatus) with an increased thyroid activity in salt water. He suggests that it requires more energy to pump out excess ions, as is necessary in seawater, than to produce a hypotonic urine, as occurs in fresh water teleosts. The results of this investigation seem to corroborate his findings because the active transport mechanisms initiated during the regulatory phase would certainly require additional metabolic energy.

In mammals the posterior pituitary hormone vasopressin influences the water-electrolyte metabolism in two ways. First, it has an anti-diuretic action, promoting reabsorption of water from the kidney tubule, and second, it promotes the

renal excretion of the sodium ion. Although all available evidence indicates that the hormones of the neurohypophysis are important in the adaptation of euryhaline teleosts to salt water (Arvy and Gabe, 1954; Fridberg, 1959; Weisel, 1958), the results of Fontaine and Raffy (1950) indicate that this action is certainly not one of water retention. The results obtained in this investigation indicate that water retention is secondary to the removal of excess sodium from the body tissues and fluids, as the tissue water content started to rise only after the active excretion of sodium was initiated. The absence of an anti-diuretic action in aquatic vertebrates is in line with the suggestion of Heller (1956) and Sawyer (1956) that the development of water retaining mechanisms, as found in amphibians and higher vertebrates, is associated with the adaptation of animals to a terrestrial life. It is possible in primitive vertebrates, such as the teleost fish, that the neurohypophyseal hormones exert their effect only on electrolyte balance. Maetz (1959) has isolated a fraction, natriferin, from teleost neurohypophysis which promotes the passage of sodium through isolated frog skin. It would be interesting to investigate the effect of natriferin and oxytocin on the extrarenal excretion of sodium.

It thus appears that the osmoregulatory mechanisms which facilitate survival of euryhaline fish in salt water are hormonal in nature and enable the animal to pump out excess sodium which would otherwise accumulate in the tissues and body fluids. The evidence indicates that the posterior pituitary hormones accompanied by an increase in thyroid activity are responsible for this adaptation. The role of interrenal hormones has yet to be demonstrated.

SUMMARY

1. Cation concentrations of Steelhead trout in fresh water were found to be:

for serum: 150 meq. Na^+/litre

3.24 meq. K^+/litre

for muscle: 8.8 meq. Na^+/kg. wet tissue

109 meq. K^+/kg. wet tissue

and a tissue water content of 77.8%.

2. Upon transfer to 60% seawater, the increase in the sodium content of the serum and muscle tissue, was accompanied by a dehydration of the tissues during the first twenty-four hours after transfer.

3. The regulatory processes, initiated after twenty-four hours in seawater, enabled the animal to excrete excess sodium ions from the blood and from the intracellular compartment. It is suggested that active transport mechanisms mobilized in the gills and in the muscle cells enabled the removal of the excess cations.

4. Since the excretion of sodium from the muscle cells was accompanied by an active deposition of potassium in the intracellular compartment, it is proposed that an active cation exchange mechanism was acting at the membranes of these cells.

5. After 110 hours in seawater, the fish were returned to a new equilibrium which was characterized by: 1) a serum cation concentration only 6% higher than the fresh water fish, 2) a muscle cation concentration 15% higher than the fresh water fish, and 3) a tissue water content lower than that of fresh water fish.

Although the serum concentrations differed only slightly from the fresh water controls, the increased electrolyte concentration in the tissues decreased the concentration gradient between the external environment and the cellular compartment, thus decreasing the osmotic influx of ions.

6. The serum potassium values did not vary significantly during the treatment, presumably due to the efficient renal excretion of this ion.

7. The possible role of the hormones of the thyroid gland, the neurohypophysis and the interrenal tissue in the initiation and continuance of the regulatory mechanisms enabling the survival of this euryhaline fish in seawater are considered.

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APPENDIX

Table 8. Analysed as to Sex Differences.
(Cation Values)

Sex	Serum meq./L serum						Muscles ion meq./kg. wet muscle							
	Na		K		Na/K		% H_2O		Na		K		K/Na	
	M	SE	M	SE			M	SE	M	SE	M	SE	M	SE
Total	150.0 (13)	1.46	3.24 (13)	.139	478 (13)	3.43	77.8 (24)	.15	8.88 (16)	.322	108.6 (16)	2.31	12.47 (16)	.512
Male fish mature	147.8 (5)	2.30 .85	3.35 (5)	.246 .968	44.9 (5)	3.55 .203	78.0 (8)	.559 15.3	8.84 (5)	1.14	101.7 (5)	5.75	12.14 (5)	1.21
Male fish immature	148.5 (5)		3.33 (2)		44.8 (2)		77.5 (5)	0.27	8.97 (5)	0.42	113.9 (5)	3.6	12.80 (5)	.79
Female fish	152.5 (6)	2.33	3.13 (6)	.255	50.3	4.61	77.8 (8)	.34	8.83 (5)	.44	109.9 (5)	2.82	12.57 (5)	.738

Table 9. Transfer to 60 % Seawater. 1. Serum Sodium and Potassium meq./L. 2. Serum Na/K Ratio.

Treatment Hours in 60 % Seawater	Sample Size N	Na Mean	± SE	K Mean	± SE	Na/K Mean	± SE
10	9	165.1	4.9	2.85	3.80		
20	3	176	7.0	4.28	1.25	47	
25	4	190	3.4	3.59	.55	55.3	7.42
36	5	193	4.9	4.21	1.08	52.1	7.65
48	3	182	19	4.84	.37	38.1	2.8
60	4	183	9.5	2.81	.37	66.8	6.3
66	4	182	4.8	3.95	.23	46.3	1.9
94	4	168	5.3	2.18	.15	77.3	8.7
110	4	160	5.6	3.29	.59	51.3	6.7
140	8	161	2.6	3.57	.30	46.8	3.1
166	8	168	6.2	4.30			
192	4	156	8.2				
216	4	151	3.8	2.38	.32	67.3	12.7
240	10	160	2.5	2.42	.20	69.7	5.6

Table 10. Transfer to 60 % Seawater. 3. Muscle Sodium and Potassium/Kg. Wet Muscle. 4. Potassium : Sodium Ratios.

Treatment Hours in Seawater	Sample Size N	Na Mean	± SE	K Mean	± SE	K/Na Mean	± SE
25	4	22.6	2.0	115	3.0	5.18	0.52
36	5	20.3	1.7	120	2.5	6.03	0.48
48	4	19.3	0.6	120	1.9	6.26	0.21
60	4	17.5	2.4	123	3.1	7.32	0.98
66	4	14.1	0.4	125	2.7	8.90	0.34
94	4	15.3	0.8	135	6.7	8.82	0.57
110	4	12.6	0.7	131	4.2	10.5	0.34
140	8	12.3	0.4	125	2.5	10.3	0.43
166	4	15.3	1.2	132	0.5	8.7	0.53

Table 11. Transfer to 60 % Seawater. 5. % Tissue Water. 6. Sodium and Potassium meq./Kg Dry Weight.

Treatment	Sample Size	% Water		Na meq./kg. Dry Muscle		K meq./kg. Dry Muscle	
Hours in Seawater	N	Mean	± SE	Mean	± SE	Mean	± SE
25	4	75.3	0.31	90.2	6.6	466	10.7
36	5	74.9	0.31	80.8	6.1	478	9.5
48	4	75.8	0.75	80.5	5.2	500	16.9
60	4	75.8	0.68	72.1	7.7	507	24.5
66	4	75.6	0.71	57.8	2.6	513	7.9
94	4	75.7	1.44	63.1	4.3	554	10.8
110	4	76.7	0.55	53.9	1.4	561	17.0
140	8	77.0	0.40	53.6	1.5	547	11.4
166	4	76.1	0.95	63.8	3.8	552	9.5