J. exp. Biol. (1981), 90, 33–41
Printed in Great Britain

STUDIES ON WATER IN BARNACLE MUSCLE FIBRES
1. THE DRY WEIGHT COMPONENTS OF FRESH FIBRES

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(*Received* 8 *April* 1980)

SUMMARY

The dry weight components comprise 25 % of the total weight of fresh muscle fibres of the giant barnacle *Balanus nubilis*. Half of the solids are water insoluble; 6 % of this material is fat, and the rest is mostly myofilament protein. Less than 3 % of the dry weight consists of inorganic ions, and these account for only a quarter of the osmotic activity in the fibre water. Seventy per cent of the osmotic activity is due to nitrogenous solutes which comprise 18 % of the dry weight. Glycine predominates, followed by TMAO + betaine, proline, glutamine + asparagine, arginine, and taurine. About 13 % of the dry weight is soluble protein and not more than 10 % is an unidentified soluble substance of high molecular weight. Thus, *Balanus* conforms with other marine invertebrates in the high content of osmotically active nitrogenous solutes in its myoplasm.

INTRODUCTION

The intracellular environment of muscle fibres varies greatly in its solute content and composition, depending upon the animal's osmotic environment and ability to regulate the osmotic and ionic composition of the blood. In those euryhaline and marine species that have been found capable of tolerating internal osmotic pressures greater than about 350 milliosmoles, the cells contain relatively high concentrations of small nitrogenous solutes (Shaw, 1958; Jeuniaux, Duchâteau-Bosson & Florkin, 1961*a*, *b*; Robertson, 1961, 1975, 1976; Awapara, 1962; Bricteux-Grégoire *et al.* 1962; Clark, 1968; Lutz & Robertson, 1971; Freel, Medler & Clark, 1973; Schoffeniels, 1976). Despite this high intracellular osmotic pressure, however, the muscles of these animals are similar in their biological properties to those of species (such as most vertebrates) that have much lower osmotic pressures (Hoyle, McNeill & Selverston, 1973; Freel, 1978).

Studies on isolated enzymes suggest that the elevation of intra-cellular osmotic pressure by small nitrogenous solutes does not have the deleterious effect upon enzyme function that can be caused by osmotically equivalent amounts of univalent ions (Clark & Zounes, 1977; Bowlus & Somero, 1979). It seems reasonable that nitro-

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genous solutes might also influence the functional state of nonsoluble solids, such as the contractile proteins of muscle. To investigate this question we have studied the muscle cells of the giant barnacle *Balanus nubilis*.

In this paper we report the intracellular composition of fresh barnacle fibres. In the following paper (Clark, Hinke & Todd, 1980) we have utilized membrane-damaged fibres, in which the solute composition could be manipulated at will, to investigate the relative effects of ions and small organic solutes. Our results suggest that these organic solutes do protect the structural integrity of nonsoluble (as well as soluble) cell proteins, and that they also influence cell volume.

MATERIAL AND METHODS

Preparation of coelomic fluid and fibre samples

Specimens of *Balanus nubilis* from Dodds Narrows off the southeast coast of Vancouver Island were maintained in aerated sea water at 10 °C in the Vancouver Public Aquarium. Prior to use in experiments, animals were transferred to the laboratory aquarium, which contained artificial sea water (Instant Ocean, Aquarium Systems Inc.) at 12 °C.

Coelomic fluid samples, taken from healthy animals which had been blotted free of sea water, were withdrawn using a hypodermic needle inserted through the membrane surrounding the scutes. A subsample was diluted with five parts of 96 % ethanol for amino acid analysis. The remainder was centrifuged at low speeds to remove debris and analysed immediately for total solid content and freezing point depression, as described below. Single muscle fibres were obtained from the bundles of scutal depressors, which were dissected in standard barnacle Ringer (Gayton & Hinke, 1968).

Estimation of total solids and dissolved solids

To determine total solid content, a fibre was severed from its tendon and blotted on filter paper until no more water could be removed before measuring wet and dry weights (constant weight at 70 °C). For estimation of the dissolved solids in the total fibre water, one or two fibres were first dissected free from each bundle, blotted and their water content determined; then the remaining fibres were carefully cleaned of fat, tendons and connective tissue, severed from their basal attachments to the shell, blotted thoroughly, minced (over ice) and packed into 2 ml ultracentrifuge tubes. Centrifugations were carried out at 0 °C in a Beckman 12–65 preparatory ultracentrifuge, at 40000 g for 1/2 h, 63500 g for 1/2 h, and 176000 g for 1 h. Wet and dry weights were estimated on all supernatants; protein analyses (Lowry, Roseborough, Farr & Randall, 1951) and osmotic pressure measurements (see below) were made on the larger samples obtained at higher forces.

Dry weights showed small, linear decreases with increasing centrifugal force. Total soluble dry weight was therefore obtained empirically by linear extrapolation to zero force, which gave a value 7 % greater than that actually measured at 40000 g (51·3 % compared with 47·8 % of total dry weight). It seems unlikely that this extrapolation seriously underestimates the soluble dry weight fraction, since it estimates the nonsoluble (mainly myofilament) proteins at 11·3 % of the total cell weight, which is similar to the 12 % estimated for rabbit muscle (Pemrick & Edwards, 1974). Loss of

protein at greater forces was also linear and accounted for a consistent fraction (56%) of the total soluble dry weight loss. As a first approximation, we therefore assumed similar centrifugation profiles for protein and nonprotein high molecular weight solutes.

Fat determination

The amount of ether extractable fat was estimated from the loss in dry weight of fibres after three extractions of 30, 15 and 10 min, each in 2 ml of anhydrous diethyl ether, followed by a further 10 min extraction in 2 ml of petroleum ether.

Osmotic pressure

The osmotic pressures of samples of sea water, coelomic fluid and fibre ultracentrifugate were determined by freezing point depression using a Fiske osmometer.

Amino acid analysis

Total ninhydrin-positive nitrogen was estimated both in single fibres and on samples of coelomic fluid by the method of Clark (1973), utilizing a glycine standard. Fibre water content was determined on fibres dissected from the same muscle bundle. The results were calculated as millimoles of ninhydrin-positive nitrogen (nin+N) per kilogram water.

For analyses of individual amino acids, ethanolic extracts of fibres or coelomic fluid were evaporated to dryness, redissolved in a known volume of 0·1 M-citrate buffer (pH 2·1) and centrifuged to remove insoluble material. Standard amino acid analyser techniques were utilized for identification of acidic, neutral and basic amino acids, and other ninhydrin reactive substances. Standards were run sequentially with each sample.

TMAO and betaine analysis

The organic bases, trimethylamine oxide (TMAO) and betaine, were analysed together using a modification of the colorimetric reineckate precipitation method described by Kermack, Lees & Wood (1955) and adapted by Robertson (1975). Because our method employs centrifugation rather than filtration to separate the reineckate salts, it is given here in detail.

Reagents are: (1) 10% aqueous trichloroacetic acid (TCA).

(2) Buffer consisting of 100 ml of 0·1 M-citric acid and 2·0 ml of 0·2 M-Na_2HPO_4 (pH 2·0–2·2), saturated overnight with an excess of ammonium reineckate (NH_4-$[Cr(SCN)_4(NH_3)_2]$.H_2O). The solution is filtered and TMAO and betaine, previously dissolved to saturation in 10% TCA, are added until a precipitate begins to form (approximately 0·02 ml is required per 100 ml of reineckate solution). The suspension is filtered, and both precipitate and filtrate are kept. The saturated filtrate should be used the same day, since the reineckate salt decomposes, giving off HCN and losing its ability to precipitate the bases.

(3) *n*-Propanol rinse solution, saturated with reineckate-base precipitate. The precipitate obtained in (2) above is rinsed with a small volume of *n*-propanol, which is discarded. The washed precipitate is then stirred with one litre of *n*-propanol for 20 min to saturate the alcohol with the base-reineckates. The excess is filtered off.

Preweighed fresh fibres containing approximately 25 μmol of TMAO + betaine are

extracted by shaking at 0 °C for 2 days in 3 ml absolute methanol. A quantitative subsample (containing approximately 20 μmol of TMAO + betaine) is evaporated to dryness in a 15 ml graduated centrifuge tube and redissolved in 0·5 ml 10% TCA. Then 10 ml of saturated reineckate reagent is rapidly added with vigorous mixing. After being shaken several times, the tube is allowed to stand for 1 h to ensure complete precipitation. After centrifugation, the supernatant is gently aspirated away with a fine-tipped pipette so as not to disturb the precipitate. With a vortex mixer, the precipitate is resuspended in 4 to 5 ml *n*-propanol reagent. After centrifugation and removal of the alcohol rinse, the precipitate is dissolved in acetone to a final volume of 10 ml. Absorbance is read at 530 nm, and no background correction is needed.

Although this method results in the loss of a small amount of the initial reineckate-base precipitate, which floats on the surface of the reagent, the amount lost is nearly constant regardless of the total concentration of substituted amine present. Hence, above 10 μmol, the absorbance is linear for standards containing up to 100 μmol TMAO + betaine. The only restriction is that the samples must contain more than 10 μmol of TMAO + betaine. The unknowns are read from a standard curve prepared simultaneously.

RESULTS

The mean water content of 64 fibres from eight barnacles was 75·1% (standard error ± 0·2). Except for one animal with a mean fibre water content of 80%, the range fell between 73·7 and 76·8%. This agrees well with other data (McLaughlin & Hinke, 1966; Hinke, 1970). In estimating the concentrations of various solids within fresh fibres, we have therefore taken the dry weight as 25% of the total weight.

We have taken the extracellular water to be 6% of the total fibre water (Gayton & Hinke, 1968; Hinke, 1969, 1970; Brigden, Spira & Hinke, 1971), and we assume that the concentrations of dissolved solids in this compartment are equal to those in the coelomic fluid. The proportion of nonsoluble solids in the clefts of the extracellular space, expressed as grams per kilogram of water, is taken to be the same as that in the intracellular space, since electronmicrographs (Gayton & Hinke, 1968; Brigden, Spira & Hinke, 1971) clearly show that the large transverse clefts are filled with a collagenous material of similar density to the myofibrils. (Assuming no insoluble solids in the clefts increases total intrafibre solids by less than 3%.)

Utilizing the above assumptions, we have calculated the total solid and the dissolved solid contents in the intrafibre water from measurements on the coelomic fluid and total fibre water, as shown in Table 1. The dissolved solids in the total fibre water were estimated for four samples collected over a 3 month period. It is of interest that half of the intrafibre solids are soluble and nearly 30% of the latter are amino acids and taurine. The proportions of individual amino acids in coelomic fluid and intrafibre water are shown in Table 2. Note the preponderance of glycine within the fibre; other important intracellular solutes are proline, asparagine and glutamine (combined), arginine (the phosphagen in crustaceans), and taurine with lesser amounts of alanine, glutamic acid, and valine.

Coelomic fluid and fibre water each have an osmolarity of about 1000 m-osmol (isosmotic with the aquarium sea water) (Table 1). The identified amino acids form only a small fraction of the osmotically active solutes in the coelomic fluid. Most of the

Table 1. *Total and dissolved solids, amino acids, and osmolarity of coelomic fluid and muscle fibres*

	Coelomic fluid	Total fibre water	Intrafibre water*
Total solids (g/kg H_2O)	48·2	333	341
Dissolved solids (g/kg H_2O)	48·2	$167 \pm 2{\cdot}3$ ($n = 4$)	175
Amino acids and taurine (g/kg H_2O)†	0·18	47·6	50·6
Osmolarity (m-osmol)	990	989 ± 29 ($n = 2$)	990
Total nin + N (mmol/kg H_2O)	$3{\cdot}18 \pm 0{\cdot}30$ ($n = 2$)	539 ± 15‡ ($n = 24$)	573
Individual amino acids (mmol/kg H_2O) minus proline†	1·32	429	456
Unidentified nin + N (mmol/kg H_2O) (obtained by difference)	1·86	110	117

* Based on a 6% extracellular water content in blotted fibres and equal proportions of nonsolute solids in extracellular and intrafibre compartments.

† Calculated from Table 2 after subtraction of proline, which reacts only negligibly with ninhydrin. An 'average' molecular weight for glutamine and asparagine was used.

‡ 24 fibres obtained from three barnacles collected over a period of 1 year.

Table 2. *Concentration of individual amino acids in coelomic fluid and intrafibre water of* Balanus nubilis

Amino acid	Coelomic fluid mmol/l	% of total	Intrafibre water mmol/kg H_2O	% of total
Alanine	0·042	3·0	18·81	3·7
ASN + GLN	0·210	15·2	46·55	9·3
Arginine	0·082	5·9	34·34	6·8
Aspartic acid	Trace	Trace	Trace	Trace
Glutamic acid	0·030	2·2	9·04	1·8
Glycine	0·120	8·7	279·26	55·5
Histidine	0·014	1·0	2·58	0·5
Isoleucine	0·024	1·7	3·34	0·7
Leucine	0·048	3·5	6·81	1·4
Lysine	0·075	5·4	3·25	0·6
Methionine	Trace	Trace	2·51	0·5
Phenylalanine	—	—	Trace	Trace
Phosphoethanolamine	0·024	1·7	—	—
Phosphoserine	0·015	1·1	—	—
Proline	0·060	4·3	46·70	9·3
Sarcosine	Trace	Trace	—	—
Serine	0·036	2·6	7·20	1·4
Taurine	0·480	34·7	26·65	5·3
Threonine	0·036	2·6	4·26	0·8
Tyrosine	0·021	1·5	3·47	0·7
Valine	0·066	4·8	8·35	1·7
Total	1·383		503·12	

difference is no doubt due to neutral salts, as is the case in other marine crustaceans (Robertson, 1949, 1961). Much of the unidentified nin + N is probably ammonia, which was not quantified owing to loss of an intermediate fraction during sample preparation. Within the fibre, however, the absolute discrepancy between the measured amino acids and the total nin + N was much greater, the unidentified fraction amounting to 20%; the nature of this fraction is discussed later.

Table 3. *Intracellular dry weight components of* Balanus nubilis *muscle fibres*

(Based on 75 % total fibre water content and 6 % of water and nonsoluble dry weight in extracellular space.)

Component	m-osmol (ϕ)	mmol/kg H_2O	g/kg H_2O	% Dry weight
Dissolved Solids				
Low molecular weight				
Ions				
Na		35–56	1·29	0·38
K		158–180	7·04	2·06
Cl		30–35	1·24	0·36
Subtotal, ions	244 (0·9)	271	9·57	2·80
Organics				
Amino acids + taurine	503 (1·0)	503	50·9	14·92
TMAO + betaine	94 (1·15)	82	9·3	2·73
Unidentified (by difference)	149 (1·0)	149 (est)	25·2	7·39
Subtotal, organics	746	734 (est)	85·4	25·04
Subtotal, osmotically active solutes	990	1005	95·0	27·8
High molecular weight				
Protein			45·0	13·2
Other			35·1	10·3
Subtotal, high molecular weight solutes			80·1	23·5
Total: All dissolved solids			175	51·3
Nonsoluble Solids				
Fat			11·0	3·2
Mg		16	0·39	0·1
Ca		1	0·16	0·05
Other (mainly myofilament protein)			154·5	45·3
Total: Nonsoluble solids			166	48·7
Grand total			341	100·0

Table 3 summarizes the intrafibre dry weight components in *Balanus* muscle. Values for the intracellular ions are obtained from the literature as follows: Na and K (McLaughlin & Hinke, 1966; Hinke, Caillé & Gayton, 1973); Mg (Page, Mobley, Johnson & Upshaw, 1971); Ca (Ashley, 1967); Cl (Hinke *et al.* 1973; Hinke & Gayton, 1971). The highest reported concentrations have been used in calculating the contribution of ions to the dry weight. Only the univalent ions are considered to contribute to the osmotic pressure. Calcium is presumably sequestered in the sarcoplasmic reticulum and some 60% of magnesium is bound to myofilaments or to cell solutes (Brinley, Scarpa & Tiffert, 1977).

The value for amino acids and taurine given in Table 3 is taken from Table 2. The value for TMAO + betaine is the mean from 10 samples, each containing 10 fibres, with a standard error of ± 2 mM; it has been calculated on the assumption that these bases do not occur in the extracellular water. TMAO was reported by Norris & Benoit (1945) to have a concentration of 50–70 mM in the whole fibre of *Balanus nubilis*, suggesting that TMAO accounts for most of the total base found here.

The following osmotic coefficients have been assigned to the soluble components: univalent ions, 0·9 (Scatchard, Hamer & Wood, 1938); amino acids and taurine, 1·0 (Robertson, 1975); TMAO and bentaine, 1·15 (mean) (Robertson, 1975). If these

solutes are all osmotically active, then there is a discrepancy of 149 m-osmol between the osmotic pressure of the supernatant (Table 1) and the identified solutes of Table 3. For convenience, this discrepancy is shown as unidentified organic solutes; their possible nature is discussed below.

The soluble high molecular weight components shown in Table 3 are mean values from four barnacles, which all agreed within 5 %. The measured protein accounts for over half of this fraction, and is 13 % of the total dry weight. The possible identity of the remaining fraction is discussed below.

The nonsoluble solids comprise half the fibre dry weight. Exhaustive extraction with ether removes 3·2 % (± 0·20 S.E.; $n = 15$) of the total dry weight. The remaining dry weight is assumed to be mainly myofilament protein, with a small quantity of other proteins, glycogen and nucleic acids (see electronmicrograph of Brigden *et al.* 1971).

DISCUSSION

The measured low molecular weight components found in *Balanus* muscle are almost identical to those observed in two decapod marine crustaceans, *Carcinus* (Shaw, 1958) and *Nephrops* (Robertson, 1961). The total ions (including Ca and Mg) are 288 mM in *Balanus*, 275 mM in *Carcinus*, and 289 mM in *Nephrops*. If we take the total nin + N from Table 1 (573 mM) as the best estimate of total amino acids and taurine, add to it the proline value from Table 2 (47 mM) (since proline reacts negligibly with ninhydrin compared to the glycine standard (Clark, 1973)), and also add 82 mM of TMAO and betaine (Table 3), we obtain a total for intracellular nitrogenous solutes in *Balanus* of 702 mM, compared with a total of 699 mM in *Carcinus* and 682 mM in *Nephrops*.

The predominant amino acids found in *Balanus nubilis* (Table 2) are almost identical with those in the closely related species, *Balanus aquila* (Brinley *et al.* 1977), and in *Nephrops* (Robertson, 1961). Furthermore, there are significant amounts of the nitrogenous bases TMAO and betaine in *Balanus* muscle, as in the muscle of all other marine crustaceans where they have been sought (Norris & Benoit, 1945; Kermack *et al.* 1955; Shaw, 1958; Robertson, 1961). It is these solutes whose function, vis-à-vis the contractile proteins, has been investigated in the following paper (Clark *et al.* 1980).

Assignment of the remaining fibre dry weight to specific categories is less certain. As noted earlier, our value for myofilament protein appears correct to within a few per cent. The major difficulty arises in assigning unidentified dissolved solids to the low or high molecular weight fractions. One cannot rely simply on the magnitude of the 'missing' osmotic activity. As pointed out by Robertson (1961), the agreement in osmolarity between freezing point depression of the coelomic fluid and the measured sum of the intracellular solutes may be more apparent than real. Not only do soluble proteins exert an unknown and possibly significant osmotic effect; it is also true that some ions are almost certainly osmotically unavailable. In *Nephrops*, all of the intracellular calcium and most of the magnesium are bound (Robertson, 1961). As noted earlier, we have placed these ions among the nonsoluble solids in *Balanus* fibres. In *Nephrops*, most of the sodium is bound (Robertson, 1961), and in *Balanus* at least half of the sodium is osmotically inactive (Hinke, 1970). On the other hand, not all of the

fibre water behaves as solvent, at least for ions (Hinke, 1970). Hence, even though in both *Nephrops* (Robertson, 1961) and *Balanus* the fraction of fluid which is physically separable from the fibre has the same total osmolarity as the coelomic fluid, its solute composition is not necessarily representative of that of the total intrafibre water.

In addition to the unidentified nin+N (Table 1), which may have included some ammonia (Clark, 1968), as well as amino acids and oligopeptides that did not redissolve during sample preparation, *Balanus* muscle probably contains a number of other small solutes such as have been found in *Nephrops* (Robertson, 1961): lactate (9 mM); inorganic phosphate (21 mM); acid soluble organic phosphates (excluding arginine-PO_4) (30 mM). All of these solutes, taken together and assigned an osmotic coefficient of 1·0, could more than account for the 'missing' osmotic activity in the supernatant fraction. For this reason, it seems likely that the nonprotein high molecular weight soluble fraction, assigned as 10% of the total dry weight in Table 3, may be an overestimate. The measurable loss of nonprotein dry weight during high speed centrifugation, however, indicates that some such compound is present, possibly as the polysaccharide moiety of a glycoprotein. On the other hand, the soluble protein or myogen in *Balanus* muscle comprises 20% of the total fibre protein, as is to be expected (Lehninger, 1975).

REFERENCES

ASHLEY, C. C. (1967). The role of cell calcium in the contraction of single cannulated muscle fibers. *Am. Zoologist* **7**, 647–659.

AWAPARA, J. (1962). Free amino acids in invertebrates: a comparative study of their distribution and metabolism. In *Amino Acid Pools* (ed. J. T. Holden), pp. 158–175. New York: Elsevier.

BOWLUS, R. D. & SOMERO, G. N. (1979). Solute compatibility with enzyme function and structure: Rationales for the selection of osmotic agents and end-products of anaerobic metabolism in marine invertebrates. *J. exp. Zool.* **208**, 137–152.

BRICTEUX-GRÉGOIRE, S., DUCHÂTEAU-BOSSON, G., JEUNIAUX, C. & FLORKIN, M. (1962). Constituants osmotiquement actifs des muscles du crab chinois *Eriocheir sinensis*, adapté à l'eau douce ou l'eau de mer. *Arch. int. Physiol. Biochim.* **70**, 273–286.

BRIGDEN, H. L., SPIRA, A. W. & HINKE, J. A. M. (1971). The extracellular space of the single fiber from the giant barnacle. *Can. J. Physiol. Pharmacol.* **49**, 801–811.

BRINLEY, F. J., JR., SCARPA, A. & TIFFERT, T. (1977). The concentration of ionized magnesium in barnacle muscle fibres. *J. Physiol.* **266**, 545–565.

CLARK, M. E. (1968). Free amino-acid levels in the coelomic fluid and body wall of polychaetes. *Biol. Bull.* **134**, 35–47.

CLARK, M. E. (1973). Amino acids and osmoregulation. In *Experiments in Physiology and Biochemistry* (ed. G. Kerkut), **6**, 81–114. New York: Academic Press.

CLARK, M. E., HINKE, J. A. M. & TODD, M. E. (1980). Studies on water in barnacle muscle fibres. II. Role of ions and organic solutes in swelling of contractile filaments in chemically-skinned fibres *J. exp. Biol.* **90**, 43–63.

CLARK, M. E. & ZOUNES, M. (1977). The effects of selected cell osmolytes on the activity of lactic dehydrogenase from the euryhaline polychaete, *Nereis succinea*. *Biol. Bull.* **153**, 468–484.

FREEL, R. W. (1978). Transmembrane activity gradients of K and Cl in the muscle fibers of osmoconforming marine decapod crustaceans. *J. exp. Biol.* **72**, 127–140.

FREEL, R. W., MEDLER, S. G. & CLARK, M. E. (1973). Solute adjustments in the coelomic fluid and muscle fibers of a euryhaline polychaete, *Neanthes succinea*, adapted to various salinities. *Biol. Bull.* **144**, 289–303.

GAYTON, D. C. & HINKE, J. A. M. (1968). The location of chloride in single striated muscle fibers of the giant barnacle. *Can. J. Physiol. Pharmacol.* **46**, 213–219.

HINKE, J. A. M. (1969). Glass microelectrodes in the study of binding and compartmentalization of intracellular ions. In *Glass Microelectrodes* (ed. M. Lavalée, O. F. Schanne and N. C. Hébert), pp. 349–375. New York: John Wiley.

HINKE, J. A. M. (1970). Solvent water for electrolytes in the muscle fiber of the giant barnacle. *J. gen. Physiol.* **56**, 521–541.

HINKE, J. A. M., CAILLÉ, J.-P. & GAYTON, D. C. (1973). Distribution and state of monovalent ions in skeletal muscle based on ion electrode, isotope, and diffusion analyses. *Ann. New York Acad. Sci.* **204**, 274–294.

HINKE, J. A. M. & GAYTON, D. C. (1971). Transmembrane K^+ and Cl^- activity gradients for the muscle fiber of the giant barnacle. *Can. J. Physiol. Pharmacol.* **49**, 312–322.

HOYLE, G., MCNEILL, P. A. & SELVERSTON, A. I. (1973). Ultrastructure of barnacle giant muscle fibers. *J. Cell Biol.* **56**, 74–91.

JEUNIAUX, C., DUCHÂTEAU-BOSSON, G. & FLORKIN, M. (1961*a*). Free amino acids in the intracellular osmoregulation of euryhaline marine worms. *Biochem. J.* **79**, 24–25.

JEUNIAUX, C., DUCHÂTEAU-BOSSON, G. & FLORKIN, M. (1961*b*). Variation de la composante amino-acide des tissus et euryhalinité chez *Perinereis cultrifera* Gr. et *Nereis diversicolor* O. F. Müller. *J. Biochem., Tokyo* **49**, 527–531.

KERMACK, W. O., LEES, H. & WOOD, J. D. (1955). Some non-protein constituents of the tissues of the lobster. *Biochem. J.* **60**, 424–428.

LEHNINGER, A. L. (1975). *Biochemistry* (2nd ed.), p. 753. New York: Worth Publishers.

LOWRY, O. H., ROSEBOROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

LUTZ, P. L. & ROBERTSON, J. D. (1971). Osmotic constituents of the coelacanth *Latimeria chalumnae* Smith. *Biol. Bull.* **141**, 553–560.

MCLAUGHLIN, S. G. A. & HINKE, J. A. M. (1966). Sodium and water binding in single striated muscle fibers of the giant barnacle. *Can. J. Physiol. Pharmacol.* **44**, 837–848.

NORRIS, E. R. & BENOIT, G. F., JR. (1945). Studies on trimethylamine oxide. I. Occurrence of trimethylamine oxide in marine organisms. *J. biol. Chem.* **158**, 433–438.

PAGE, E., MOBLEY, B. A., JOHNSON, M. & UPSHAW, J. E. (1971). Magnesium in single skeletal muscle cells of *Balanus*. *J. gen. Physiol.* **57**, 188–201.

PEMRICK, S. M. & EDWARDS, C. (1974). Differences in the charge distribution of glycerol-extracted muscle fibers in rigor, relaxation and contraction. *J. gen. Physiol.* **64**, 551–567.

ROBERTSON, J. D. (1949). Ionic regulation in some marine invertebrates. *J. exp. Biol.* **26**, 182–200.

ROBERTSON, J. D. (1961). Studies on the chemical composition of muscle tissue. II. The abdominal flexor muscles of the lobster *Nephrops norvegicus* (L). *J. exp. Biol.* **38**, 707–728.

ROBERTSON, J. D. (1975). Osmotic constituents of the blood plasma and parietal muscle of *Squalus acanthias* L. *Biol. Bull.* **148**, 303–319.

ROBERTSON, J. D. (1976). Chemical composition of the body fluids and muscle of the hagfish *Myxine glutinosa* and the rabbit-fish *Chimaera monstrosa*. *J. Zool., Lond.* **178**, 261–277.

SCATCHARD, G., HAMER, W. J. & WOOD, S. E. (1938). Isotonic solutions. I. The chemical potential of water in aqueous solutions of sodium chloride, sulfuric acid, sucrose, urea and glycerol. *J. Amer. chem. Soc.* **60**, 3061–3070.

SCHOFFENIELS, E. (1976). Adaptations with respect to salinity. *Biochem. Soc. Symp.* **41**, 179–204.

SHAW, J. (1958). Osmoregulation in the muscle fibres of *Carcinus maenas*. *J. exp. Biol.* **35**, 920–929.