WATER AND ION BALANCE IN THE PROSOBRANCH LIMPET

ACMAEA SCUTUM

by

HERBERT HENRY WEBBER

B.Sc., University of British Columbia, 1963

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FACULTY OF GRADUATE STUDIES

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HERBERT HENRY WEBBER

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WATER AND ION BALANCE

IN THE PROSOBRANCH LIMPET ACMAEA SCUTUM

ABSTRACT

The major aspect of this study was to evaluate the effect of changes in external salinity on the concentrations of Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++} in the blood, urine, and foot muscle cells of Acmaea scutum. To estimate intracellular ion values, measurements of muscle tissue ion values and the extracellular volume (as represented by the inulin space) were made.

As well, aspects of the water balance of A. scutum have been studied; the effect of changes in external salinity on water content of whole animal, and muscle cells has been documented; and the water content of animals maintained at a constant salinity has been studied.

The results showed that ion values of the blood, except for K^+, were the same as respective ion values of external salinities. At all salinities the concentration of K^+ in the blood was greater than sea water K^+ values. Dialysis experiments showed the K^+ gradient was not due to a Donnan equilibrium.

All urine ion values were the same as ion values of external salinities. The K^+ gradient observed between blood and sea water did not exist between urine and sea water.

Extracellular volume of foot muscle changed linearly with change in external salinity (16.7% at 50% sea water and 31.0% at 125% sea water). This change in extracellular space indicated that cellular volume changes with changes in external salinity.

Intracellular ion values were different than blood ion values. Intracellular estimates of Na^+ and Cl^- were, at all salinities tested, close to or not significantly different from zero. Intracellular K^+ estimates were, at all salinities, much higher than blood K^+ values. Intracellular K^+ values also changed significantly with changes in external salinity. Over a range of salinities from 50 to 125% sea water intracellular K^+ values appeared to

increase linearly. Intracellular values of Ca^{++} and Mg^{++} were lower than corresponding values of experimental salinities. At a given salinity the sum of intracellular ion values was much lower than the sum of blood ion values.

Results on seasonal distribution of ions and water of foot muscle for animals from a constant salinity showed that, over an 18 month period, muscle ion and water values varied significantly. As well, water content values of whole animal for the same field samples varied significantly.

Water content of whole animal also changed significantly with changes in external salinity. For A. scutum from a marine environment changes in water content caused by changes in experimental salinities were maintained for up to one week immersion. Whole animal water content for A. scutum from an estuarine environment however, returned to starting water values after 48 hr. immersion in experimental salinities. Whole animal water content data for field samples from an estuarine environment indicated little volume regulation. Over a range of environmental salinities from 18 to 82% sea water, water content ranged from 89.0 to 77.0%.

As well as showing changes in total body water with changes in external salinity, A. scutum demonstrated large changes in water content at a given constant salinity. Changes in water content at a constant salinity resulted from sea water entering the blood space from the external environment. When the molecules inulin and amaranth were dissolved in experimental salinities and the water uptake response tested, these molecules also entered the blood space from the external salinities.

The ion values of blood, urine, and muscle cells were similar to values of these parameters recorded for other gastropod molluscs. Water content values from experiments on the effect of change in external salinity also agreed with data from studies on other molluscs. The water uptake response of A. scutum, however, was not in accordance with measurements on change in blood volume at constant salinities for other gastropod molluscs. A definite example of a gastropod that can take sea water from the external environment into the blood space must now be added to the literature

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INTRODUCTION

Prosobranch limpets of the genus Acmaea are common mem-
bers of the intertidal fauna of the Pacific coast of North
America. Acmaea scutum, which ranges from Alaska to San Pedro,
California (Test, 1945), is, on the southern intertidal coast
of British Columbia, the most common and widely distributed
of all species of Acmaea.

During tidal exposure, Acmaea encounters air desiccation,
and increased and decreased salinities due to evaporation and
rain fall. As well, in the area of Vancouver, British Columbia,
a number of species of Acmaea, including A. scutum are found
in the continuously lowered salinities of an estuarine environ-
ment.

An aquatic animal has three primary fluid spaces: the
blood volume, the intracellular fluid, and the urine volume.
The important considerations given to these fluid spaces in
adaptations to changes in external salinity are the total
osmotic pressure, ionic values, and water content.

In the Prosobranch Gastropods, data on the total osmotic
pressure, ion values, and water content for these three fluid
spaces are incomplete, and generalizations are based on rel-
atively few species.

The data on total osmotic pressure of blood for Proso-

branchs indicate that, with one exception, these Gastropods are unable to regulate the osmotic pressure of the blood. That is, the osmotic pressure of the blood is the same as that of the environmental salinity. The one exception is found in the genus _Littorina_. Todd (1964) found that three species of this genus were able to regulate the osmotic pressure of the blood in salinities lower than 50% sea water. In the genus _Acmaea_, Segal and Dehnel (1962) found that _Acmaea limatula_, an ecological equivalent of _A. scutum_ (Test, 1945), was not able to regulate the total osmotic pressure of the blood over a range of salinities from 25 to 125% sea water.

Data on the osmotic pressure of urine for marine Proso-branchs is available only for _Littorina_. Todd (1964) found that the osmotic pressure of urine in three species of this genus was the same as the osmotic pressure of the blood.

No values are available for the osmotic pressure of the intracellular fluid of Prosobranchs and, in fact, this measure-ment has been made on very few invertebrate tissues. Within the molluscs, Potts (1952) found the osmotic pressure of single muscle fibers of _Mytilus edulis_ to be within 1.5% of that of the blood.

Data on ion distributions in the blood volume, intracellular space, and urine volume for Prosobranchs are more incomplete than values for total osmotic pressure. Potts and Parry (1964)

and Robertson (1964) have summarized the information on blood

ion values of Prosobranchs. Basically, the blood values of K^+

and Ca^{++} were higher, and values of $SO_4^=$ lower, than the con-

centration of these ions in the external sea water; blood and

sea water concentrations of Na^+ and Cl^- were virtually the

same. No data are available for ion values of urine or for

the intracellular space of marine Prosobranchs.

In this study, the Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++} values of

the blood, urine, and cellular component of the foot muscle

have been determined for Acmaea scutum over a range of experi-

mental salinities from 50 to 125% sea water.

Changes in tissue ionic concentrations are accompanied

by changes in tissue water content. Historically, it was

thought that all membranes were semipermeable and acted as

osmometers. Now it is realized that all membranes are permeable

to salt ions as well as water. Although showing permeability

to ions and water, molluscs such as the marine pulmonate,

Onchidium (Dakin and Edmonds, 1931); the mussel, Mytilus

(Maloef, 1937); and the Opistobranch, Aplysia (van Weel, 1957)

showed very little volume regulation when exposed to changes

in external salinity. In this study, the water content of

the soft body parts and the cells of the foot muscle has been

determined for A. scutum which were exposed to a range of

salinities from 50 to 125% sea water. As well, the total water

content has been compared for limpets from a marine and estuarine environment.

During the course of this study it became evident that the water content of A. scutum showed great variation at a constant salinity. This aspect of water balance of molluscs, especially Gastropods and Eulamellibranchs, has been the subject of much interest. The functioning of the hydrostatic skeleton in expanding the siphon and foot of Eulamellibranchs and the foot of Gastropods is dependent upon the amount of fluid in body spaces (Chapman and Newell, 1947; Chapman, 1958). At different times it was believed that these animals could incorporate sea water from the external environment in the functioning of the hydrostatic skeleton. However, the present day view has been that, with the exception of one family of Gastropods (Naticidae), a constant blood volume in the circulatory system was sufficient to explain the large expansions of the siphon and foot musculature (Morris, 1950; Chapman, 1958; and Brown and Turner, 1962). For the exception, Morris (1950) described in Uber (Polinices) strangei, a system of acquiferous ducts that were separate from the blood system and apparently took up water during the expansion of the foot. Although the hydrostatic skeleton of Acmaea scutum does not function in expanding the foot in the same manner as Pectinibranch Gastropods, data are presented showing that this limpet is capable of taking

up large amounts of sea water from the external environment

into the blood system.

MATERIAL AND METHODS

Collecting Areas

Acmaea scutum used in this study were collected from two

areas. The first, Jordan River on the south west coast of

Vancouver Island, British Columbia, had a constant salinity

of 31.8‰ and a temperature range of 8°C in winter months to

13°C in summer months. The second collecting area, Whytecliff

Park, was some 20 miles north west of Vancouver, British Colum-

bia; this area was an estuarine habitat with salinity fluctu-

ation between 6.4‰ and 25.5‰ according to the stage of the

tidal cycle and the amount of the fresh water discharge from

the Fraser River. Temperatures at Whytecliff Park ranged from

5°C in winter months to 20°C in summer months.

No effort was made to collect animals from a standardized

intertidal zone. It was found, however, that A. scutum of

the size range used for experiments (5-10g) were most often

found in zone three as described by Ricketts and Calvin (1962).

Animals were maintained in the laboratory at 10°C without

food and with no regular photoperiod regime. Approximately

25 animals were kept in plastic trays of 5 liter capacity.

A. scutum from Jordan River were held at 100% sea water,

while animals from Whytecliff Park were held in 75-80% sea

water. A. scutum used in experiments were maintained in hold-

ing salinities for no longer than five weeks.

Collection and Measurement of Samples

Blood Samples: Blood samples were taken from the ventral sinus through an incision in the foot muscle. For cation analysis, aliquots of 50 or 100 microliters of unaltered blood were diluted with glass distilled water to a volume of 25ml. For Cl^- samples, aliquots of 50 or 100 microliters were placed in 4 ml of 0.1N HNO_3 and 10% acetic acid. Na^+, K^+, and Ca^{++} were measured on a Zeiss PF-5 flame photometer. K^+, and Ca^{++} standards had Na^+ added in the same concentration as unknowns to adjust for Na^+ interference. Ca^{++} and Mg^{++} were determined simultaneously by titration with E.D.T.A. using eriochrome black T as an indicator. Mg^{++} concentration was obtained by subtracting the Ca^{++} value from the flame photometer from the combined $Ca^{++}-Mg^{++}$ value obtained by titration. Blood Cl^- values were determined on the high rate of a Buchler-Cotlove chloridometer.

Urine samples: Urine samples were collected in the following manner. Animals were removed from the experimental salinity, the mantle caused to retract by tactile stimulation, and water in the nuchal cavity and space between the shell and foot removed by aspiration. The head was then reflected with a spatula to make visible the urinary pores. Gentle pressure was applied to the foot to cause fluid to be extruded

through the right urinary pore. This fluid was collected on a clean plastic film. Only fluid that was observed to come from the right urinary pore was used for analysis. Generally between 0.5 and 1.0 ml of urine was collected. Because of this relatively large collected volume of urine, contamination by sea water adhering to the surface of the nuchal cavity and mantle was believed to be negligible. Aliquot and dilutions for urine analysis were the same as for blood samples. Methods of measurement of urine cations and Cl^- were the same as for blood ions. Urine samples taken for inulin analysis were 50 microliters and were analysed as described for blood samples below in the section on estimation of extracellular space.

Dialysis samples: Samples for dialysis experiments on blood and urine were taken as described above. The method for dialysis experiments was adapted from Robertson (1949). Approximately 0.2 ml of blood or urine were dialysed for 24 hr. against the experimental sea water from which the animals were taken. Sampling of dialysates over a time series indicated dialysis was complete by 24 hr. Aliquots and dilutions for dialysates were the same as described for blood samples. Methods of measurement of dialysate cations and Cl^- concentrations were the same as for blood ions.

Muscle ions: The method of extracting ions from muscle tissue was an adaptation of a technique described by Cotlove

(1963). Sections of foot muscle approximately 0.5 mm thick were taken from live animals. Between 95 and 100 mg of this muscle tissue were placed in 10 ml of glass distilled water, and the ions extracted for 24 hr. Duplicate 2 ml aliquots were used for Cl^- determination. 0.5 ml of 1.5N HNO_3 + 50% acetic acid were added to each aliquot and muscle Cl^- determined on the low rate of a Buchler-Cotlove Chloridometer. Four milliliter aliquots were made up to 25 ml with glass distilled water for cation determinations. Muscle cations were determined by the same method as described for blood cations.

The method of distilled water extraction for muscle Cl^- was compared with the method of Cotlove (1963) for 5-15 μeq of Cl^- using NaOH and 4% $ZnSO_4$ for digestion. The muscle Cl^- of 17 A. scutum from 100% sea water was extracted using the NaOH-$ZnSO_4$ technique for 5-15 μeq of Cl^- as given in Cotlove (1963). Likewise, the muscle Cl^- of 20 A. scutum from 100% sea water was extracted using distilled water. The Cl^- content of muscle by NaOH-$ZnSO_4$ extraction and by distilled water extraction were not significantly different when compared by t-test ($p > .05$). Distilled water extraction was used for determination of muscle cations and Cl^- in this study because the NaOH-$ZnSO_4$ technique did not provide an opportunity to determine cation concentrations.

Whole animal water content: For measurement of the total

body water of <u>A</u>. <u>scutum</u> the soft body parts were removed from the shell and blotted with absorbent tissue to remove excess water. The weight of the soft body parts was recorded within approximately 30 sec of the removal from the shell. The soft body parts were dried for 24 hr at $100°C$. Water content was taken to be the difference between wet and dry weight and was expressed as a percentage. All weights were determined on a Mettler balance to $±0.0005$ g.

<u>Muscle</u> <u>water</u> <u>content</u>: Samples for determination of muscle water were taken as described above for muscle ion samples. The weight range of tissue for muscle water determinations, however, ranged from 50 to 150 mg. The water content of muscle tissue was expressed as a percentage.

<u>Extracellular</u> <u>space</u>: The blood space of <u>A</u>. <u>scutum</u> was equilibrated with inulin in two ways. First, 0.1 ml of 5% inulin was injected into the visceral sinus and the animals allowed to equilibrate for 6 hr. Secondly, animals were held in experimental salinities for 24 hr; then the animals were squeezed gently to expell water and returned for a further 24 hr in the same salinity containing 4 g/l of inulin. The increase in volume shown by the animal over 24 hr in salinities containing inulin resulted in the limpets having an equilibrated blood inulin concentration of around 2 g/l. For each animal, a 50 microliter aliquot of blood and between 95 and

100 mg of muscle tissue were taken as described above. Blood
samples were assayed for inulin directly. Muscle samples were
placed in 10 ml of glass distilled water for 24 hr for extrac-
tion of inulin. Extraction for time periods greater than 24
hr did not increase the yield of inulin. Although the effi-
ciency of distilled water extraction of inulin from the muscle
tissue of A. scutum is not known, Schultz et al. (1966) found
that extraction of C^{14} inulin from rabbit ileum in 8 mM $LiSO_4$
gave at least a 95% recovery. Estimation of inulin concentra-
tion was by the anthrone method of Young and Raisz (1952).
Duplicate 2 ml aliquots were used for inulin analyses of
muscle samples. Samples were read on a Beckman DU spectro-
photometer at 625 mμ using anthrone reagent as a blank.

Experimental Salinities

One hundred per cent sea water was arbitrarily defined as
sea water of 31.8‰ salinity and ion concentration of:

	Na^+	Cl^-	$SO_4^=$	K^+	Ca^{++}	Mg^{++}
mEq/l	433.0	497.2	68.6	10.1	25.6	97.9.

The sea water of the Jordan River collecting area, al-
though 100% salinity, had a lower Ca^{++} concentration (19.6
compared to 25.6 mEq/l). To prepare 100% sea water NaCl,
Na_2SO_4, KCl, $CaCl_2$, and $MgCl_2$ were added to a more dilute
sea water available in a closed circulating sea water system.
To prepare 125% sea water, 200% sea water prepared in the same

manner as 100% sea water was diluted with glass distilled
water. Fifty and 75% sea water were prepared by adding glass
distilled water to either 100 or 200% sea water.

<u>Methods for Water Uptake Experiments</u>

<u>Measurements of volume change</u>: Animals were maintained
in the experimental salinity for 24 hr before use. The method
of volume measurement was as follows. The animal was taken
from the experimental salinity and the foot was compressed
gently with absorbent tissue to expell water. Most of the
water expelled on gentle pressure came through the right
urinary pore. The volume of the animal was determined by
displacement of water. The displaced water was weighed. The
method was accurate to ±0.05 ml. The animal was then placed
back into the experimental salinity with dorsal surface of
the shell against the substrate, and was maintained in this
position for the desired time period. The volume of the
animal was again determined after gently shaking to remove
water from the nuchal cavity and space between the foot and
shell. The increase in volume was determined by subtracting
the starting volume from the final volume. The volume of the
soft body parts alone was determined by estimating the volume
of the shell separately and subtracting this value from the
volume of the soft body parts plus shell.

<u>Blood amaranth samples</u>: Samples for the determination of

blood amaranth concentrations were taken from the visceral

sinus through an incision in the foot muscle. Approximately

100 μliters were taken up in a capillary tube and transferred

to a Beckman spectrophotometer microcell with 50 μliter cap-

acity and 10 mm path length. The absorbency of unaltered

blood samples was determined at 520 mμ on a Beckman DU spectro-

photometer using the respective experimental sea water as a

blank.

Statistical Methods

All statistical methods used were taken from Steele and

Torrie (1960) or Yates (1960). Student's t-tests were consid-

ered to be two tailed. When results, expressed as percentages,

were from a binomial distribution, all statistical tests were

performed on an arcsin transformation of percentage data.

Analyses of variance were considered to be model I and the

error mean square used as a denominator in F tests. The

analysis of covariance used is outlined on page 312 of Steele

and Torrie (1960). Unless otherwise stated, the level of

significance in discussion of results was $p=0.01$. The results

of statistical tests given in tables have been expressed in

the following manner: P=NS referred to tests that were not

significantly different at the $p=0.05$ probability level;

P=0.05 referred to tests that were significant at the $p=0.05$

probability level but not at the $p=0.01$ probability level;

P=0.01 referred to tests that were significantly different

at the $p=0.01$ probability level.

RESULTS

I   Ion Balance of Blood, Urine, and Muscle

Blood Ion Concentrations from Field Samples

Acmaea scutum were collected from Jordan River at approxi-
mately monthly intervals from June 1964 to December 1965.   Ten
animals were placed in field sea water under laboratory condi-
tions for 24 hr.   Blood samples were taken and analysed for
Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++}.   The means of each determination
were pooled for statistical analysis.   Table 1 gives the re-
sults of this experiment.   The blood concentrations of Na^+,
Cl^-, Ca^{++}, and Mg^{++} were not significantly different from the
concentration of the respective ion in environmental sea water.
The concentration of blood K^+ however, was significantly greater
than the concentration of sea water K^+.

To determine if the magnitude of the concentration gradi-
ent of K^+ between blood and sea water varied at different
times of the year, an analysis of variance was performed on
the blood K^+ data.   The probability obtained, $p > 0.1$, indicated
that there was no significant difference in the magnitude of
the K^+ concentration gradient for field samples of A. scutum
collected approximately monthly from June 1964 to December
1965.

TABLE 1

Concentration of blood ions from $\underline{A}$. $\underline{scutum}$ field samples (Jordan River), collected approximately monthly from June 1964 to December 1965. Individual samples of 10 animals have been pooled and tested for significance against environmental sea water concentrations. Sea water concentrations represent measurements taken at each sample collection and were treated statistically as a constant.

	Na^+	Cl^-	K^+	Ca^{++}	Mg^{++}
Blood Concentration (mEq/L)	432.3	496.7	11.8	19.7	97.8
Sea Water Concentration	433.0	497.2	10.1	19.6	97.8
Blood/Sea Water Ratio	0.998	0.998	1.17	1.00	0.998
n	90	90	100	90	90
Standard Error	0.56	0.78	0.46	0.09	0.54
Probability	N.S.	N.S.	0.01	N.S.	N.S.

Response of Blood Ions to Changes in External Salinity

For field samples of A. scutum from Jordan River (with a constant environmental salinity of 100% sea water), there was no difference in concentration between blood and sea water for the ions Na^+, Cl^-, Ca^{++}, and Mg^{++}, but there was a small gradient between the concentration of blood and sea water K^+. To test if the concentrations of these ions in the blood showed a similar pattern with changes in experimental salinity A. scutum collected from Whytecliff Park were placed in 50, 75, and 125% sea water for time periods of 1.5, 3, 6, 12, and 48 hr. The sample size at each salinity and time period was 10 animals. The experiment was repeated for Jordan River animals. Jordan River animals were also placed in each salinity for a time period of one week. The experiment was conducted from June through August 1963.

Response of blood Na^+, Cl^-, Ca^{++}, and Mg^{++}: Figure 1 gives the generalized response curves of blood Na^+, Cl^-, Ca^{++}, and Mg^{++} for Whytecliff and Jordan River animals to experimental salinities. Blood ion values for animals from both environments the constant salinity of Jordan River and the estuarine condition of Whytecliff Park, showed similar responses to changes in experimental salinity. When exposed to 50, 75, and 125% sea water, the concentration of blood Na^+, $Cl-$, Ca^{++}, and Mg^{++} approached the concentration of the respective ion in the new

FIGURE 1

Response of blood Na^+, Cl^-, Ca^{++}, and Mg^{++} values of A. scutum to changes in experimental salinity. At each point the concentration of each ion was converted to a percentage of the experimental salinity and the average used.
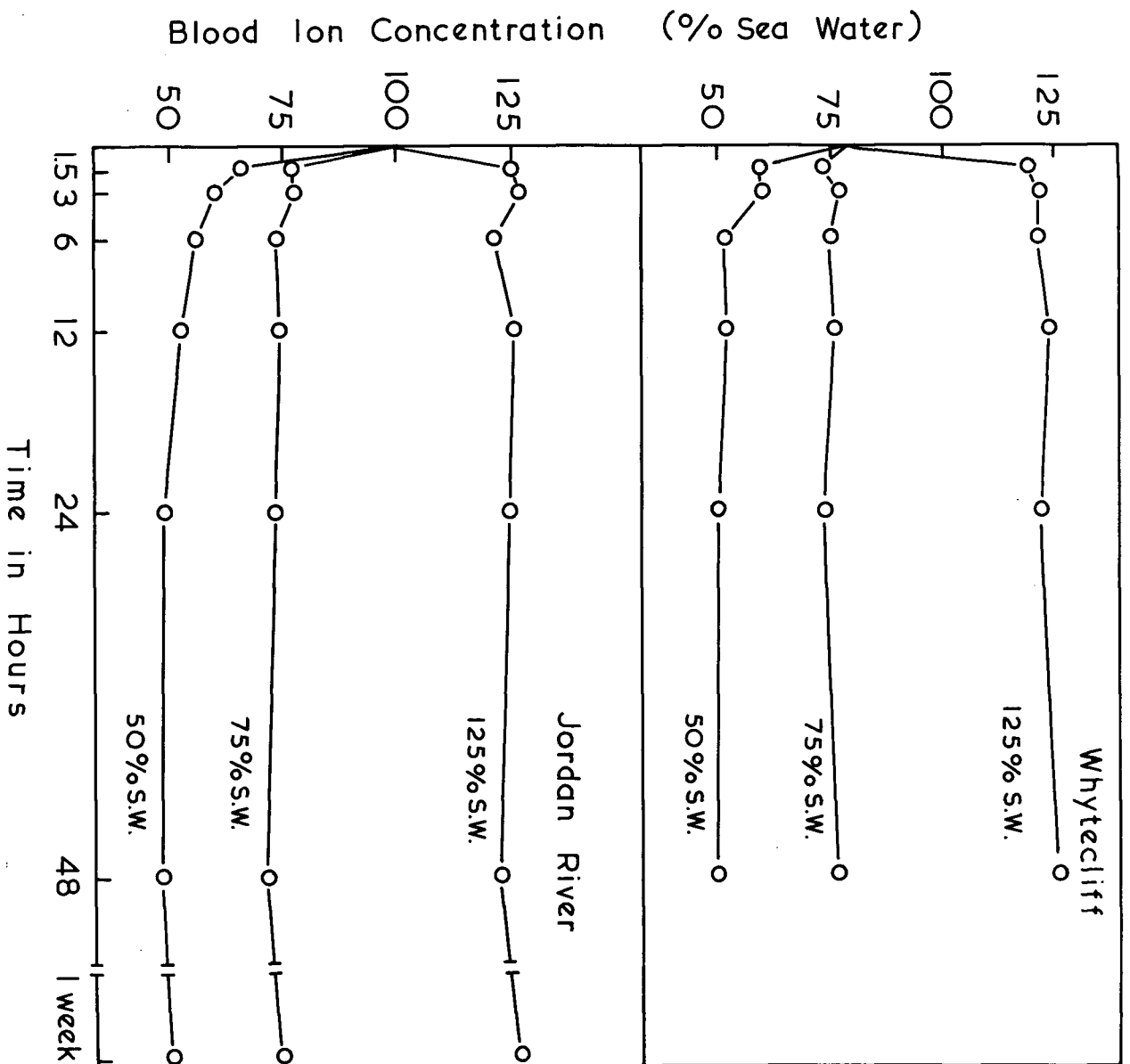
salinity. The time to equilibrium was primarily dependent on the magnitude of difference between the holding and experimental salinity.

Table 2 gives the blood values of Na^+, Cl^-, Ca^{++}, and Mg^{++} for various time periods of immersion in experimental salinities.

Table 3 indicates that there is no defined pattern for any particular ion to be consistently faster or slower in reaching equilibrium with sea water.

Table 4 gives blood-sea water ratios of Na^+, Cl^-, Ca^{++}, and Mg^{++} for each experimental salinity. The ratios for all ions at each salinity were not significantly different from one except for Ca^{++} at 50 and 75% sea water for Whytecliff animals.

Response of blood K^+: Figure 2 shows the response of blood K^+ to 50, 75, and 125% sea water. As with Na^+, Cl^-, Ca^{++}, and Mg^{++} there was an initial decrease of blood K^+ concentration in 50 and 75% sea water, and an initial increase in 125% sea water. The values of blood K^+ levelled off after a period of approximately 3 hr in 75% sea water, 6 hr in 125% sea water, and 12 hr in 50% sea water.

Assuming that K^+ was similar to Na^+ in equilibrium response with time, K^+ blood concentrations which corresponded to equilibrated Na^+ blood values (Table 2) were pooled for each

TABLE 2

Effect of salinity and time on the blood concentration of Na^+, Cl^-, Ca^{++}, and Mg^{++}. Results are from animals collected from an estuarine and marine environment. Concentrations are expressed in mEq/L. The means represent determinations from 10 animals. t-tests comparing blood concentrations with experimental sea water were performed on each sample until the blood concentration was statistically insignificant from sea water.

| | | Time in Hours | | | | | | | | | | |
| | | 1.5 | | 3 | | 6 | | 12 | | 24 | | 48 | 1 week |
	Salinity	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	$\bar{x}$
Na^+	50	J.R. 294.4	.01	264.0	.01	251.7	.01	219.6	NS	225.2		227.2	219.5
		Whyt 233.0	.01	237.5	.01	208.6	NS	223.5		213.0		217.2	
	75	J.R. 348.2	.01	346.2	.01	327.7	NS	315.0		325.2		320.0	325.0
		Whyt 317.0	NS	322.8		321.6		327.5		318.3		321.5	
	125	J.R. 524.0	.01	523.5	.01	540.5	NS	536.0		527.0		537.5	542.0
		Whyt 530.5	.01	537.0	NS	532.0		533.0		520.5		537.5	
Cl^-	50	J.R. 336.5	.01	295.0	.01	284.5	.01	252.3	NS	242.3		246.3	247.5
		Whyt 279.4	.01	283.1	.01	240.6	NS	247.7		254.4		247.1	
	75	J.R. 380.3	NS	379.0		370.9		363.7		368.3		363.0	366.2
		Whyt 374.5	NS	367.3		362.1		366.8		368.0		367.1	

				Time in Hours										
			1.5		3		6		12		24		48	1 week
	Salinity		$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	P	$\bar{x}$	$\bar{x}$
Cl⁻	125	J.R.	629.0	NS	632.4		614.8		616.0		626.0		613.0	614.0
		Whyt	586.8	.01	595.1	.01	601.3	.01	620.0	NS	614.3		630.2	
	50	J.R.	16.5	.01	14.7	.01	13.9	NS	12.3		11.6		10.6	13.1
		Whyt	16.7	.01	16.4	.01	14.6	NS	13.7		15.2		13.2	
Ca⁺⁺	75	J.R.	19.3	NS	19.8		18.0		19.9		18.0		17.5	19.2
		Whyt	18.3	.01	21.3	NS	20.7		20.0		18.6		21.9	
	125	J.R.	31.7	NS	32.3		30.2		31.9		31.9		32.2	31.9
		Whyt	30.8	.01	32.2	NS	31.3		32.7		31.7		34.2	
	50	J.R.	65.2	.01	62.4	.01	59.7	.01	53.9	.01	50.9	NS	51.7	51.4
		Whyt	65.7	.01	69.0	.01	58.3	.01	50.3	NS	47.4		55.8	
Mg⁺⁺	75	J.R.	75.6	NS	74.4		72.9		72.3		70.1		70.7	72.6
		Whyt	78.1	NS	79.1		76.9		79.5		76.4		72.4	
	125	J.R.	124.6	.01	131.9	.01	116.9	.01	128.2	.01	123.2	NS	116.3	132.9
		Whyt	119.6	NS	120.4		121.7		____		122.1		122.9	

TABLE 3

Time required in hours for blood Na^+, Cl^-, Ca^{++}, and Mg^{++} values to equilibrate with the respective ions in 50, 75, and 125% sea water.

	Na^+	Cl^-	Ca^{++}	Mg^{++}
Jordan River				
50% S.W. (dilution of 1/2)	6-12	6-12	3-6	12-24
75% S.W. (dilution of 1/4)	3-6	1.5-3	0-1.5	0-1.5
125% S.W. (concentration of 1/4)	3-6	0-1.5	0-1.5	0-1.5
Whytecliff				
50% S.W. (dilution of 1/4)	3-6	3-6	3-6	6-12
75% S.W. (dilution of 1/10)	0-1.5	0-1.5	1.5-3	0-1.5
125% S.W. (concentration of 1/2)	1.5-3	6-12	1.5-3	0-1.5

TABLE 4

Equilibrated values (mEq/l) of blood ions in experimental
salinities. Blood/sea water ratios were calculated and
tested for significance by t-test.

	Salinity		$\bar{x}$	n	blood/sea water ratio	P
Na^+	50	J.R.	222.9	40	1.03	NS
		Whyt	215.6	40	1.00	NS
	75	J.R.	322.7	50	0.99	NS
		Whyt	321.4	60	0.99	NS
	125	J.R.	536.6	50	0.99	NS
		Whyt	532.0	60	0.98	.05
Cl^-	50	J.R.	247.1	40	0.99	NS
		Whyt	247.4	40	0.99	NS
	75	J.R.	368.5	60	0.99	NS
		Whyt	367.6	60	0.99	NS
	125	J.R.	620.7	70	1.00	NS
		Whyt	621.5	30	1.00	NS
Ca^{++}	50	J.R.	12.3	50	0.96	NS
		Whyt	14.2	40	1.10	.01
	75	J.R.	18.8	70	0.98	NS
		Whyt	20.5	50	1.03	.01
	125	J.R.	31.7	70	0.99	NS
		Whyt	32.4	50	1.01	NS
Mg^{++}	50	J.R.	51.3	30	1.05	NS
		Whyt	51.1	30	1.04	NS
	75	J.R.	72.7	70	0.99	NS
		Whyt	77.1	60	1.05	NS
	125	J.R.	124.1	30	1.01	NS
		Whyt	121.3	50	0.99	NS

FIGURE 2

Effect of salinity and time of immersion on the blood
concentration of K^+ for <u>A</u>. <u>scutum</u> from a marine (Jordan
River) and estuarine (Whytecliff) environment. Each
point is the mean of determinations on 10 animals.
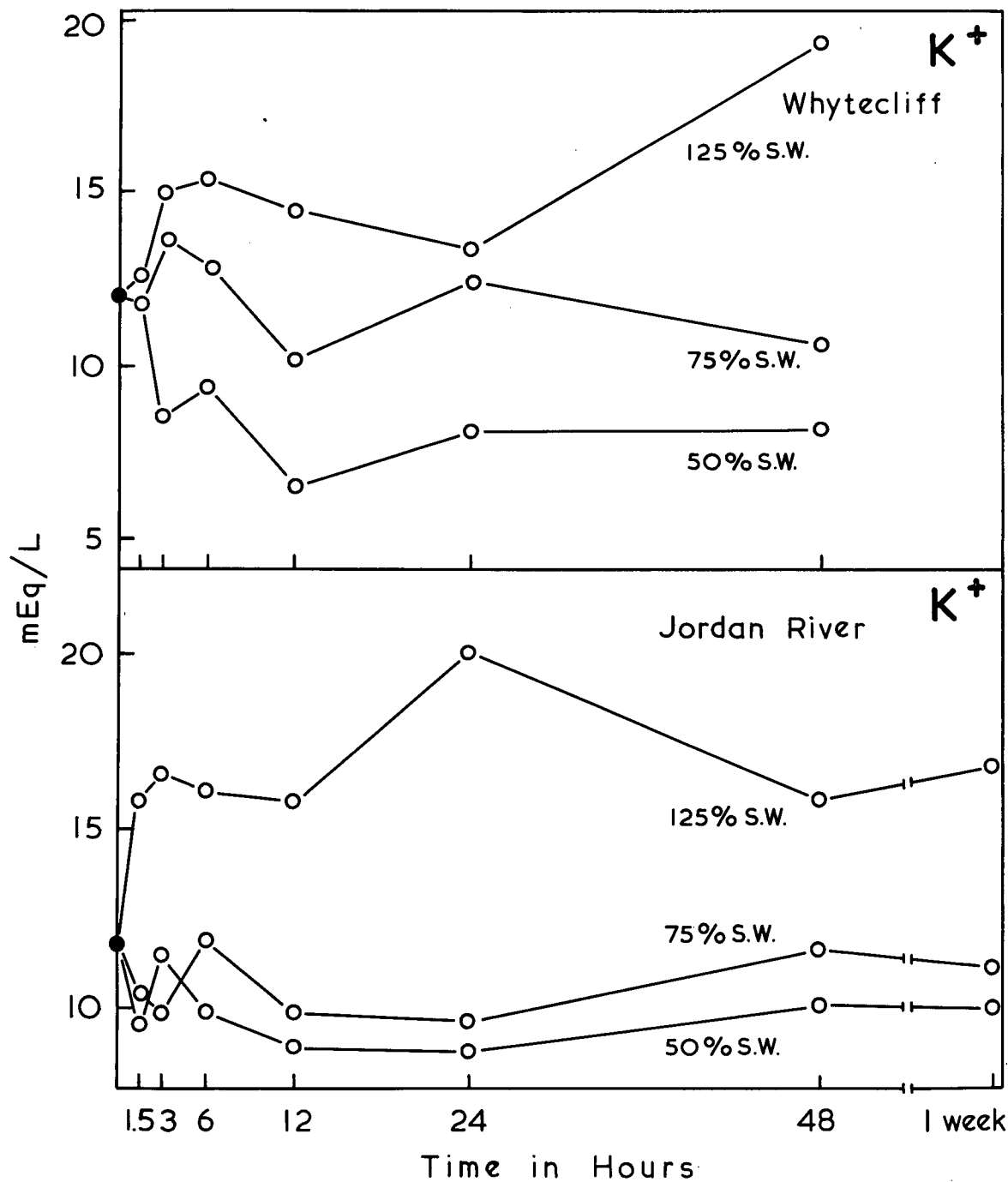
salinity. The results are expressed in Table 5. Clearly, at each salinity, the concentration of blood K^+ was significantly higher than the concentration of K^+ in the corresponding salinity.

To test for significance of difference in blood K^+ concentration between means of animals from 50, 75, and 125% sea water, analyses of variance were performed separately on Jordan River and Whytecliff blood K^+ data. Tukey's w, derived from the analysis of variance was 4.03 mEq/l for Whytecliff blood K^+ data, and 4.32 mEq/l for Jordan River blood K^+ data. Comparing only those means that were assumed to be in equilibration with the external sea water, the following levels of significance were obtained. For Jordan River blood K^+ data all means for 50% sea water differed significantly from corresponding means at 125% sea water. Means from 75% sea water did not differ significantly from means at 50% sea water. Except for the time interval of 6 hr, means from 75% sea water differed significantly from corresponding means at 125% sea water. For Whytecliff blood K^+ data, means from 50% sea water differed significantly from corresponding means at 125% sea water. Means from 50% sea water did not differ significantly from corresponding means at 75% sea water. Finally, for Whytecliff animals means at 75% sea water did not differ significantly from corresponding means at 125% sea water, except for

TABLE 5

Summary of blood K^+ data. The means were pooled from values
assumed in equilibrium with experimental salinities. Probabil-
ity values are results of t-tests on concentration differences
between blood and sea water K^+ values for each salinity.

	Sal	$\bar{x}$ mEq/l	n	Ratio B/S.W.	P
J.R.	50	9.36	40	1.87	0.01
	75	10.88	50	1.43	0.01
	125	16.94	50	1.35	0.01
Whyt	50	8.05	40	1.61	0.01
	75	11.97	60	1.57	0.01
	125	15.56	50	1.24	0.01

the time period of 48 hr.

Urine Ion Concentrations

Data on the blood values of Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++} over a range of experimental salinities from 50 to 125% sea water for animals from a marine and estuarine environment showed that at all salinities blood K^+ values were higher than corresponding sea water K^+ values, and that blood Ca^{++} of animals from an estuarine environment at 50 and 75% sea water was greater than respective sea water Ca^{++} values. To ascertain if these ions showed a similar response in urine, Jordan River animals were placed in each of 50, 75, and 125% sea water for sampling at time intervals of 1.5, 6, 12, 24, and 48 hr. Urine and blood samples were taken from the same animals. The sample size was five animals.

Effect of salinity change on urine Na^+, Cl^-, Ca^{++}, and Mg^{++}: Figure 3 and Table 6 show that the urine values of Na^{++}, Cl^-, Ca^{++}, and Mg^{++} followed the same general response curve to changes in external salinity as has been described for these ions in blood.

There are, however, some small but significant differences between blood and urine Na^+ and Cl^- from animals in 50% sea water. Table 6 shows that, in 50% sea water, urine Na^+ and Cl^- were significantly greater than blood Na^+ and Cl^-. This gradient was most noticeable at 1.5, 6, and 12 hr time periods.

FIGURE 3

Effect of salinity and time of immersion on blood and urine Na^+ values for Jordan River animals. Each point is the mean of determinations on five animals.
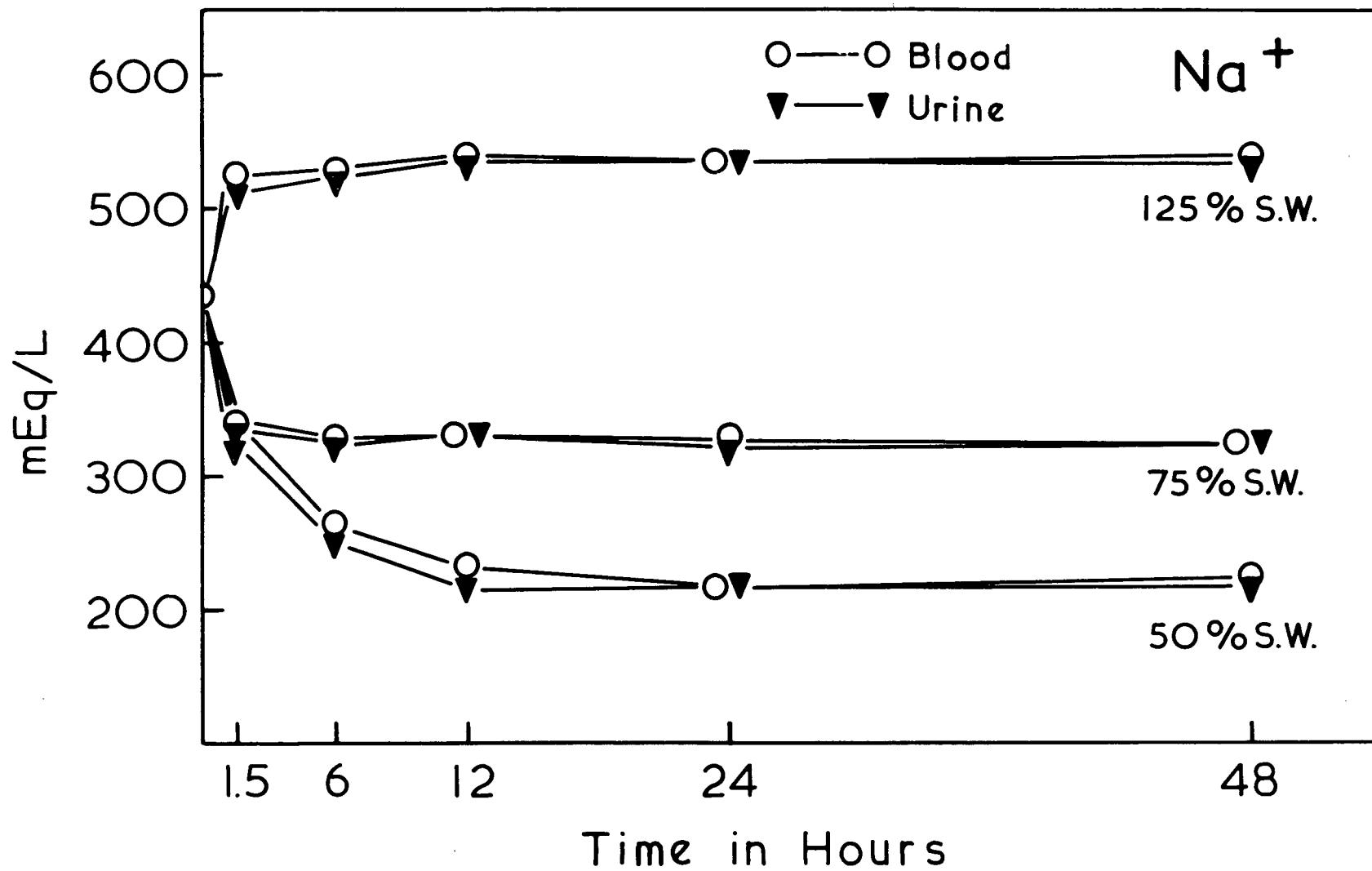
TABLE 6

Effect of salinity and time on blood and urine ions for Jordan River A. scutum. Each value is the mean of 5 determinations and is expressed in mEq/l. Differences between blood and urine concentrations were compared by paired sample t-test.

			Time in hours					t-tests	
	Salinity		1.5	6	12	24	48	n	P
Na$^+$	50	blood	324.4	251.5	217.1	216.5	218.0	25	.01
		urine	337.4	260.0	229.5	215.1	223.4		
	75	blood	330.5	322.5	322.4	325.7	320.2	25	NS
		urine	335.0	325.8	326.4	325.7	322.2		
	125	blood	514.2	525.1	535.0	540.5	535.5	25	NS
		urine	526.9	529.7	540.0	538.2	538.5		
Cl$^-$	50	blood	376.2	274.4	258.4	244.8	245.6	25	.01
		urine	388.6	292.0	262.8	248.0	299.2		
	75	blood	_____	364.8	367.8	_____	374.0	15	NS
		urine	_____	367.2	368.6	_____	372.4		
	125	blood	590.8	615.6	618.8	618.8	618.8	25	NS
		urine	592.8	615.6	618.8	620.0	617.6		
Ca^{++}	50	blood	18.4	15.2	13.7	13.2	12.4	25	NS
		urine	19.8	15.7	13.3	13.0	12.4		
	75	blood	18.9	18.6	20.8	18.3	19.3	25	.05
		urine	18.9	18.4	20.2	18.4	18.8		
	125	blood	31.4	31.1	33.0	30.9	31.4	25	NS
		urine	31.3	30.2	32.3	30.3	31.4		
Mg^{++}	50	blood	77.1	56.3	49.4	46.3	46.6	25	NS
		urine	84.2	53.3	53.3	48.4	45.7		
	75	blood	75.3	75.4	70.8	69.9	74.5	25	NS
		urine	77.3	76.6	71.5	74.3	73.5		
	125	blood	105.1	126.4	121.5	117.6	121.1	25	NS
		urine	106.0	131.3	124.8	115.7	119.6		

For Na^+ and Cl^- at 75 and 125% sea water and for Ca^{++} and Mg^{++} at 50, 75, and 125% sea water there was no significant difference between blood and urine concentrations.

Effect of salinity change on urine K^+: As was shown above, blood K^+ values were always greater than the corresponding K^+ concentrations of sea water. Urine K^+ (Fig 4) however, showed a similarity to blood Na^+ and Cl^- (Table 2) in the response to change in salinity. That is; after a period of adjustment (between 1.5 and 3 hr for 75 and 125% sea water, and 6 to 12 hr for 50% sea water), urine K^+ concentrations approached the K^+ concentrations of corresponding salinities. The results of statistical analysis on urine K^+ data were as follows. First, using a paired sample t-test, the concentration of blood K^+ was significantly greater than the concentration of urine K^+ at 50, 75, and 125% sea water. Secondly, to determine if the concentration of K^+ in the urine was the same as the K^+ concentration of the experimental salinity, the urine K^+ values for means that were equilibrated to the experimental salinities were pooled (for 75 and 125% sea water means from 1.5 to 48 hr were pooled; and for 50% sea water means for 12, 24, and 48 hr were pooled). Comparison by t-test showed that, at each salinity, the urine and sea water concentrations of K^+ were not significantly different.

FIGURE 4

Effect of salinity and time of immersion on blood and urine K^+. Each point is the mean of determinations on five animals.
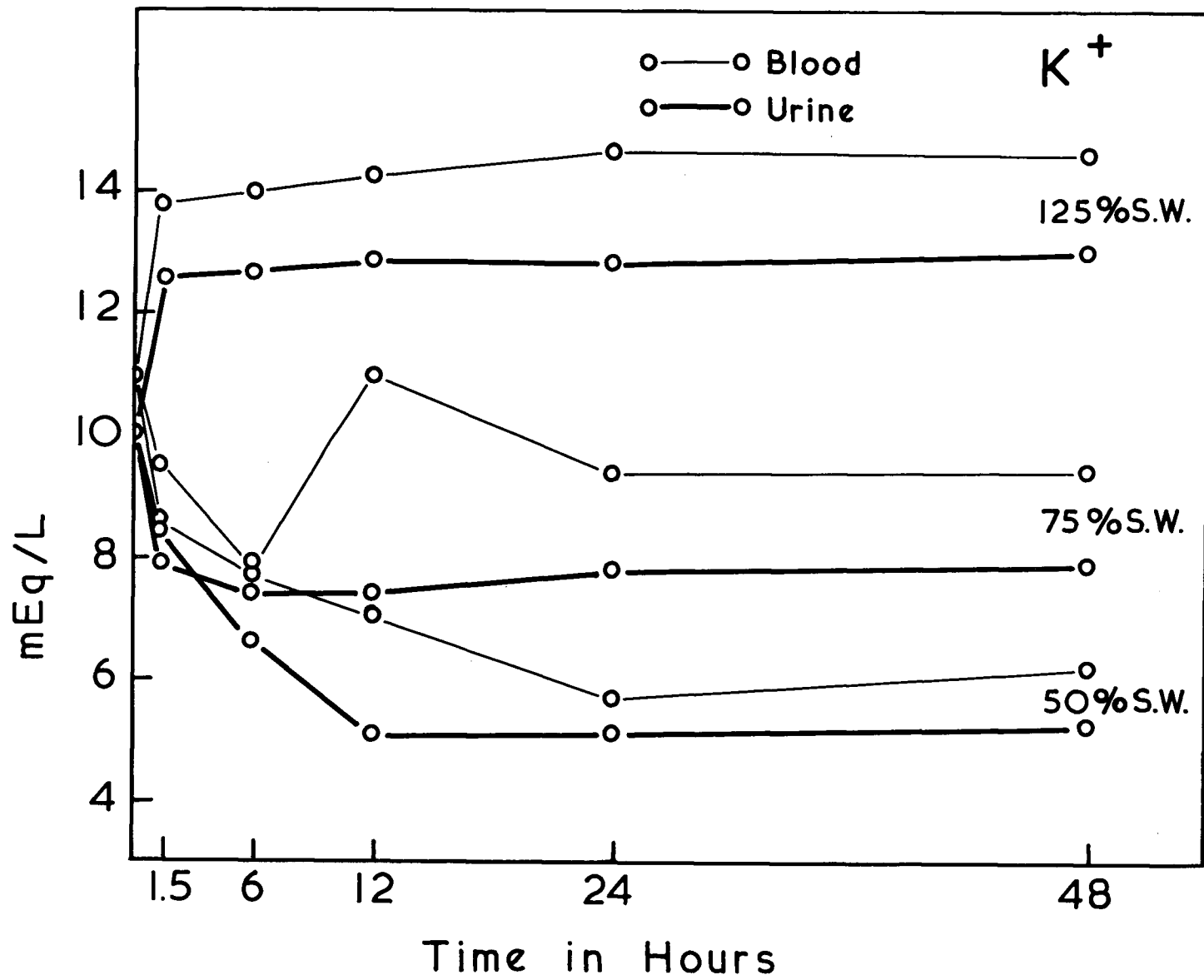
Dialysis of Blood and Urine at Experimental Salinities

Data, provided above on ion values of blood and urine showed three situations in which concentration gradients existed at experimental salinities. First, blood K^+ values were always greater than corresponding urine and sea water K^+ values (Fig 2, Fig 4). Secondly, during equilibration to a change in experimental salinity, blood and urine concentrations of Na^+, Cl^-, Ca^{++}, and Mg^{++} were different from sea water values of these ions (Fig 3, Table 6). Finally, during equilibration to 50% sea water, urine values of Na^+ and Cl^- were greater than blood values (Fig 3, Table 6). By dialysing blood and urine samples against the respective experimental salinity it is possible to determine if the concentration gradients observed were due to Donnan equilibriums.

Samples for dialysis of blood and urine were taken from the same animals from which samples for blood and urine ion analyses were taken. Table 7 shows that dialysate values of blood and urine ions did not differ. As well, dialysate concentrations of blood ions did not differ from sea water ion values. With urine dialysates only Mg^{++} at 50% sea water, differed from sea water ion concentrations. This gradient between the dialysates of urine Mg^{++} and the Mg^{++} values at 50% sea water is not considered biologically meaningful.

The gradient between the concentration of K^+ in blood and

TABLE 7

Effect of dialysing blood and urine against respective experi-
mental salinities. Concentrations are in mEq/L. t-tests were
used to compare blood and urine dialysates, and blood and urine
dialysates with sea water.

	Sal	Blood	n	urine	n	S.W.	n	Blood-urine	Blood-S.W.	Urine-S.W.
		Dialysis Values							t-tests	
Na^+	50	218.4	25	218.4	25	216.6	25	NS	0.05	NS
	75	326.3	24	325.0	25	324.3	25	NS	0.05	NS
	125	538.5	25	540.7	25	541.6	25	NS	NS	NS
Cl^-	50	248.2	25	249.6	25	249.9	15	NS	NS	0.05
	75	374.1	15	376.5	15	374.6	15	NS	NS	NS
	125	619.9	25	621.9	25	619.5	15	NS	NS	0.05
K^+	50	5.0	25	5.1	25	5.0	25	NS	NS	NS
	75	7.7	24	7.7	25	7.5	25	NS	NS	0.05
	125	12.9	25	12.9	25	12.7	25	NS	NS	NS
Ca^{++}	50	12.6	25	12.6	25	12.7	25	NS	NS	NS
	75	19.3	24	19.4	25	19.3	25	NS	NS	NS
	125	32.2	25	32.1	25	32.0	25	NS	NS	NS
Mg^{++}	50	49.5	25	50.4	20	49.5	25	NS	NS	NS
	75	74.3	24	76.8	25	78.5	25	0.05	NS	NS
	125	122.9	25	125.6	25	121.9	25	0.05	NS	0.01

sea water was abolished in all three salinities by dialysis.

The concentration of the blood dialysate was not significantly

different from the K^+ concentration of the experimental sal-

inity in which the animal was immersed.

Ion Concentration of Foot Muscle

In order to estimate the intracellular ionic values of

the foot muscle, two measurements had to be made. These were

the ion concentration of foot muscle tissue, and the extent of

the blood space of this muscle tissue. Two sets of muscle ion

data were collected from Jordan River A. scutum. First, muscle

ion values for field samples were taken from June 1964 through

December 1965. Secondly, the effect of experimental salinities

on the distribution of muscle ions was determined.

Muscle ion values for Jordan River field samples: Figure

5 shows that the ion content of foot muscle for field samples

from Jordan River varied with time. Tukey's w, calculated

from analysis of variance data for each ion, was used for the

minimum difference needed for significance. Values of Tukey's

w (mEq/Kg wet weight tissue) for foot muscle ion data were as

follows:

Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}
37.1	14.3	39.6	19.3	12.8

It is clear that all ions of the foot muscle showed signifi-

cant variation over the time of collection of field samples

FIGURE 5

Values of Na^+, Cl^-, K^+, Ca^{++}, Mg^{++}, and H_2O for the foot muscle tissue of $\underline{A}$. $\underline{scutum}$ field samples. Animals were collected from Jordan River over a period of 18 months. Each point is the mean of determinations on 10 animals. The concentrations of muscle Na^+ and K^+ in mEq/Kg water are also plotted.
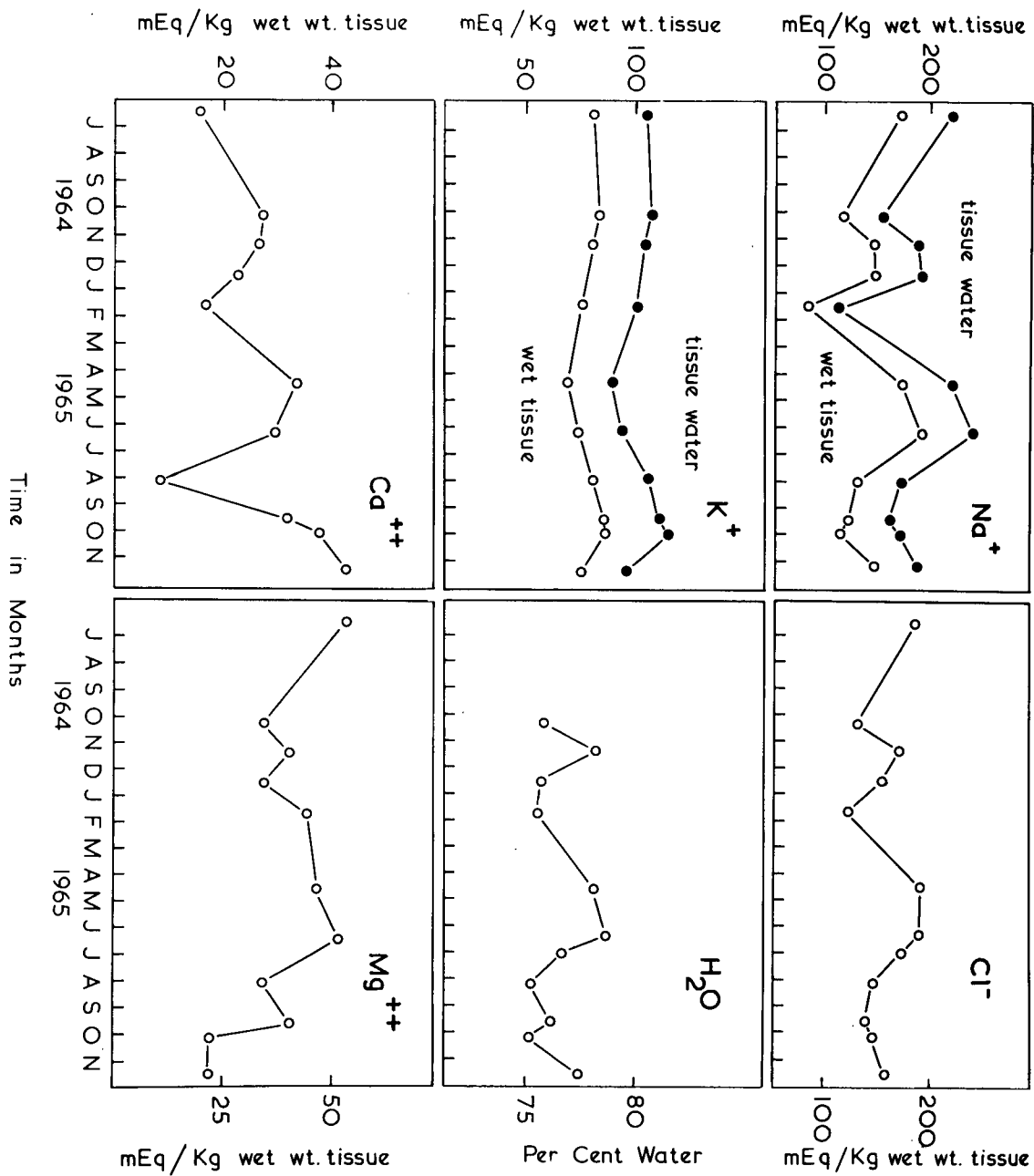
from an envrionment of constant salinity (100% sea water).

To evaluate the possibility that seasonal muscle ion variation was only a reflection of variation in water content of the muscle tissues, the concentration of muscle K^+ and Na^+ was expressed as mEq/Kg of muscle H_2O, and plotted with muscle Na^+ and K^+ expressed in mEq/Kg wet weight tissue. This is shown in Figure 5. The similarity of the two curves eliminates the possibility that differences in muscle water content was an important factor in the seasonal variation of muscle Na^+ and K^+ values.

Comparing muscle and blood ion concentrations from Jordan River field samples (salinity of 100% sea water), the concentrations of muscle Na^+, Cl^-, and Mg^{++} were lower than blood concentrations. Muscle Ca^{++} was approximately the same concentration as blood Ca^{++} and muscle K^+ was higher than blood K^+ concentration (Fig 5, Table 2)

Response of muscle ions to experimental salinities: Data on the effect of experimental salinities on muscle ion values were obtained from A. scutum collected from Jordan River in July 1964. The experiment was repeated on A. scutum collected from Jordan River in December 1964. Tukey's w was calculated for each ion from a three way analysis of variance in which data from summer and winter animals were combined.

December muscle ion data: Figure 6 shows the effect of

FIGURE 6

Effect of salinity and time on foot muscle values of Na^+ Cl^-, K^+, Ca^{++}, Mg^{++}, and H_2O for Jordan River animals collected in December 1964. Each point is the mean of determinations on 10 animals.
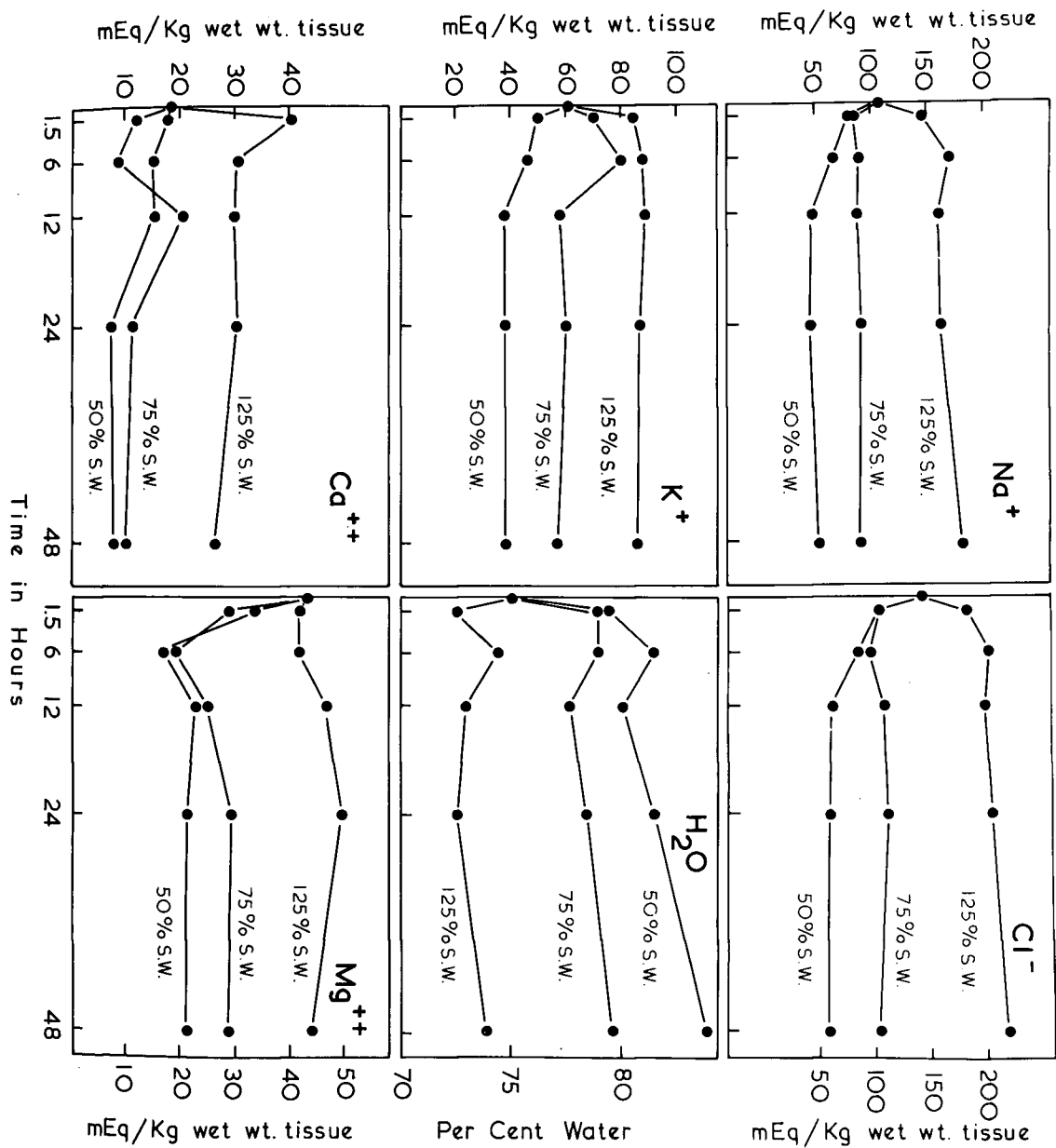
experimental salinities on muscle ion concentrations. Values
of Tukey's w (mEq/Kg wet weight tissue) for each ion were:

$$Na^+ \quad Cl^- \quad K^+ \quad Ca^{++} \quad Mg^{++}$$

$$34.8 \quad 39.5 \quad 13.3 \quad 15.4 \quad 13.2.$$

There are three important features to note from the muscle
ion data of Figure 6. First the times to equilibration for
muscle ions (Fig 6) were not appreciably different from times
to equilibration for blood ions (Table 3). Secondly, at a
given salinity, muscle ion concentrations (Fig 6) were different
from blood ion concentrations (Table 2). Muscle Na^+, Cl^-, and
Mg^{++} values were lower than blood Na^+, Cl^-, and Mg^{++} values.
Muscle K^+ values were higher than blood K^+ values. Muscle
Ca^{++} values, however, were approximately the same as blood
Ca^{++} values. The third point of importance in the muscle
ion data of Figure 6 is the significance of differences in
concentration of a given ion at different experimental sal-
inities. Using Tukey's w, the equilibrated values of muscle
Na^+, Cl^-, and K^+ were significantly different at each experi-
mental salinity. For muscle Ca^{++} and Mg^{++}, equilibrated
values at 50 and 75% sea water were not significantly different.
Values of Ca^{++} and Mg^{++} at 125% sea water, however, differed
significantly from values at 50 and 75% sea water.

Comparison of December and July muscle ion data: Muscle
ion values of Jordan River animals collected in July 1964 are

are given in Table 8. The difference between equilibrated

muscle ion values for each ion and salinity for December and

July data are given in Table 9. The only significant diff-

erences between July and December muscle data were for Na^+

and Cl^- at 125% sea water. July values for these two ions

were greater than December values. Basically then, there was

no difference in muscle ion values at experimental salinities

for animals collected from Jordan River in July and December

1964. A comparison of muscle ion values for field samples

collected from Jordan River in July and December 1964 (Fig 5)

also shows that muscle ion values for these two times of the

season were not significantly different.

Extracellular Volume of Foot Muscle

The ionic concentration of muscle tissue consists of a

contribution from the blood space and a contribution from the

intracellular portion. In order to estimate the contribution

of the blood space, the extracellular volume of muscle tissue

as represented by the inulin space was determined. A. scutum

collected from Jordan River during October and November 1965

were used to estimate the inulin space of foot muscle. Animals

were equilibrated in experimental salinities for 24 hr before

use. Twenty animals in each of 50 and 125% sea water and 19

animals in 100% sea water were used. For statistical analysis,

the calculated per cent inulin space was regressed on the

TABLE 8

Effect of salinity and time on the muscle concentration of Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, and water. The data were collected from Jordan River $\underline{A}$. scutum in July 1964. Ion concentrations are in mEq/Kg wet weight tissue, and water concentrations in % of wet weight tissue. Tukey's w was used to determine equilibrated means.

	Sal	Time in hours 1.5	6	12	24	48	Tukey's w	equilibrated $\bar{x}$
	50	114.6	66.8	55.9	63.8	59.9		61.6
Na^+	75	107.9	107.7	91.4	100.5	102.3	34.8	102.0
	125	228.5	247.7	206.6	240.1	181.8		220.9
	50	123.0	73.3	64.8	74.5	64.0		69.1
Cl^-	75	115.4	126.5	103.3	103.9	118.6	39.2	113.5
	125	249.5	273.7	225.9	270.2	192.1		242.2
	50	52.8	46.3	46.7	41.9	40.4		43.8
K^+	75	69.0	54.8	65.7	67.8	62.8	13.3	64.0
	125	81.8	70.9	77.9	78.5	103.4		82.0
	50	12.0	12.9	16.7	8.4	3.0		10.6
Ca^{++}	75	12.3	9.3	11.7	13.1	9.3	15.4	11.1
	125	17.6	18.2	29.1	18.9	25.8		21.9
	50	31.2	26.2	31.6	24.2	19.9		26.6
Mg^{++}	75	56.9	33.0	33.0	32.7	36.8	13.2	38.5
	125	54.2	55.5	59.2	53.5	50.8		54.6
	50	80.3	83.8	82.4	83.0	84.8		83.5
H_2O	75	80.8	79.8	79.4	81.3	80.4	2.6	80.3
	125	74.4	75.5	76.9	75.0	73.9		45.1

TABLE 9

Differences in concentration (mEq/Kg wet weight tissue) of muscle ions and water for Jordan River animals sampled in July and December 1964. Time periods in equilibration with the experimental sea water were used to calculate the differences. Tukey's w, the minimum difference needed for significance, is also included.

	Na^+		Cl^-		K^+	
salinity	difference	w	difference	w	difference	w
50	-8.5	34.8	3.5	39.2	2.9	13.3
75	14.7	34.8	11.3	39.2	-1.3	13.3
125	56.6	34.8	40.8	39.2	-4.9	13.3

	Ca^{++}		Mg^{++}		H_2O	
salinity	difference	w	difference	w	difference	w
50	-4.6	15.4	3.6	13.2	2.5	2.6
75	-1.3	15.4	12.3	13.2	1.7	2.6
125	-9.3	15.4	10.1	13.2	1.9	2.6

corresponding experimental salinity. The slope from the resulting equation, Y=17.6 + 0.130X, was significantly different from zero. The extracellular volumes of foot muscle at 50, 75, 100, and 125% sea water were calculated from the regression equation and are listed in Table 10. The per cent extracellular space increased with salinity from 16.7% per Kg wet weight tissue at 50% sea water, to 31% per Kg wet weight tissue at 125% sea water.

A second method of estimating extracellular space is to assume all chloride of total muscle analysis to be extracellular. In this case Manery (1954) proposed the following equation.

$$H_2O_{Cl^-}^{ecs} = \left[\frac{T_{Cl^-}}{B_{Cl^-}}\right] \left[H_2O_B\right] \left[rCl^-\right]$$

where

$H_2O_{Cl^-}^{ecs}$ = chloride space, mEq/Kg muscle tissue

T_{Cl^-} = tissue chloride concentration, mEq/Kg muscle tissue

B_{Cl^-} = blood chloride concentration, mEq/l

H_2O_B = blood water content, taken to be 980 g/l

rCl^- = Donnan distribution ratio

The Donnan distribution ratio accounts for ion concentration differences between the plasma and extracellular space of vertebrate muscle tissue. The concentration differences

TABLE 10

Per cent extracellular volume of foot muscle tissue. Estimates
are the inulin space (measured) and the Cl^- space (calculated
using the equation from Manery, 1954).

		Salinity		
% E.C.V.	50	75	100	125
Inulin space	16.7	21.0	25.9	31.0
Cl^- space	23.3	27.6	28.2	32.4

are due to the protein content of the plasma. In <u>A</u>. <u>scutum</u>
the plasma and extracellular space are one and the same, and
rCl^- was assumed to be one. Using the Manery equation es-
timates of extracellular volume were obtained at 50, 75, 100,
and 125% sea water and are given in Table 10. Values at 100
and 125% sea water were similar to inulin space values. How-
ever, chloride space values at 50 and 75% sea water were
higher than values from inulin space.

<u>Estimation of Intracellular Ion Concentrations</u>

<u>Method of estimation</u>: In order to estimate intracellular
ion concentrations two sets of data were used. These were the
values for per cent extracellular space of foot muscle (as
represented by the inulin space) and data for total ion
content of foot muscle. For 100% sea water, concentrations
of ions of 30 animals collected during October and November
1965 were used. For 50, 75, and 125% sea water, the total
muscle ion data used to calculate intracellular concentrations
were chosen from animals collected in December 1964 to min-
imize error due to seasonal variation.

For each salinity, means of total muscle ion concentra-
tions were pooled for time periods equilibrated to each sal-
inity. For 50 and 125% sea water means were pooled for 12,
24, and 48 hr. Intracellular concentrations were estimated
for each ion at each salinity in the following manner:

$$M_i = M_t - E_{cs}(B_{100})$$

Where for a given ion

M_i = intracellular concentration, mEq/Kg muscle tissue

M_t = concentration of total muscle, mEq/Kg muscle tissue

E_{cs} = % extracellular space

B_{100} = blood concentration (mEq/l) divided by 100.

(Intracellular water content was estimated by the same formula, using per cent values for concentrations.)

The intracellular component of a kilogram of muscle tissue changes with salinity because of different values of extra-cellular space and water content. Therefore, the intracellular values at each salinity were converted to mEq/Kg cell water.

Confidence intervals, expressed as ±2 standard error units for p=0.05 probability level, and ±3 standard error units for the p=0.01 probability level, were calculated on each estimate of intracellular concentration using the following method as outlined in Yates (1960, p 160).

a. The variance of per cent extracellular space times blood concentrations of ions and water.

$$\text{Variance } (X_1 k) = \text{Var } X_1 k_1^2$$

where the variance of X_1 was calculated from the analysis of variance data on the regression of per cent extracellular space on salinity. The formula used (Steele and Torrie, 1962) was:

$$s\hat{y}^2 = sy^2.x \left[\frac{1}{n} + \frac{(X-\bar{x})^2}{x^2}\right]$$

where

$s\hat{y}^2$ = estimate of variance of extracellular space

$sy^2.x$ = error mean square from analysis of variance of regression

X = salinity at which Y is extimated

$\bar{x}$ = mean of X

x^2 = corrected X term from regression.

k_1 represents the blood concentration of an ion in mEq/l of blood divided by 100. The blood water concentration was assumed to be 100%; and for intracellular water variance calculations, k_1 was set at 1.

b. Variance of total muscle concentrations of ions and water:

$$\text{Variance } X_2 = EMS/r$$

where

EMS = error mean square from analysis of variance

r = number of replicates

c. The conversion factor applied to intracellular concentrations per Kg of wet weight tissue to bring the values to Kg of cell water was defined as a constant k_2.

d. Variance of intracellular estimates

$$\text{Variance } (X_1 - X_2 k_1)k_2 = (\text{Var } X_1 + \text{Var } X_1 k_2)k_2^2$$

where k_2 and the variance of X_1 and $X_2 k_1^2$ are defined above.

Intracellular estimates: The estimates of intracellular ion content, with 0.05 and 0.01 confidence intervals, are expressed in Table 11.

Na^+ and Cl^-: The estimates of intracellular Na^+ and Cl^- at all salinities were close to zero. Using the probability level of p=0.01, only estimates of Na^+ at 75 and 100% sea water and Cl^- at 75% sea water were significant from zero.

K^+: The intracellular estimates of K^+ were, at all salinities, significant from zero. Likewise, estimates of intracellular K^+ values from 50, 75, 100, and 125% sea water were significantly different from each other. As Figure 7 shows, the response of intracellular K^+ concentration to salinity appeared to be linear.

Ca^{++}: Estimates of the concentration of Ca^{++} indicated a small but significant intracellular concentration. At the 0.01 probability level, the estimates for 50 and 75% sea water were significantly different from the estimate of intracellular Ca^{++} from 100 and 125% sea water.

Mg^{++}: Mg^{++} also showed a small significant intracellular concentration of approximately 20mEq/Kg cell water in all but 125% sea water. At the 0.01 probability level the estimate of intracellular Mg^{++} was significantly different from zero at 50, 75, and 100% sea water, but was not significant from zero at 125% sea water. At the 0.05 probability level estimates

TABLE 11

Concentrations of total muscle ions (mEq/Kg wet tissue), cellular ions (mEq/Kg cell water), water content of muscle (per cent wet tissue), and cellular water (per cent tissue water). Confidence intervals are ± 2 (p=0.05), and ±3 (p=0.01) standard deviation units.

| | Na^+ | | | | Cl^- | | | | K^+ | | | |
| | total | | cellular | | total | | cellular | | total | | cellular | |
Salinity	$\bar{x}$	n	$\bar{x}$	C.I.	$\bar{x}$	n	$\bar{x}$	C.I.	$\bar{x}$	n	$\bar{x}$	C.I.
50	48.9	30	19.7	±19.3 ±28.9	59.2	30	27.2	±21.8 ±32.7	38.9	30	58.7	± 7.0 ±10.4
75	88.1	40	34.6	±21.8 ±32.7	105.2	40	46.8	±24.7 ±37.0	63.9	40	108.4	± 7.8 ±11.8
100	134.5	30	45.7	±26.3 ±39.4	142.9	30	28.8	±29.8 ±44.7	82.0	30	162.0	± 9.2 ±13.8
125	168.7	40	2.1	±35.5 ±53.2	205.8	40	31.1	±40.2 ±60.4	87.9	40	198.2	±10.8 ±16.1

Salinity	Ca^{++} total $\bar{x}$	n	Ca^{++} cellular $\bar{x}$	C.I.	Mg^{++} total $\bar{x}$	n	Mg^{++} cellular $\bar{x}$	C.I.	H_2O total $\bar{x}$	n	H_2O cellular $\bar{x}$	C.I.
50	10.2	30	12.5	± 7.9	21.7	30	20.8	± 7.0	81.7	30	79.3	±4.8
				±11.8				±10.5				±7.2
75	12.6	40	15.0	± 8.9	25.5	40	17.6	± 7.9	78.6	40	73.1	±2.4
				±13.4				±11.9				±3.6
100	26.1	30	39.8	±10.5	36.4	30	22.6	± 9.4	74.8	30	65.5	±2.0
				±15.7				±14.1				±3.0
125	29.4	40	46.0	±12.2	45.3	40	17.4	±11.7	73.4	40	57.7	±4.0
				±18.3				±17.5				±6.0

FIGURE 7

Estimates of intracellular K^+ and water values at experimental salinities. Values were taken from Table 11. Vertical lines represent confidence intervals (p=0.01) around each mean.
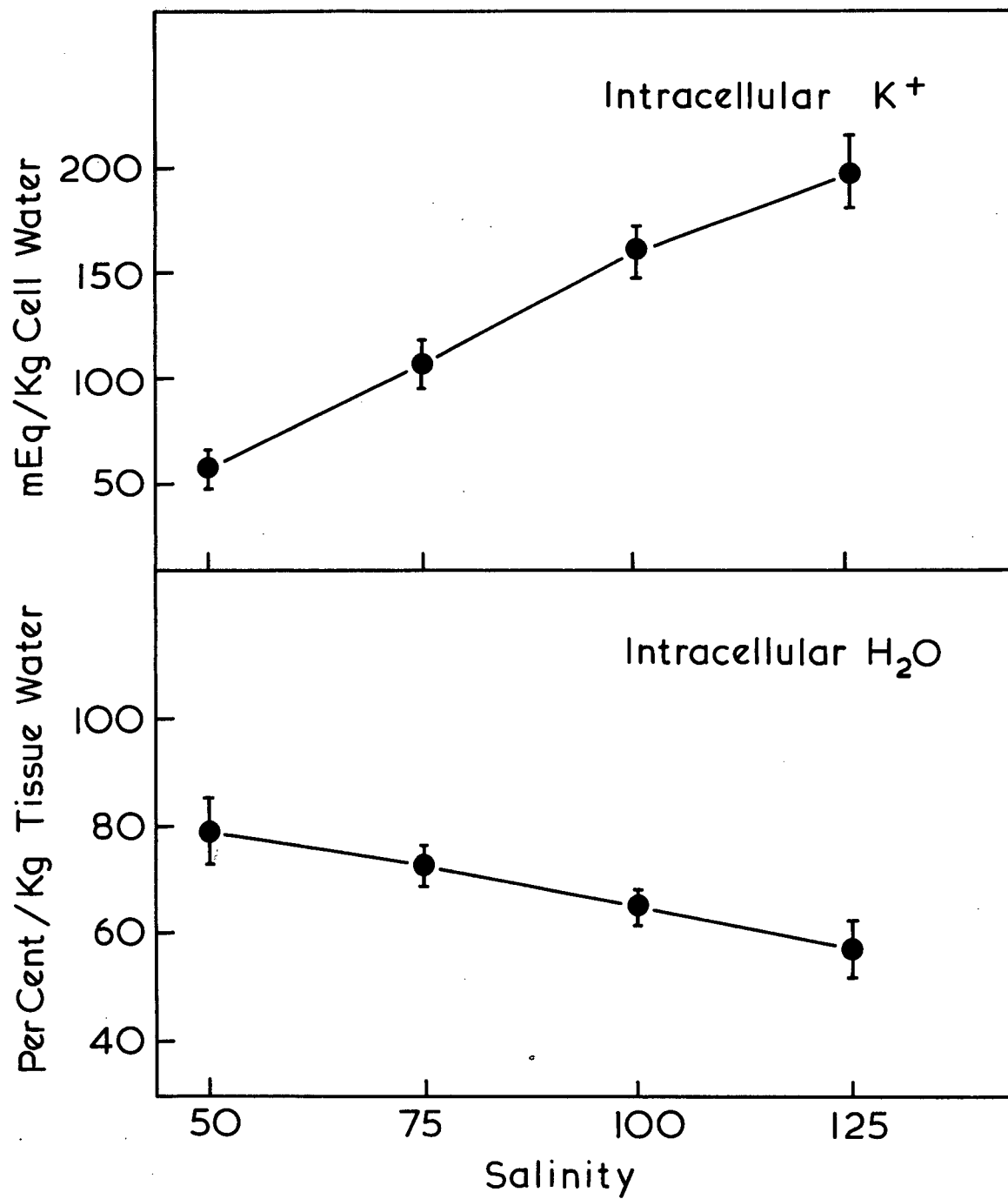
for intracellular Mg^{++} at all salinities were significantly greater than zero.

II Water Balance of Acmaea scutum

Data for field samples from the constant salinity environment of Jordan River showed that the distribution of a given ion in the foot muscle was significantly variable over an 18 month period. The water content of whole animal and muscle tissue was measured for field samples from Jordan River to determine if variation similar to that of muscle ions occured.

Significant changes in the ion content of muscle tissue and muscle cells also resulted from changes in experimental salinity. To determine if water content showed similar changes, the effect of change in experimental salinity on water content of muscle tissue and muscle cells was documented. As well, the effect of change in salinity on whole animal water content was studied.

Water Content of Whole Animals

Jordan River field samples: The mean water content was determined from 10 A. scutum collected approximately monthly from June 1964 through December 1965, and placed in field sea water for 24 hr under laboratory conditions. To establish the relationship between percentage water and size of animal,

the water content of soft body parts was regressed on dry

weight for each field sample of 10 animals. The resulting

slopes were tested for homogeneity by a procedure outlined

in Steele and Torrie (1960, p 319). The differences in

slopes between field samples were not significant. The data

for water content of A. scutum from each field sample were

then pooled and the common slope calculated. The resulting

equation was Y=0.30 + 3.42X. Using this slope, the water

content of animals of different weight was calculated. A

limpet with dry weight of 0.20 g would have a water content

of 0.98 g, while a limpet of dry weight 0.50 g would have a

water content of 2.01 g. When these data were converted to

percentages, an animal with dry weight of 0.02 g would have

had a water content of 83.1%, whereas a limpet with dry

weight of 0.50 g would have had a water content of 80.1%.

Thus, the soft body parts of large limpets had relatively

less water than small limpets. Since no effort was made at

each sample collection to take animals of the same weight the

possibility existed that differences in percentage water were

due only to differences in size of animals collected. To

eliminate variation in water content due to differences in

dry weight of sample means, an analysis of covariance was

applied to field water data. Homogeneity of variance between

samples was assumed. As mentioned above, sample slopes were

found to be homogeneous. The analysis of covariance indicated significant differences in water content between treatment means. Figure 8, part A, shows the absolute water content of whole animal adjusted to the mean dry weight of samples (0.570 g). Confidence intervals, (p=0.01), plotted around each mean, show that over the period of time in which field samples were collected total water content of A. scutum which varied from 77.5 to 82.0 showed significant differences.

The per cent water of muscle for samples of 10 animals from field samples is also plotted in part B of Figure 8. The similarity of the curves of muscle water content and whole animal water content indicates that the possibility of relative changes in amounts of various tissues through the time of the experiment (i.e. visceral gland or gonad) was not the primary factor accounting for the variation with time of the whole animal water content.

Response of whole animal water content to experimental salinities: A. scutum for total water determination were collected from Whytecliff Park in May 1963 and from Jordan River in July 1963. Ten animals from each collecting area were placed in each of 50, 75, and 125% sea water for time intervals of 1.5, 3, 6, 12, and 48 hr. In addition, samples from Jordan River were placed in the three salinities for a time period of one week. Experiments on the effect of long

FIGURE 8

Water content of whole animal and of foot muscle tissue
of Jordan River field samples collected from July 1964
through December 1965. In part A, the water content of
whole animals has been adjusted to the mean dry weight
of 0.570 g by analysis of covariance. Vertical lines
represent confidence intervals (p=0.01). In part B, total
body water for animals with dry weight of 0.570 g is
expressed as a percentage. Part B also shows unadjusted
percentage values of muscle water.
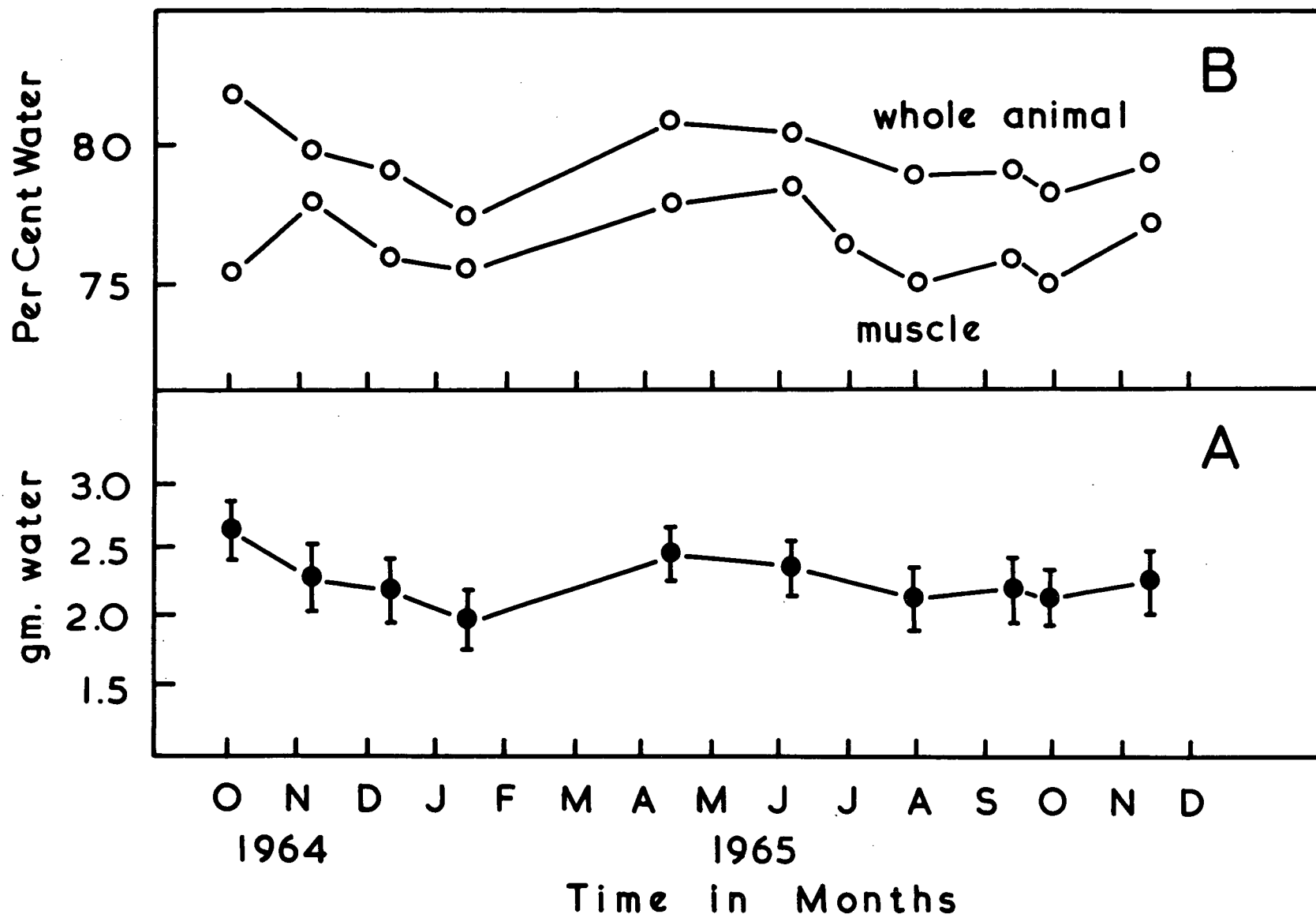
term immersion in 50 and 125% sea water on total body water were also conducted. Animals were collected from Whytecliff Park in June 1963 and maintained in 50% sea water with periodic sampling, for a total of 85 days. A similar experiment in which animals from Whytecliff were collected in November 1963 and maintained in 50 and 125% sea water with periodic sampling for a total of 100 days was also performed. It has been established for A. scutum from Jordan River field samples that, as the size of the animal increased, the relative water content decreased. Therefore, the water content data from experimental salinities were also adjusted by analysis of covariance. Jordan River and Whytecliff data were analysed separately. As well, it was assumed that slopes for a given salinity were homogeneous, thus data for 50, 75, and 125% sea water were analysed separately. For comparison of treatment means between different salinities and habitiats, means of water content were expressed using a common dry weight for all salinities and both habitats. Confidence intervals (p=0.01) were applied to the treatment means adjusted to the common dry weight for all experiments.

Change in water content over one week immersion in experimental salinities: Figure 9 shows the absolute and per cent water content for A. scutum with a dry weight of 0.430 g from Jordan River and Whytecliff. It is evident that limpets

FIGURE 9

Water content of soft body parts for $\underline{A}$. $\underline{scutum}$ from a
marine (Jordan River) and estuarine (Whytecliff) environ-
ment. In parts A means of water content have been ad-
justed to the average dry weight of 0.430 g. Vertical
lines represent confidence intervals (p=0.01). In
parts B, water content of animals with dry weight of
0.430 g, is expressed as a percentage.
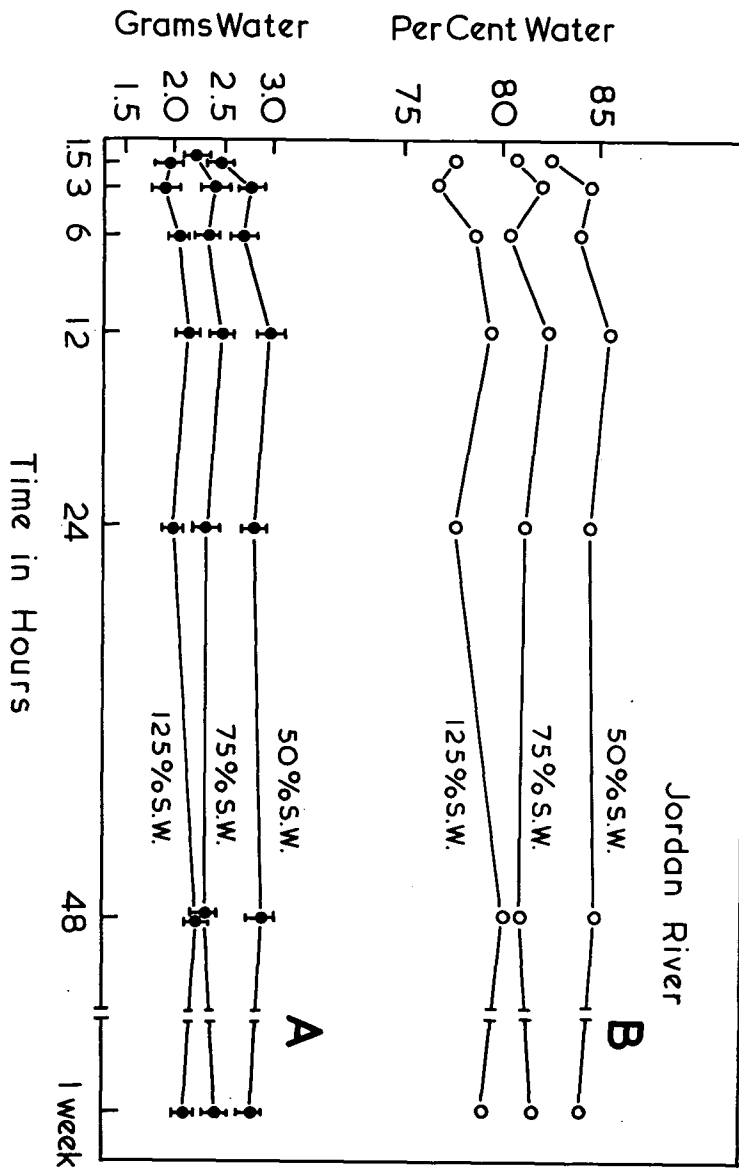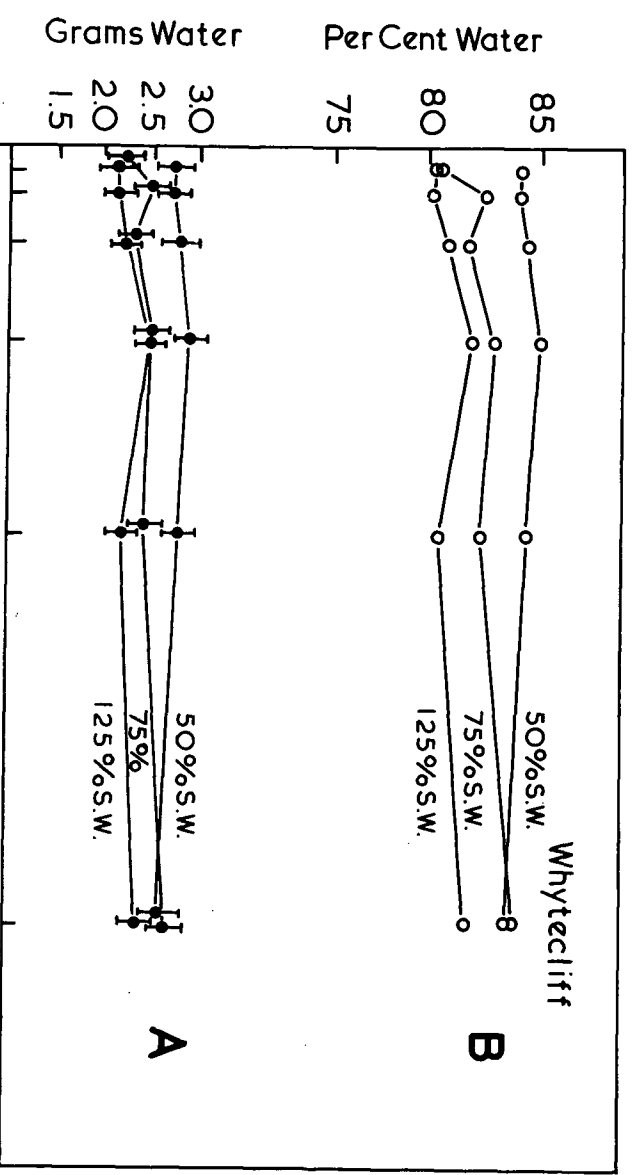
showed changes in water content according to the experimental

salinity.

Considering time periods of immersion in experimental sal-

inities up to 24 hr for both estuarine (Whytecliff) and marine

(Jordan River) animals, the water content of limpets in 125%

sea water was significantly lower than the water content of

animals in 50% sea water. The water content of A. scutum in

the intermediate salinity of 75% sea water for time periods

up to 24 hr showed a different pattern for Jordan River and

Whytecliff animals. For Jordan River animals values of water

content at 75% sea water from 3 to 24 hr were significantly

different from values of water content for animals from 50

and 125% sea water. For Whytecliff animals at time periods

up to 24 hr, however, the values of water content at 75% sea

water were not significantly different from water content

values of animals in 125% sea water except at 3 hr.

Water content values for time periods of immersion in

experimental salinities for time periods from 24 hr to one

week are also shown in Figure 9. Whytecliff limpets showed

a different response from Jordan River animals. At 48 hr

there was no significant difference in water content for

Whytecliff animals at 50, 75, and 125% sea water. For Jordan

River animals at 48 hr, however, water content at 50% sea

water was significantly different from the water content of

animals at 125% sea water. Water content of animals in 75%

sea water at 48 hr differed significantly from that of 50%

sea water but not from that at 125% sea water.

Water content values for Jordan River animals at one week

are also given in Figure 9. Values for 50, 75, and 125% sea

water were all significantly different.

Effect of extended holding periods in 50 and 125% sea water:

Values of total water content of Whytecliff animals in 50 and

125% sea water for time periods from one week to 100 days are

given in Figure 10. For animals collected in the winter,

water content values at one week, and subsequent sampling

times up to 100 days, at 50 and 125% sea water, were not sig-

nificantly different. For animals collected in the summer

(Fig 10), and placed in 125% sea water, there was no signif-

icant change in water content from 7 to 85 days.

Effect of an Estuarine Environment on Water Content of A.

scutum

To determine if A. scutum from the estuarine environment

of Whytecliff Park regulated total water content in naturally

changing salinities, the following experiment was performed.

Ten animals from Whytecliff Park were collected every two

weeks from November 1963 to July 1964. A sample of the surf-

ace water was taken at the time of collection and the animals

maintained for 24 hr under laboratory conditions in this

FIGURE 10

Effect of prolonged immersion in 50 and 125% sea water
on water content of soft body parts for A. scutum from
an estuarine environment (Whytecliff). In parts A,
water content, adjusted to the average dry weight of
0.430 g, has p=0.01 confidence intervals (vertical lines).
In parts B, water content of animals with dry weight
of 0.430 g is expressed as a percentage.
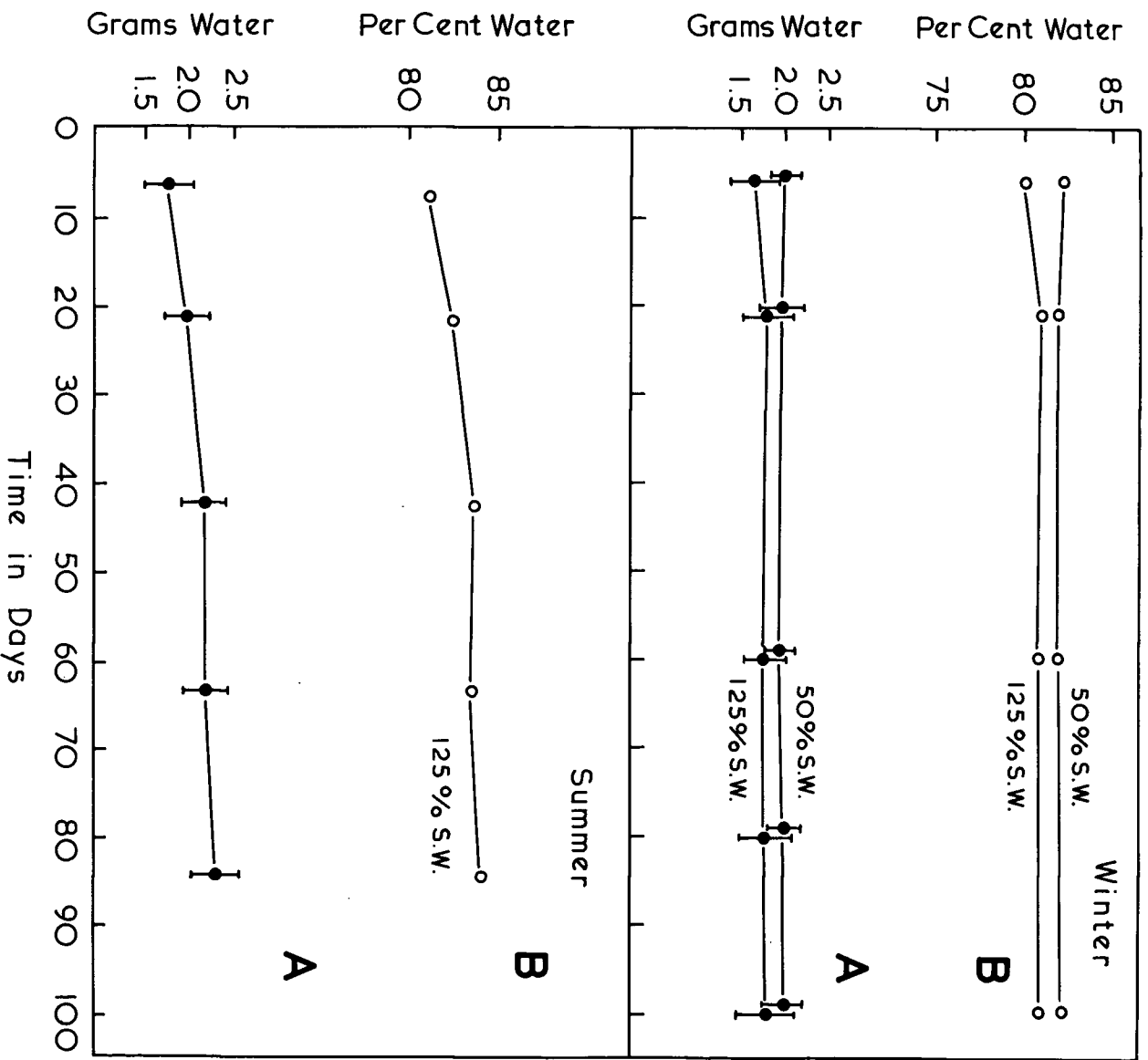
field sea water. Values of total water content, expressed

as a percentage, along with salinities of the field sea water

are shown in Figure 11. It is evident that, as environmental

salinity decreased, the water content of the animal increased.

To test the significance of this relationship, per cent water

for each animal was regressed on the respective field salinity.

The b value, -0.156, was tested for significance by analysis

of variance of regression. The probability obtained, $p < 0.005$,

indicated that water content and environmental salinity showed

a significant linear relationship over a range of salinities

from 18.8 to 88.8% sea water, thus demonstrating a lack of

volume control for field samples from an estuarine environ-

ment.

<u>Muscle Water Values</u>

<u>Jordan River field samples</u>: The concentration of water

in the foot muscle of <u>A</u>. <u>scutum</u> collected from Jordan River

from June 1963 to December 1964 is shown in Figure 5. Tukey's

w, calculated for field water data, was 2.6%. It is clear

that, like muscle ion values, the muscle water content showed

significant variation with time from an environment of con-

stant salinity.

<u>Response of muscle water to experimental salinities</u>:

Muscle samples to determine the effect of change in experi-

mental salinity on water content were taken from the same

FIGURE 11

Per cent water content of field samples from an estuarine
environment (Whytecliff). Each water content point is
the mean of determinations on 10 animals. The field
  salinity in which each sample was immersed is also
shown.
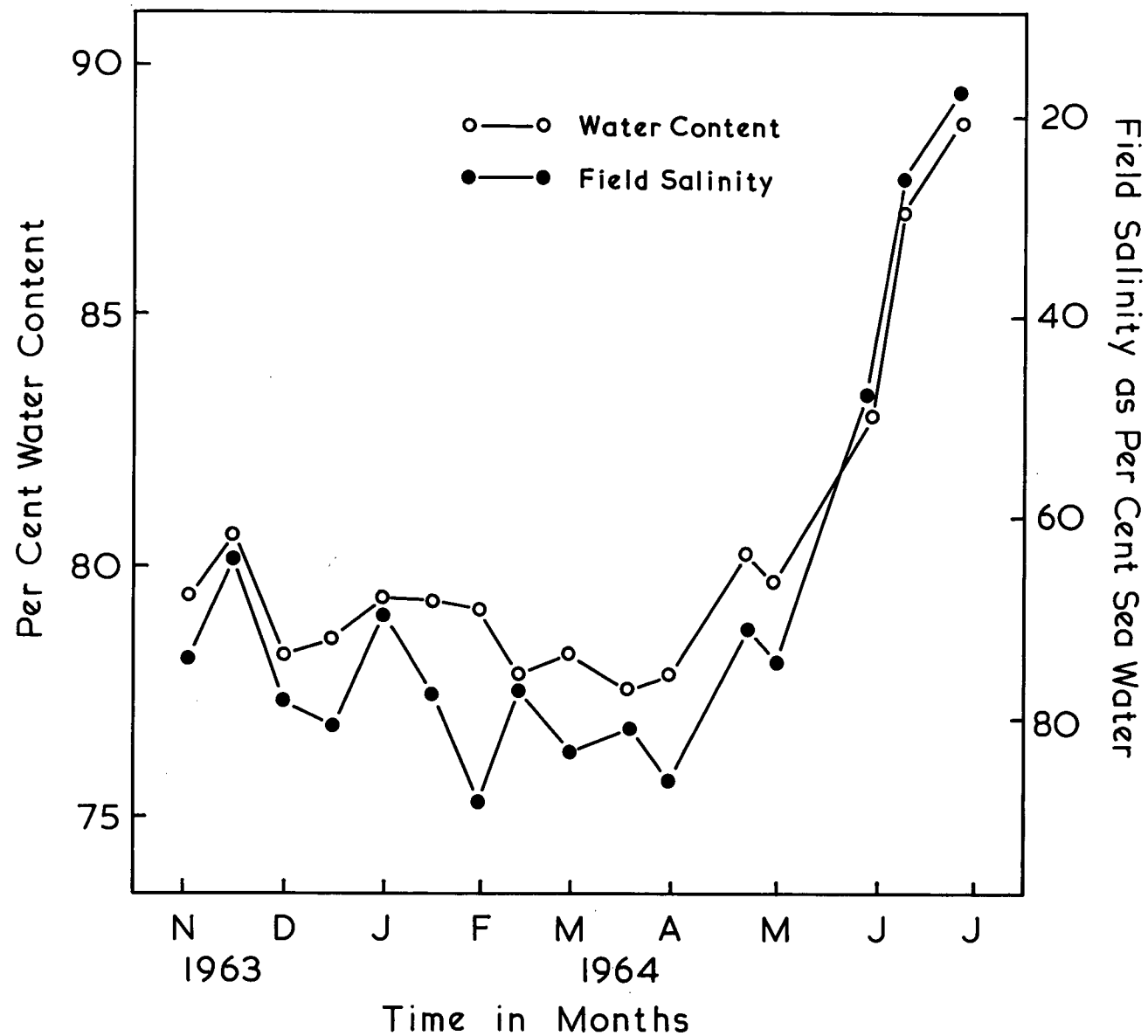
animals as muscle samples for ion analyses. Figure 6 shows

the effect of experimental salinities on muscle water data

collected from Jordan River in December 1964. Tukey's w was

2.6%. The response of muscle water (Fig 6) to changes in

external salinity was similar to the response of total body

water (Fig 9). Muscle water content increased significantly

in 50 and 75% sea water and decreased significantly in 125%

sea water. However, muscle water values were lower than

corresponding values for total body water.

Comparing muscle water concentrations at the experimental

salinities, all means at 125% sea water were significantly

different from means at 50 and 75% sea water. Comparing

means at 50 and 75% sea water, only values at 24 and 48 hr

were significantly different.

Table 10 gives the muscle water content at experimental

salinities for Jordan River animals collected in July 1964.

For July and December muscle water data, Tukey's w was used

to determine values equilibrated to each salinity. Table 11

shows that the differences between muscle water values at

each experimental salinity for July and December data were

not significant.

Intracellular Water Values

The method of estimation of intracellular muscle ion

values is described above. Total muscle water content was

estimated for the same animals from which muscle ion values
were estimated. Intracellular water concentrations were
estimated using the same method as for the estimation of
intracellular ion values. Table 11 gives intracellular estim-
ate of water for salinities ranging from 50 to 125% sea water.
Like intracellular K^+, intracellular water showed significant
changes in different salinities. Values of cellular water at
50 and 75% sea water were not significantly different. Nor
were values at 100 and 125% sea water significantly different.
However, estimates of intracellular water at 50 and 75% sea
water differed significantly from estimates at 100 and 125%
sea water. Figure 7 shows that,like intracellular K^+, intra-
cellular water values appeared to show a linear response to
changes in experimental salinity.

III Characteristics of the Water Uptake Response

It has been shown above that the water content of the
whole animal changes in different experimental salinities.
A further aspect of the water balance of A. scutum is the
change in water content of the whole animal at a constant
salinity.

Increase in Volume at Constant Salinity

A. scutum, collected from Jordan River in July and Aug-
ust of 1965 were used for measurements of increases in volume

of soft body parts at a constant salinity. Samples of ten animals were used at each time period. Measurements were made at 1.5, 6, 12, 24, and 48 hr at 50% sea water; 1.5, 3, 6, 12, 24, and 48 hr at 100% sea water; and 3, 6, 12, 24, and 48 hr at 125% sea water. To test if, at each salinity, the starting volume of animals had the same water content relative to dry weight, the water content of the soft body parts was regressed on the dry weight of animals at each salinity. The regression slopes, with the probabilities of significance, are given in Table 12. The significant slopes indicate that at each salinity there was a linear relationship between water content of the initial volume and the dry weight of animals.

Each experimental salinity contained 0.025 g/l of the dye amaranth. To determine if the presence of the dye affected water uptake, data from experiments on A. scutum collected from Jordan River in December 1964 were used. The results of t-tests on increase in volume in salinities with and without dye showed that the presence of amaranth in experimental salinities did not significantly alter the ability of animals to increase the volume of soft body parts.

Table 13 gives the results of the increase in water content in 50, 100, and 125% sea water for A. scutum collected from Jordan River in July and August 1965. Part A shows that A. scutum increased the volume of soft body parts at a

TABLE 12

Regression equations of initial water content plotted against
dry weight for A. scutum used in experiments on uptake of
water at a constant salinity. P values indicate the signif-
icance of the regression slopes.

Salinity	Regression equation	P
50	Y = 1.36 + 5.32X	.01
100	Y = 0.81 + 4.23X	.01
125	Y = 0.68 + 4.50X	.01

TABLE 13

Increase in volume of soft body parts of _A_. _scutum_ in experimental salinities after animals had been immersed for 24 hr. Part A is the number of animals of a sample of 10 showing an increase in soft body parts of $\geq 5\%$. Part B is the mean increase in volume (per cent) for those animals showing an increase of $\geq 5\%$. Part C is the blood concentration of amaranth (mg/l) in the blood after the increase. Experimental salinities contained amaranth at a concentration of 0.025 mg/l.

	Part A						Part B					
Sal	number of animals out of 10 showing increase in volume						means of increase in volume (per cent)					
	Time in hours						Time in hours					
	1.5	3	6	12	24	48	1.5	3	6	12	24	48
50	3	–	7	10	10	10	9.7	–	23.3	40.7	46.3	54.1
100	7	9	10	10	10	10	70.7	71.7	86.2	98.2	100.3	100.5
125	–	9	10	9	10	10	–	72.8	77.8	83.4	120.0	100.3

Part C

Blood concentration of amaranth

		Time in hours					
Salinity		1.5	3	6	12	24	48
50	dye conc.	.011	–	.007	.009	.006	.007
	n	1		4	7	6	7
100	dye conc.	.013	.012	.012	.011	.014	.010
	n	4	7	7	8	7	9
125	dye conc.	–	.012	.011	.010	.011	.010
	n		7	10	7	8	7

constant salinity. Part B gives the average percentage increase in volume at each time period for those animals that showed an increase of $\geq 5\%$. The results indicated that for 100 and 125% sea water, the time at which all animals showed an increase, and the values of the per cent increase were similar. However, at 50% sea water the time at which all animals showed an increase was longer than at 100 and 125% sea water, and the magnitude of the increase was one half that of the value for 100 and 125% sea water.

The variation in per cent increase at a given time and salinity was great. For example, at 24 hr the per cent increase ranged from 44.6 to 157.3 for 100% sea water, from 67.4 to 184.8 for 125% sea water, and from 8.5 to 77.3 at 50% sea water. This large variation indicated that only large observed differences could be considered significant. The primary purpose of this experiment was to demonstrate that A, scutum could show an increase in water content of soft body parts when held at a constant salinity.

Nature of the Water Uptake Response

Salt concentration of water taken up: For limpets equilibrated to a given salinity, blood samples, taken from animals that were not at maximum volume, had the same ionic values as samples taken from animals that were at maximum volume. Since the blood concentration of ions remained constant with

the increase in volume of soft body parts, this increase must

have been achieved by taking up both the ions and the water

from a given salinity.

Uptake of amaranth: It is evident then, that the water

uptake response involved the passage of sea water from the

external environment into the blood space. To test if A.

scutum could take into the blood space molecules with a larger

size than the ions of sea water, the dye amaranth and the

carbohydrate inulin were dissolved in experimental salinities.

When A. scutum showed an increase in volume, it was evident

that dye from the experimental salinity entered the blood

system of the animal. Part C of Table 13 gives the average

dye concentration of the blood at each salinity and time

period. To ascertain if the concentration of dye in the blood

increased linearly with the increase in volume of an animal,

the blood concentration of amaranth was regressed on per cent

volume increase for 50, 100, and 125% sea water. The slopes:

50%-0.000022, NS; 100%-0.000055, NS; and 125%-0.000003, NS

showed that for each salinity there was no linear relation

between the per cent water taken up and the blood concentration

of amaranth. An inspection of Table 13 however, indicates an

overall relation between blood concentration of amaranth and

increase in volume. At 100 and 125% sea water, the soft body

parts increased by 100%, indicating the volume of water taken

up was equal to the starting volume of the animals. The dye concentration of blood at 100 and 125% sea water was approximately one half that of the experimental sea water (0.025g/l). For 50% sea water, the per cent increase was approximately 50% of the starting volume. The dye concentration of animals showing a 50% increase in volume was approximately one fourth that of the experimental sea water. For 50, 100, and 125% sea water then, it appears that the increase in water content of soft body parts was facilitated by taking in a volume of water as well as the dye amaranth. The resulting blood concentration of dye was due to the diluting effect of the water content of the starting volume. It was not possible to analyse more critically the relationship between water and dye uptake, because the intracellular concentration of amaranth was not known.

Uptake of inulin: It has been established that A. scutum can increase the volume of soft body parts at a given salinity by uptake of the experimental sea water. As well, animals take up the dye amaranth in an approximate proportion to the amount of water taken up. To determine if this limpet could also take up the molecule inulin from experimental salinities the following experiment was performed. The starting volumes of 30 A. scutum from 100% sea water were measured and the animals placed in 100% sea water with inulin concentrations

of 4 g/l. Ten animals were removed after 6 hr and the volume and blood inulin concentrations determined. The remaining 20 animals were removed after 24 hr and the volume and blood inulin concentration measured. As well, 10 animals were treated in the same way in each of 50 and 125% sea water, the time period of immersion being 24 hr. The results of this experiment are given in Table 14. In both 50 and 125% sea water the increase in volume due to uptake of sea water resulted in a blood inulin concentration that was greater than one half the concentration of inulin in the experimental salintiy. In 100% sea water, after an increase in volume of just over 100%, the blood concentration of inulin was around 2.8 g/l compared with 4 g/l in the experimental salinity. At 100% sea water, if the starting volume was considered as a water space, the doubling of the volume by uptake of water and 4 g/l inulin would result in a final blood inulin concentration of 2.0 g/l. However, since the inulin probably did not penetrate intra-cellularly, it would be expected (and confirmed in Table 14) that dilution by body fluids would result in a blood inulin concentration of greater than one half that of sea water.

As with the amaranth data, the concentration of blood inulin was regressed on the percentage increase for animals in 100% sea water. The resulting slope, b=0.0015, was not significantly different from zero.

TABLE 14

Blood inulin concentration (g/l) after A. scutum had shown an increase in volume of soft body parts by taking up sea water from the external salinity. Each experimental salinity contained an inulin concentration of 4 g/l.

salinity	% increase	n	inulin conc.	n
50	57.7	10	2.40	10
100	113.8	30	2.85	29
125	74.1	10	2.97	10

Effect of ligation of the head:To determine if the source

of water uptake in A. scutum was from the mouth area, the

starting volume of animals from 100% sea water was recorded,

and the neck (posterior to the tentacles), was ligated with

linen thread. Ten ligated animals were placed in 100% sea

water for 24 hr and the volume recorded. The average increase

in volume of the 10 animals was only 0.22 ml which corresponded

to an increase of 10%. The procedure was repeated on a second

10 animals and the average volume change after 6 hr was 0.19

ml (an increase in volume of <10%). The ligations of these

animals were then removed and the animals were left a further

24 hr in 100% sea water. There was no change in volume of

these animals after the ligations were removed for the 24 hr

period. Ligation of the head then abolished the water uptake

response. As well, animals that had the ligation removed

were also unable to demonstrate a water uptake response.

Since it appeared that the water might be taken up around

the mouth area, histological cross sections of the head, cut

at between 10 and 15 μ were examined. No pores or channels

into the blood space through which the large quantities of

water involved in the water uptake response could pass were

observed. It is proposed that the sea water is taken up by

the gut or radula sac and then passed into blood spaces. How-

ever, no direct evidence is available to support this hypothesis.

DISCUSSION

Ionic Regulation of Blood Ions

Ionic regulation of body fluids has been defined by Robertson (1949) as "...the maintenance in a body fluid of concentrations of ions differing from those of a passive equilibrium with the external medium...". Thus, an analysis of a blood sample is compared with a second that has been dialysed against the experimental salinity in which the animal was immersed. Differences between the dialysed and undialysed blood sample represent a gradient due to ionic regulation. Differences between the dialysate and sea water represent a passive equilibrium due to Donnan equilibrium. With the blood of $\underline{A}$. $\underline{scutum}$, dialysis experiments showed no concentration differences due to Donnan equilibrium; thus, gradients described between blood and sea water were considered to be ionic regulation.

Field samples: The data on the blood concentration of Na^+, Cl^-, Ca^{++}, and Mg^{++} of $\underline{Acmaea}$ $\underline{scutum}$ from an environment with constant salinity of 100% sea water showed that there was no ionic regulation of these ions. These results are similar to data presented for other Gastropod molluscs by Robertson (1964), except that the four species described by Robertson showed slight regulation of one or more of the ions

Na^+, Ca^{++}, or Mg^{++}.

Blood K^+ of A. scutum, however, showed a small but significant concentration gradient between blood and sea water. This ionic regulation of blood K^+ agrees with findings on other Gastropod molluscs. Robertson (1964) showed blood-sea water ratios for K^+ ranging from 1.14 for the Prosobranch Neptunea antiqua to 1.42 for the Prosobranch Buccinum undatum. The value obtained for A. scutum from 100% sea water was 1.17.

The degree of ionic regulation of A. scutum was similar to other Gastropod molluscs in that K^+ was the primary ion to be regulated. A. scutum is, however, lowest on the scale of ability to regulate ionically in Gastropods studied thus far in that there was no regulation of Na^+, Cl^-, Ca^{++}, or Mg^{++}.

Effect of change in experimental salinity: The response of blood ions of A. scutum to changes in external salinity was similar to the response of total osmotic pressure of Acmaea limatula as described by Segal and Dehnel (1962). Over a range of 25 to 150% sea water, A. limatula showed no regulation of the total osmotic pressure of the blood. In this study A. scutum showed no regulation of the ions Na^+, Cl^-, or Mg^{++} over a range of 50 to 125% sea water. This lack of ionic regulation in experimental salinities was found for animals from both an environment of constant 100% sea water and an estuarine environment.

Blood K^+ values however, were greater than sea water K^+ values over a range of salinities from 50 to 125% sea water. The greatest concentration gradient between blood and experimental salinity was found for limpets in 50% sea water. This increase in gradient of blood K^+ from 100 to 50% sea water agrees with results for blood K^+ of _Mytilus edulis_. Potts (1954) found the K^+ blood-sea water gradient to be 1.18 in 100% sea water (_A_. _scutum_ was 1.17 in 100% sea water). In 50% sea water the K^+ blood-sea water ratio for _Mytilus_, calculated from Potts (1954), was 1.42 compared with 1.87 and 1.61 for _A_. _scutum_ in 50% sea water.

Ion Concentrations of Urine

_Na_$^+$, _Cl_$^-$, _Ca_$^{++}$, _and Mg_$^{++}$: Since the blood concentration of Na^+, Cl^-, Ca^{++}, and Mg^{++} did not differ from sea water over a range of salinities from 50 to 125% sea water, it would be expected that the concentration of these ions in urine would be the same as the blood concentration. The results of urine analysis bear this out; in experimental salinities of 50, 75, and 125% sea water, the equilibrated urine and blood concentrations of Na^+, Cl^-, Ca^{++}, and Mg^{++} were the same.

_K_$^+$: The concentration gradient of K^+ observed between blood and sea water was abolished between urine and sea water. That is, the concentration of urine K^+ was the same as the sea

water K^+ concentration. It appears that there is a reabsorp-

tion of K^+ ions by the kidney.

The only other marine molluscs on which urine analysis

data are available are the Cephalopods. Data for the squid,

Sepia officinalis, and the octopus, Eledone cirrosa, are

summarized by Robertson (1964, p 290). Cephalopods showed a

much greater degree of ionic regulation of body fluids com-

pared with marine Gastropods therefore comparison of renal

function in ionic regulation must be made cautiously. How-

ever, like both Sepia and Eledone, the kidney of Acmaea

appeared to reabsorb K^+.

Urine-blood gradient during equilibrium: A. scutum placed

from 100% to 50% sea water showed a urine-blood concentration

gradient for Na^+ and Cl^- up to 24 hr immersion. For both

ions the urine concentration was greater than the blood con-

centration. These data may be interpreted in at least two

ways. When the limpets were placed in lowered salinities,

the kidney may have secreted Na^+ and Cl^- to promote the

equilibration of the blood with the external salinity. Or,

the concentration difference may have been due to a time lag

in the formation of the urine, that is, the urine at sampling

may have been formed at a previous time when the blood con-

centration was greater. The supporting data for these state-

ments, however, are not conclusive. This urine-blood gradient

can only be offered as an observation requiring more conclus-

ive data.

Muscle Ions

It has been established that there was no regulation of

Na^+, Cl^-, Ca^{++}, and Mg^{++} and only a slight regulation of K^+

in the blood of A. scutum. Any ionic gradients then, between

cells of the bounding membranes and the external sea water

were basically the same as ionic gradients between blood and

cells of the foot muscle.

Muscle tissue ion values: The sum of the concentrations

of Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++} of whole muscle tissue was

lower than the sum of the same ions of the blood at all salin-

ities tested (50, 75, 100, and 125% sea water). Specifically

Na^+, Cl^-, Ca^{++}, and Mg^{++} tissue values were much lower than

respective blood values, while, total muscle K^+ was much

higher than blood K^+. The increased muscle K^+ was offset by

the much reduced Na^+, Cl^-, and Mg^{++} concentrations which

resulted in the muscle tissue having a lower sum of ion

concentrations compared with blood.

This lower concentration of total muscle ions compared

with blood is a characteristic of all tissues, and has been

described for other molluscs by Krogh (1939), Fox (1941),

Hayes and Pelluet (1947), Potts (1958), and Robertson (1965).

The conclusion drawn from the relative differences in concentra-

tion between tissue and blood ions is that, relative to blood concentration, the intracellular concentration of Na^+, Cl^-, Ca^{++}, and Mg^{++} is low while K^+ is very high. However, quantification of intracellular ion concentration requires an estimate of the extracellular space and consequently a measure of the ionic values of this extracellular fluid.

Extracellular space of foot muscle: The extracellular volume of A. scutum foot muscle was estimated by measuring the inulin space of the muscle tissue. The percentage muscle tissue estimated to be extracellular space ranged from 17% in 50% sea water to 31% in 125% sea water. The large difference between 50 and 125% sea water indicated a significant change in cellular volume between the two salinities. Potts (1958) found the extracellular space of various muscle of Mytilus in 100% sea water to vary from 19.3% for the fast adductor to 29.5% for the byssus retractor. Comparing the byssus retractor to A. scutum foot muscle, both considered "slow" muscles, the values of extracellular space for 100% sea water were similar, 29.5 and 25.9% respectively. At 50% sea water however, the differences between byssus retractor and A. scutum foot muscle were great, 25.3% compared with 16.7%. This indicates that foot muscle cells of Acmaea scutum showed a greater volume change in 50% sea water than did byssus retractor cells of Mytilus.

The extracellular volume of muscle tissue may also be estimated using an equation assuming that all chloride is extracellular (Manery, 1954). Using this equation it was found that at 100 and 125% sea water the chloride space was similar to the inulin space. In 50 and 75% sea water however, estimates of chloride space were much higher than estimates derived from inulin space.

The discrepancy in the two estimates might be due to the following: in the case of the Manery equation the assumption that all muscle chloride is extracellular is probably incorrect. Conway (1957) and Cotlove and Hogben (1962) have provided evidence that Cl^- is distributed intracellularly in vertebrate muscles. As well, Deffner and Hafter (1960) found a Cl^- concentration in squid (_Loligo pealeii_) nerve axoplasm of 168.6 mEq/Kg water. Although extrapolation from the above findings to _Acmaea_ foot muscle must be accepted with caution, the inulin space was felt to be the better estimate of extracellular space.

Intracellular ion estimates at 100% sea water: At 100% sea water the muscle cells of _A. scutum_ contained a K^+ concentration (162 mEq/Kg cell water) that was much greater than any other ion measured. Na^+ was 46, Ca^{++} 40 and Mg^{++} 23 mEq/Kg cell water. All of these values were significantly different from zero. The intracellular Cl^- concentration

was not significantly different from zero. Compared to the blood concentration of all ions measured, cellular K^+ was much greater, Ca^{++} about the same, and Na^+, Cl^-, and Mg^{++} much lower.

Potts (1958), in measuring the cellular concentration of ions in Mytilus, found differences with the kind of muscle used. For comparison with A. scutum foot muscle, data from the byssus retractor muscle of Mytilus was used. As well, comparison was made with the mantle muscle of Sepia as described by Robertson (1956). The intracellular ion data for the three animals were similar. K^+ had the highest intracellular concentration (Mytilus, 153; Acmaea, 162; and Sepia, 189 mEq/Kg cell water). Na^+ and Cl^- concentrations were much lower than cellular K^+ values or corresponding Na^+ blood values. The cellular concentration of Mg^{++} was similar in Acmaea and Sepia and agreed with the Mg^{++} concentration of the fast adductor cells of Mytilus. However, there were some differences: the cellular Na^+ content of Mytilus byssus retractor was higher than the cellular Na^+ of Acmaea and Sepia (95 compared with 46 and 31 mEq/Kg cell water). Likewise, the muscle cells of Mytilus and Sepia contained significant concentrations of Cl^- whereas Acmaea cells did not. The cellular Ca^{++} content of Acmaea was higher than Sepia mantle muscle and the fast adductor of Mytilus. These diff-

erences were not large and did not alter the fact that the

foot muscle cells of $\underline{A}$. $\underline{scutum}$ had ionic concentrations

similar to those found in cells of most other animals. That

is: the intracellular concentrations of Na^+, Cl^-, K^+, Ca^{++},

and Mg^{++} were much different than the fluid surrounding

the cells; the intracellular K^+ concentration was greater

than any other ion; and Na^+ and Cl$^-$ had low intracellular

concentrations.

Effect of salinity change on cell ion concentrations:

The only ions to show a consistent intracellular concentra-

tion change over a range of experimental salinities from 50

to 125% sea water were K^+ and Ca^{++}. From 100 to 50% sea

water the intracellular concentration of both ions decreased

by a factor greater than one half. As well, an increase in

external salinity from 100 to 125% sea water resulted in a

smaller proportionate increase for both ions than the

decrease in concentration noted from 100 to 75% sea water.

The cellular concentration of Na^+, Cl^-, and Mg^{++} did not

show any consistent change at 50, 75, 100, and 125% sea

water. For all salinities, cellular Na^+ and Cl$^-$ were less

than 47 mEq/Kg cell water and, in some cases, the values

were not significant from zero. Cellular Mg^{++} showed a

concentration of 18 to 23 mEq/Kg cell water over the

range of experimental salinities. Potts (1958) gives

data for the effect of 50% sea water on cellular concentra-
tions of various muscle tissues of <u>Mytilus</u>. He found that
cellular K^+ of the fast and slow adductors and the byssus
retractor muscle showed a decrease in concentration of less
than one half from 100 to 50% sea water. For example, the
byssus retractor muscle cells had a K^+ concentration of 153
mEq/Kg cell water in 100% sea water and 112 mEq/Kg cell water
in 50% sea water. On the other hand, the cellular concentra-
tions of Na^+ and Cl^- of <u>Mytilus</u> muscle cells showed a decrease
from 100 to 50% sea water that was greater than one half.
Although differences existed in the response of intracellular
ions to changes in external salinity for <u>Acmaea</u> and <u>Mytilus</u>
muscle it is evident that increased and decreased experimental
salinities resulted in changes in cellular concentrations of
ions.

If cellular K^+ were in ionic equilibrium with nondiffus-
ible organic ions, and Na^+ were extruded by active processes,
then it might be expected that intracellular K^+ and extra-
cellular Cl^- would be in Donnan equilibrium. That is,
$\frac{(K_i^+)}{(K_o^+)} = \frac{(Cl_o^-)}{(Cl_i^-)} = r$. The K^+ and Cl^- ratios for each salinity
are given in Table 15. The variance of each ratio was calcu-
lated as shown in Yates (1960, p 198), and the p=0.01 confi-
dence intervals, expressed as ±3 standard deviation units,

TABLE 15

Ratios of concentration of K^+ and Cl^- inside and outside fibers of $\underline{A}.$ $\underline{scutum}$ foot muscle.

salinity	$\dfrac{K_i^+}{K_o^+}$	$\dfrac{Cl_o^-}{Cl_i^-}$
50	6.2 ±1.4	9.1 ±10.8
75	9.9 ±2.1	7.9 ±19.8
100	13.7 ±2.6	17.2 ±26.7
125	11.7 ±1.7	20.0 ±38.7

are also given. The values for the K^+ and Cl^- ratios at each
salinity were quite different indicating that the distribution
of these two ions did not fit a Donnan equilibrium. However,
the large variation in the Cl^- ratios expressed by the confi-
dence intervals did not permit any conclusions. For other
molluscs, (Sepia and Eledone, Robertson, 1956; and Mytilus,
Potts, 1958), the K^+ and Cl^- values of muscle tissue were not
in Donnan equilibrium.

Water Balance

Permeability to water and salts: Changes in external
salinity resulted in significant changes in water content of
whole animal, muscle tissue, and muscle cell water. Decreases
in experimental salinity resulted in increases in tissue water
content. The question then arises: are changes in water
content due to passage of water alone across membranes or,
instead, by passage of water and ions? Data from many invert-
ebrate animals indicate that both ions and water are exchanged
when animals are exposed to changes in salinity (Prosser and
Brown, 1961; Potts and Parry, 1964). Many workers have
proposed formulae in order to estimate the contribution that
ions play in equilibration of water content to new salinities
(Hukuda, 1932; Adolph, 1937; Gross, 1954; and Karandeeva,
1965). However, these formulae are but indirect estimations
of the role ions play in permeability studies and do not

impart information that is as useful as that obtained by measuring ion fluxes with radioisotopes (as in the work done with Na^+ on Mytilus edulis, Potts, 1959). No data on ion fluxes are available for A. scutum; however, it is evident that this animal is similar to other animals studied in that membranes are permeable to salt ions as well as water. Estimates of intracellular water showed that from 100 to 50% sea water there was an increase in cell water from 65.5 to 79.3%. If the cell was semipermeable and allowed only water to pass through the membrane the effect of decreasing the salinity by one half would result in the cell water space doubling and in a cell water volume of greater than 100%.

Volume regulation: Even though it is evident that muscle cell membranes of A. scutum were permeable to salts as well as water, little volume regulation of cellular or whole animal water existed. As mentioned, the intracellular water content showed a large change in hydration with change in salinity ranging from 57.7% in 125, 65.5% in 100, and 79.3% in 50% sea water. The difference in per cent cell water between 100 and 50% sea water for A. scutum was much greater than that found in Mytilus edulis - 70.5% cell water in 100 and 74.7% cell water in 50% sea water (calculated from Potts, 1958).

Whole animal water content also showed little regulation

in experimental salinities at time periods of immersion of less than 24 hr. Between 1.5 to 3 hr immersion, water content increased in 50 and 75% sea water and decreased in 125% sea water. The values of water content in these salinities remained relatively constant between 3 and 24 hr immersion. Similar results have been found for the Gastropods Onchidium (Dakin and Edmonds, 1931) and Aplysia (van Weel, 1957).

Field data for A. scutum also indicate very little, if any, volume control. Animals, collected periodically from a brackish water environment with salinity ranging from 89 to 19% sea water (Fig 8), showed a linear relationship between total body water and environmental salinity. Thus, over a wide range of salinities, water content linearly increased with decrease in salinity indicating no volume control.

Data on total water content for immersion of A. scutum in experimental salinities for periods greater than 24 hr were not entirely consistant with the idea of lack of volume control. For animals from a marine environment (100% salinity) there was no indication of return to base line water content at one week immersion (Fig 6). However, for animals collected from the estuarine environment, the water content for 50 and 125% sea water showed a tendency to return to base line values by 48 hr (Fig 6). Over a period from 2 to 100 days, water content of estuarine animals at 50 and 125% sea water was not

significantly different. It is possible that, in prolonged exposure to salinity stress, animals from the estuarine habitat are able to regulate volume to some degree.

In considering the response of total water content to salinity stress, a further difference between estuarine and marine animals is found by comparing the magnitude of volume change for 50 and 125% sea water for time periods up to 48 hr immersion (Fig 6). The difference between 50 and 125% sea water for estuarine animals was approximately 5% whereas for marine animals the difference was around 8%. Again it is possible that estuarine animals were better adapted in responding to changes in environmental salinity.

Seasonal Variation

Data on total muscle Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++} as well as whole animal and muscle water content, collected over a year's period from an environment of constant 100% sea water, showed that the concentration of these ions varied significantly with time. Results also showed that the variation in whole water content was not due to relative changes in the amount of different tissues with season, or simply to changes in water content of muscle. Seasonal measurements of extracellular space were not made, so it cannot be shown conclusively whether the variation was due to changes in intracellular ion and water concentrations or changes in the extracellular space.

Indirect evidence is available on the nature of the seasonal variation. Measurements of total muscle K^+ is a reasonable estimate of the intracellular concentration of this ion. This is due to the small contribution made to the total muscle K^+ by the blood component. The curve of muscle K^+ (Fig 9) for seasonal measurements showed a much smaller variation than Na^+, Cl^-, or H_2O. This indicated a fairly stable intracellular concentration of at least K^+. The probable source of variation was then the change with season of the extracellular space. Although it has not been documented in Acmaea, it is probable that the foot muscle acts as a site of storage of metabolic products. Giese and Araki (1962) and Tucker and Giese (1962) have shown that the foot tissue of the molluscs Katherina, Mopalia, and Cryptochiton had considerable quantities of lipid, protein, and glycogen. In these animals there is some variation seasonally in the concentration of these products although correlation of variation in concentration with breeding condition was not definitive. It is possible that in A. scutum the seasonal variation of total muscle ions and water is due to changing levels of stored metabolic products resulting in changes of extracellular space.

Water Uptake Response

Implication to hydrostatic skeleton and foot expansion: A

hydrostatic skeleton has been defined by Chapman (1958) as

"...a fluid mechanism which in one way or another provides a

means by which contractile elements can be antagonized."

Hydrostatic skeletons in Eulamellibranch and Gastropod molluscs

are of particular importance. As well as maintaining body

form the hydrostatic skeleton in burrowing Eulamellibranchs

functions in the expansion of the foot and siphons, and in

many Gastropods is used for expansion and retraction of the

foot from the shell. In both these groups of molluscs, the

development of concepts of the function of the hydrostatic

skeleton has raised a controversy over the question of whether

these molluscs were able to take up sea water into the blood

space in order to facilitate the expansion of the foot. A

brief history of this controversy is supplied by Morris (1950).

At the present time it is accepted that Eulamellibranchs

have sufficient fluid in the circulatory system to account

for movement of the foot and siphons (Truemen, 1954; and

Chapman and Newell, 1956). Until the present, it was also

believed that all Gastropods, with the exception of the

Naticidae, contained sufficient fluid in the circulatory

system to fully extend the foot (Morris, 1950; Chapman, 1958;

Brown and Turner, 1962; and Brown, 1964). Within the Naticidae

Morris (1950), working on the moon snail _Uber_ (Polinices)

strangei, presented data which indicated that the expansion

of the foot was due in part to uptake of sea water into aquiferous cavities in the foot. The mechanism of water uptake into the aquiferous ducts was not known. As well, although it was believed the aquiferous ducts were separate from the circulatory system, the evidence was not definitive.

There must now be added to the literature a definitive case of a Gastropod that can take up sea water into the circulatory system from the external environment. Evidence provided in this study demonstrates conclusively that Acmaea scutum was capable of taking into the circulatory system large quantities of sea water. An A. scutum that had excess water removed by gentle pressure could, between 1.5 and 6 hr, take up a volume of sea water approximately equal to the starting volume of the soft body parts of the animal.

Although no data are available it is probable that the large change in body fluid in A. scutum has a pronounced effect on the functioning of the hydrostatic skeleton. The importance of the correct volume of fluid for the proper functioning of the hydrostatic skeleton has been shown for the Polychaete Arenicola (Chapman and Newell, 1947) and for the anemone Metridium (Batham and Pantin, 1950).

Hypothesis of mechanism of water uptake response: A possible explanation of the mechanism of water uptake in Acmaea scutum can be proposed. Limpets ligated in the neck

region failed to show the water uptake response. However,
it must be noted that animals that had the ligation removed
failed to demonstrate the water uptake response. It is prob-
able that ligation destroyed either a nervous control of the
water uptake response or membrane structures involved with
the uptake. If the water uptake response was abolished by
rupture of a membrane structure then the following may occur.
Histological examination has shown that the only visible
opening in the mouth area was that which leads to the gut and
radula sac. Sea water might be pumped into the intestine and
across the gut membrane, or it might be pumped into the radula
sac and across the radula sac membrane. In this hypothesis,
entry through the radula sac is favoured for the following
reasons. The endothelium of the gut was much thicker than
the epithelium of the radula sac. Moreover, Acmaea scutum
had a blood vessel that encircled the radula sac in the neck
area. This blood vessel broke away from the radula sac post-
eriorly and ran directly to the heart. This vessel in Acmaea
scutum was similar to the radular artery that occurs in the
limpet Patella vulgata (Graham, 1964).

Based on the assumptions and data above the following
hypothesis on the mechanism of water uptake is proposed. Sea
water is pumped by the mouth and buccal mass into the radula
sac, across the radula sac membrane and into the radula art-

ery. From the radula artery the sea water is distributed to the rest of the circulatory system. It must be stressed that no direct evidence is available to prove or disprove this hypothesis.

Whatever the mechanism of the water uptake response may be, the membrane surface across which the sea water passes into the circulatory system is characterized by a large minimum pore size. Data on A. scutum showed that the molecules inulin and amaranth were taken up into the circulatory system at approximately the same rate as the water molecules and salt ions of sea water. Inulin and amaranth have an effective molecular radii of 12 $\overset{\circ}{A}$ and 7 $\overset{\circ}{A}$ respectively, sizes that are much larger than the water molecule or salt ions.

Blood Volume

The blood volume of molluscs has been studied by Prosser and Weinstein (1950) and Martin et al. (1958). Martin et al. found a great variability between species ranging from 79.3% for the Opistobranch , Aplysia, to 5.8% for the octopus, Octopus honcongenis. As well, there was a great deal of variation within species. For example, the blood volume of the chiton Cryptochiton stelleri ranged from 33.1 to 59.4% for 17 animals. This large intraspecific variation was felt by Martin et al. to be a reflection of "...the inability of these animals to regulate their blood volume with a high degree of

stability...". Although no direct measurement of blood volume has been made on <u>Acmaea</u> <u>scutum</u> it is evident that the large quantities of sea water that can be taken into the circulatory system from the external environment affect greatly the blood space of <u>Acmaea</u> <u>scutum</u>. It is also evident that this influx of sea water must affect greatly the function of the circulatory system in the transfer of metabolic products, the role in respiration, and the role in excretion.

<u>Ecological Implications of Water Uptake Response</u>

At low tide, <u>A</u>. <u>scutum</u> is often found directly exposed on rock surfaces. During this exposure it would be advantageous for the limpet to maintain under the shell a volume of water that is as large as possible. Adaptations to this end have been described for the genus <u>Acmaea</u>. Shotwell (1950) showed for a number of species of <u>Acmaea</u>, including <u>A</u>. <u>scutum</u>, that smaller animals had relatively larger shell volumes compared with larger animals. Smaller animals were found to be distributed throughout the tidal zone, but larger animals were found only in the lower tidal zone. Segal (1956), working with <u>Acmaea</u> <u>limatula</u>, also found that animals in the high tide zone had a greater water holding capacity. Segal showed that the greater volume of the high shell represents a larger extravisceral space in the animal; that is, the space of the nuchal cavity and the volume between the foot and the shell.

Segal and Dehnel (1962) gave data showing that A. limatula which had the extravisceral water removed demonstrated a more rapid concentration of total osmotic pressure of the blood when desiccated, compared with animals that had the extravisceral water intact. In Acmaea it appears that the extravisceral water acts as an osmotic buffer, and that animals showing the greatest exposure time have the largest extravisceral water.

Segal (1956) also found that A. limatula from high levels had shells that were thicker and heavier than animals with the same soft body weight from lower levels. He believed that the heavier shell played a role in reducing the desiccating effect of exposure.

Data available for A. scutum give a further aspect to the adaptation to desiccating during intertidal exposure. The ability to take sea water into the circulatory system is an important adaptation in that it results in a larger blood volume to act as an osmotic buffer.

SUMMARY

1. The values of Na^+, Cl^-, K^+, Ca^{++}, and Mg^{++} have been measured in the blood, urine, and muscle tissue of $\underline{A}$. <u>scutum</u> over a range of salinities from 50 to 125% sea water.

2. Blood ion values were determined for limpets from a marine and estuarine environment. Only K^+ showed a concentration gradient between blood and sea water. At all salinities, blood K^+ values were higher than K^+ values of sea water.

3. Urine ion values were determined for $\underline{A}$. <u>scutum</u> from a marine environment. After equilibration to experimental salinities there was no difference between urine and sea water ion values.

4. During equilibration to 50% sea water, urine values of Na^+ and Cl^- at 50% sea water for time periods of immersion up to 12 hr were higher than blood values of these ions.

5. For $\underline{A}$. <u>scutum</u> field samples collected over an 18 month period from an environment of constant salinity, muscle ion concentrations changed significantly. As well, muscle ion values changed significantly with changes in experimental salinity.

6. To determine intracellular ion values of foot muscle, data on total muscle ion concentrations and extracellular volume as represented by the inulin space was used. Extracellular space of foot muscle ranged from 16.7% in 50% sea

water to 31.0% in 125% sea water.

7. The intracellular ion values of Na^+ and Cl^- were close to zero; Ca^{++} and Mg^{++} concentrations were lower than blood concentrations and K^+ values were much higher than blood K^+ values. With changes in external salinity, intracellular K^+ concentrations changed significantly from 58.7 mEq/Kg cell water in 50% sea water to 198.2 mEq/Kg cell water in 125% sea water.

8. Like muscle ion values, water content of whole animal and muscle tissue for field samples from an environment with constant salinity showed significant concentration changes over an 18 month period.

9. Whole animal water content for A. scutum from both a marine and estuarine environment increased significantly with decrease in experimental salinity. For marine animals there was no tendency for water content of animals in experimental salinity to return to base line values in time periods of immersion up to one week. For estuarine animals, water content in experimental salinities returned to base line values by 48 hr.

10. Total body water content of field samples from an estuarine environment showed a linear response with changes in environmental salinity indicating poor volume control of water content. Total body water ranged from 89.0% at 18% sea

water to 77.0% at 82% sea water.

11. Estimates of intracellular water content of foot muscle cells of A. scutum from a marine environment showed that changes in experimental salinity resulted in significant changes in cellular water content. Cellular water content of foot muscle ranged from 57.7% at 125% sea water to 79.3% at 50% sea water.

12. As well as showing changes in total body water at experimental salinities A. scutum showed large changes in water content of soft body parts at a given constant salinity. The changes in water content at a constant salinity resulted from sea water entering the blood space from the external environment.

13. During this water uptake response A. scutum could also take the molecules inulin and amaranth into the blood space from the external sea water when these molecules were dissolved in experimental salinities.

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