THE INORGANIC AND AMINO ACID COMPOSITION OF SOME LAMELLIBRANCH MUSCLES

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INTRODUCTION

The electrolyte composition of vertebrate striated muscle has been the subject of many investigations, and some of the principles governing the distribution of electrolytes between the muscle fibres and the plasma are fairly well understood. In comparison with the plasma the sarcoplasm of striated muscle contains a low concentration of sodium and chloride and a high concentration of potassium. This unequal distribution is a dynamic equilibrium maintained by the active extrusion of sodium from the cell. The distribution of potassium and chloride is very close to a Donnan equilibrium across the potential gradient produced by the sodium extrusion and by a concentration of indiffusible anions inside the fibres. Less is known about ionic equilibrium in invertebrate muscle, but a recent detailed investigation of the fibres of the muscles of *Carcinus maenas* suggests that the conditions are similar to those found in the vertebrates (Shaw, 1955). Gross analyses of *Loligo* muscle (Manery, 1939) would fit into the same picture if allowance is made for a small quantity, about 5%, of extracellular fluid.

Vertebrate smooth muscles (Manery & Bale, 1941; Wilkins and Cullen, 1933) contain a much higher proportion of sodium and chloride than striated muscle but this may be due, in part at least, to a higher proportion of extracellular fluid between the fibres. Bulbring & Born, quoted by Holman (1957), estimate that the extracellular fluid in the taenia coli muscle of the guinea-pig amounts to 36% of the total wet weight. A large number of analyses of invertebrate smooth muscle have been published, but as the analyses are rarely accompanied by measurements of the proportion of extracellular fluid it is difficult to draw any firm conclusions about the intracellular concentrations of ions. The analyses of the whole muscle usually show a relatively high concentration of sodium and chloride, sometimes amounting to as much as half the concentrations of the same ions in the blood (Singh, 1938; Krogh, 1939, p. 60; Steinbach, 1940), but it is not clear if this is accounted for by a high proportion of extracellular fluid or if the conditions of ionic equilibrium in invertebrate smooth muscle differ from those found in striated muscle.

To throw further light on this problem, measurements have been made of the concentrations of the chief ions—sodium, chloride and potassium—in the sarcoplasm of a variety of lamellibranch muscles. The lamellibranchs form a convenient

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source of substantial quantities of muscle uncontaminated by other tissues. Unfortunately, the small size of lamellibranch muscle fibres, usually about 8 μ in diameter, makes it impracticable to analyse individual fibres; instead, analyses have been made of the whole muscle and the volume of contaminating blood has been estimated by inulin. The muscles which have been analysed are the anterior byssus retractor and the fast and slow portions of the adductor muscle of *Mytilus edulis*, and the fast and slow portions of the adductor muscle of *Pecten maximus*. The fast adductor of *Pecten* is striated and generally similar in physical properties to crab or frog muscle (Abbott & Lowy, 1956), and therefore provides a convenient link with better known muscles. The freshwater species *Anodonta cygnaea* has a very low concentration of ions in the blood and in the muscle, and analyses have been made of the two portions of the adductor muscles of this species. In order to widen still further the range of conditions, analyses have been made of *Mytilus* adductors and byssus retractors when the animals are adapted to 50% sea water, and of *Anodonta* adductors when the animals are adapted to 18% sea water.

Inorganic ions account for only a fraction of the osmotic pressure of the tissues of the marine species, so measurements have been made of the concentrations of phosphate compounds and free amino acids in order to define more closely the differences between the marine and freshwater species.

The fluxes of sodium through the muscle cells of the ventricles of *Mytilus* and *Anodonta* have also been measured and will be described in a later paper, but for convenience the results of the analyses of these two tissues are included here.

METHODS

The animals were kept in well-aerated tanks between 10° and 16° C. Both *Mytilus* and *Anodonta* gain sodium and lose potassium from the tissues when kept under laboratory conditions, so animals were used within 1 week of arrival, unless otherwise stated.

After excision and cleaning the muscles were blotted to remove surface fluid. The water content was taken as the loss of weight after drying overnight at 105° C.

Sodium and potassium were estimated by flame photometry using an 'EEL' flame photometer. Measurements were made at maximum dilution, 0·05–0·1 mM./l. The standard solutions used contained both sodium and potassium in similar concentrations to the samples analysed. When small quantities of muscle (5–50 mg.) were dry-ashed with concentrated sulphuric acid significant quantities of sodium were lost at 500° C. Complete solution without loss was obtained by evaporating to dryness on a water-bath with 0·1 ml. concentrated nitric acid and then dissolving in 5 or 10 ml. of water. Calcium was estimated by precipitating as oxalate and titrating with ceric sulphate (Robertson & Webb, 1939). Magnesium was precipitated as hydroxyquinolate and also titrated with ceric sulphate (Robertson & Webb, 1939). Chloride was estimated by the microdiffusion method of Conway (1947). Sulphate was estimated gravimetrically as barium sulphate after removing the protein with picric acid. Phosphate was estimated colorimetrically by the method of Fiske & SubbaRow (1925). Total free amino acids were estimated by

the method of Van Slyke, MacFadyen & Hamilton (1941). In this method the carbon dioxide evolved on decarboxylation with ninhydrin, indane-trione hydrate, is absorbed by barium hydroxide. Both the carboxyl groups in aspartic acid are decomposed by this method, so the relative amount of aspartic acid was estimated by paper chromatography. Two-dimensional chromatograms were run in phenol/water and butanol/acetic acid and water, and the spots developed with ninhydrin. After development the spots were extracted with 70% acetone and the relative intensities measured by a 'Spekker' absorptiometer. The method is not of high accuracy, but the aspartic acid is only about 5% of the total amino acids in *Mytilus*, so the correction is small. The method of Van Slyke *et al.* does not estimate taurine which was reported to occur in large quantities in *Mytilus* (Kelly, 1904). Taurine was removed from the muscle by repeated extraction with 70% alcohol and precipitated as barium sulphate, after boiling in bromine water for 4 hr. in alkaline solution. The method is not specific as other alcohol soluble compounds containing sulphur, such as isethionic acid, might also be oxidized to some extent. The chromatograms showed that amino acids containing sulphur were only present in small quantities. Hydroxycarboxylic acids were separated on a column of silica gel and estimated as described by Isherwood (1946), but the quantities found were very small.

The volume of extracellular fluid was estimated by injecting a suitable quantity of a 20% solution of inulin in sea water (or in dilute sea water) into a whole animal, and estimating the concentrations of inulin in the blood and in the muscle the following day. The inulin was estimated by the colorimetric method of Roe, Epstein & Goldstein (1949) using an 'EEL' absorptiometer. The blood was collected by hypodermic from the ventricle. The inulin was removed from the muscle by soaking 50–100 mg. portions of muscle, not exceeding 1 mm. in thickness, in a suitable saline for 5 hr., with frequent shaking. Extraction was 95% complete in an hour.

RESULTS

The adductor muscles of *Pecten* and *Mytilus* are large enough to allow several determinations of sodium, potassium and chloride to be made on one animal. Tables 1 and 2 show the results of a number of such analyses. Each figure for sodium and potassium is the mean of the analyses of three separate samples of muscle. Each chloride figure is the mean of two separate determinations. The water contents of the muscles are given in Table 3.

The ventricle of *Mytilus*, and to some extent the byssus retractor, are rather small for simultaneous determinations of sodium, potassium and chloride, so only the mean values and standard errors of a large number of separate determinations of these quantities are given (Table 4).

The sodium and chloride content of *Anodonta* muscle is very variable but much of the variation is clearly related to the variation in the blood composition. When expressed as a percentage of the concentration in the blood, the sodium and chloride results are more consistent (Table 5).

Table 1. *Sodium, potassium and chloride concentrations in the adductor muscle and the blood of* Pecten maximus

(mM./kg. total water.)

Animal	Fast adductor			Slow adductor		
	Na	K	Cl	Na	K	Cl
1	77	138	—	176	128	189
2	82	166	70·7	176	143	—
3	72	154	58·0	125	146	145
4	50	157	59·5	146	149	166
5	72	160	85·4	170	131	222
6	78	136	71·6	149	113	171
7	59·5	144	57·3	133	141	157
8	—	—	60·6	—	—	156
Mean (±standard error)	70±5	151±5	66·2±4	154±7	136±5	172±10
Blood	490	12·5±0·3	573			

Table 2. *Sodium, potassium and chloride concentrations in the adductor muscle of* Mytilus edulis

(mM./kg. total water.)

Animal	Fast adductor			Slow adductor		
	Na	K	Cl	Na	K	Cl
1	150	131	146	166	108	194
2	143	131	212	180	123	242
3	183	129	179	178	134	215
4	132	129	174	180	118	212
5	174	107	183	205	108	247
6	165	122	227	217	99	254
Mean (±standard error)	158±9	125±4	187±13	188±9	115±6	227±13

Table 3. *Water content of lamellibranch muscles*

(% total weight.)

	Fast adductor	Slow adductor	Ventricle	Byssus retractor
Pecten	76·3±0·6 (20)	74·6±0·8 (15)	—	—
Mytilus	75 ±0·6 (17)	75·6±0·6 (14)	80·8±0·6 (14)	78·4±0·5 (16)
Mytilus, 50% sea water	78·1±0·6 (8)	78·9±0·3 (8)	—	80·7±0·4 (8)
Anodonta, fresh water	83·7±0·6 (6)	85·1±0·4 (6)	87·8±0·8 (8)	—
Anodonta, 18% sea water	75·8±1·0 (4)	79·2±1·0 (4)	—	—

Mean ± standard error (no. of observations).

The muscles of the marine species show very wide but systematic variations in their sodium, potassium and chloride content. The muscles may be arranged in a series on the basis of their inorganic content: *Pecten* fast adductor, *Pecten* slow adductor, *Mytilus* fast adductor, *Mytilus* ventricle, *Mytilus* slow adductor, *Mytilus* byssus retractor. In this series the sodium and chloride contents of the muscles

Table 4. *Sodium, potassium and chloride content of the anterior retractor byssus, the ventricle and the blood of* Mytilus edulis

(mM./kg. total water.)

	Na	K	Cl
Retractor byssus	208 ± 10 (18)	107 ± 3 (18)	281 ± 4 (12)
Ventricle	181 ± 11 (16)	120 ± 3 (14)	190 ± 10 (12)
Blood	490	12·5 ± 0·2 (12)	573

Mean ± standard error (no. of observations).

Table 5. *Concentrations of sodium, potassium and chloride in the adductor muscles and the ventricle of* Anodonta cygnaea

(mM./kg. total water. The sodium and chloride are also expressed as % of the blood concentration.)

	Na		K	Cl	
	mM./kg. total water	% of blood concn.	mM./kg. total water	mM./kg. total water	% of blood concn.
Fast adductor	6·6	45 ± 2·9 (6)	18·4 ± 1·0 (8)	3·86	36·7 ± 4·3 (6)
Slow adductor	9·2	63 ± 4·0 (6)	12·4 ± 1·0 (8)	6·82	64·2 ± 3·9 (6)
Ventricle	9·5	65 ± 1·7 (8)	10·5 ± 0·6 (12)		
Blood	14·7 ± 1·3	(8)	0·45 ± 0·014 (14)	10·7 ± 1·0	(6)

Mean ± standard error (no. of observations).

are steadily increasing and the potassium contents of the muscles are steadily falling. It is also the order of increasing time of stress relaxation (or, more generally, decreasing speed of muscle) where this is known (Abbott & Lowy, 1956, 1957). The fastest muscle, the fast portion of *Pecten* adductor, is similar to *Carcinus* striated muscle in that it contains little sodium or chloride, whereas the slowest muscle, the byssus retractor, contains almost half as much sodium and chloride as the blood.

The concentrations of ions found in the *Anodonta* muscles are necessarily much lower than in the marine species because the blood has a total osmolar concentration of only 4–5% of sea water, but otherwise the picture is similar. The slower muscle contains the greater quantities of sodium and chloride, amounting in this case to more than half of the blood concentration.

The intracellular concentrations of sodium and chloride

The whole muscle contains considerable quantities of extracellular fluid which must be allowed for when calculating the intracellular concentrations of the ions. The extracellular fluid has been assumed to be identical with the space accessible to inulin (Table 6). The sodium, potassium and chloride contents of the blood of *Anodonta* are given in Table 5. The sodium and chloride content of the blood of *Pecten* and of *Mytilus* is assumed to be the same as that of the sea water in which they had been kept before analysis. The potassium content of the blood of *Pecten*

Table 6. *Inulin space in lamellibranch muscle expressed as % of total water content*

	Fast adductor	Slow adductor	Ventricle	Byssus retractor
Pecten	6·0±0·26 (19)	24 ±0·6 (18)	—	—
Mytilus	19·3±0·9 (16)	24·5±1·3 (14)	26·0±3·4 (4)	29·5±1·2 (12)
Mytilus, 50% sea water	14·1±0·9 (7)	21·3±2·0 (7)	—	25·3±1·1 (6)
Anodonta, fresh water	14·4±1·2 (13)	27·4±1·4 (12)	30·5±2·5 (5)	—
Anodonta, 18% sea water	41·5±1·6 (11)	46·4±2·9 (7)	—	—

Mean ± standard error (no. of observations)

and *Mytilus* is rather higher than that of sea water, and was measured at the same time as the potassium content of the muscle (Tables 1 and 4). The mean sodium content of the blood of *Anodonta* is rather lower than a previously published figure by the author (Potts, 1954). This is probably because Birmingham tap water has a much lower sodium content than Cambridge tap water.

Table 7. *Concentrations of sodium, potassium and chloride in some lamellibranch muscles*

	(1) Whole muscle (mM./kg. total water)			(2) Blood space (mM./kg. water)			(3) Sarcoplasm (mM./kg. fibre water)		
	Na	K	Cl	Na	K	Cl	Na	K	Cl
Pecten									
Fast adductor	70±5	151±5	66·2±4	29·4±1	1	34·4±1	43±5	160±5	34±4
Slow adductor	154±7	136±5	172±10	88±3	2	104±3	81±10	163±6	83±13
Mytilus									
Fast adductor	158±9	125±4	187±13	94±4	2	111±5	79±12	152±5	94±17
Slow adductor	188±9	115±6	227±13	120±6	3	140±7	90±15	148±8	115±20
Byssus retractor	212±9	112±3	276±4	145±6	4	169±7	95±16	153±4	152±11
Ventricle	181±10	120±4	190±9	127±17	3	149±19	73±26	158±5	56±29
Mytilus, 50% sea water									
Fast adductor	58·0±6	91·4±5	60·0±4	34·5±2	0·7	40·5±3	27±7	106±6	23±6
Slow adductor	82·5±5	76·6±6	74·6±4	52·2±5	1·1	61·2±6	38±9	96±8	17±9
Byssus retractor	79·8±6	85·5±4	92·5±6	62·0±3	1·4	72·6±3	24±9	112±5	27±9
Anodonta, fresh water									
Fast adductor	6·6±0·4	18·4±1·0	3·9±0·5	2·1±0·3	0·06	1·54±0·23	5·3±0·6	21·3±1·2	2·4±0·6
Slow adductor	9·2±0·6	12·4±1·0	6·8±0·4	4·0±0·3	0·12	2·9±0·3	7·2±0·9	16·9±1·4	5·4±0·7
Ventricle	9·5±0·2	10·5±0·6	—	4·5±0·4	0·14	—	7·2±0·7	14·9±0·9	—
Anodonta, 18% sea water									
Fast adductor	64·6±2·1	26·1±0·4	48·1±2·0	35±1·8	0·3	31±1·8	50±4·8	44±2·0	29±4·6
Slow adductor	65·8±3·1	20·0±0·4	59·2±1·7	39±2·7	0·3	35±2·6	50±7·6	45±2·9	55±5·8

Mean ± standard error.

The intracellular concentrations of sodium, potassium and chloride (Table 7, column 3) are calculated from the analyses of the whole muscles (Table 7, column 1) and from the quantities of ions in the inulin spaces of the muscle (Table 7, column 2). The results are expressed in mM./kg. fibre water. The intracellular concentrations of sodium and chloride are calculated from the small difference between two large

and variable quantities, and so are peculiarly subject to error. However, the results are fairly consistent, and show that as the speed of the muscle increases, the intracellular sodium and chloride fall and, to a lesser extent, potassium rises. In the fast adductor of *Pecten* the intracellular concentrations of sodium and chloride are less than one-tenth of the concentrations of the same ions in the blood, and in this respect the muscle is similar to vertebrate striated muscle. In the slow adductor muscles, especially the slow adductor of *Anodonta*, the relative intracellular concentrations of chloride are very much higher.

Other intracellular constituents

The total osmolar concentration inside the muscle fibres of *Anodonta* is only about one-twentieth of that of the marine species. Fuller analyses have been made of the ventral adductors of *Mytilus* and *Anodonta* to define more clearly the differences between the marine and freshwater species.

The calcium, magnesium and sulphate concentrations in *Mytilus* (Table 8) are

Table 8. *Concentrations of calcium, magnesium and sulphate in the fast portions of the adductor muscles of* Mytilus *and* Anodonta

(Each figure is the mean of four determinations.)

	Blood (mM./kg. water)	Whole muscle (mM./kg. total water)	Sarcoplasm (mM./kg. fibre water)
Magnesium			
Mytilus	56	38	34
Anodonta	0·2	3·9	4·5
Calcium			
Mytilus	12·6	8·55	7·3
Anodonta	8·4	11·5	12·0
Sulphate			
Mytilus	30·7	13·0	8·8

broadly similar to those found in the muscles of other marine animals. Shaw (1955) reported a calcium concentration of 5·4 mM./kg. fibre water in the muscles of *Carcinus maenas*. The magnesium concentration in the ventral adductor of *Mytilus* is about twice as great as in *Carcinus*, 34 mM./kg. fibre water compared with 16 mM./kg. fibre water, but the concentration of magnesium in the blood is correspondingly higher, 56 mM./kg. water compared with 21·2 mM./kg. water. The concentration of magnesium in *Anodonta* blood, 0·02 mM./kg. water, is exceedingly low, but the muscle still contains 5·2 mM./kg. fibre water. Florkin & Duchâteau (1950) reported a magnesium concentration of 7·1 mM./kg. fibre water; the fibre water was calculated on the assumption that all the chloride was extracellular and the portion of the adductor analysed was not specified.

Phosphagen phosphate, inorganic phosphate and adenosine triphosphate have been measured in both *Mytilus* and *Anodonta* (Table 9). The highest concentration of phosphate in whole muscle is found in the fast portion of *Mytilus* adductor. Some of the difference between the two portions of the adductor is due to the

greater proportion of blood in the slow muscle, but the difference is not abolished even when allowance is made for this extracellular fluid. The freshwater animal contains about half the phosphate concentration of the marine animal in spite of its very much lower blood concentration. The phosphate compounds were conserved more effectively than any other measured variable when the Unionidae became adapted to fresh water.

Table 9. *Phosphate content of* Mytilus *and* Anodonta *muscle*

(Each figure is the mean of four determinations.)

	Fast adductor	Slow adductor	Byssus retractor
	Whole muscle (mM./kg. total water)		
Mytilus			
Arginine phosphate	13·4	7·8	13·5
Adenosinetriphosphate	4·9	4·9	4·7
Inorganic phosphate	13·3	12·8	6·3
Anodonta			
Arginine phosphate	8·4	6·3	—
Adenosinetriphosphate	1·7	0·95	—
Inorganic phosphate	6·9	3·9	—
	Sarcoplasm (mM./kg. fibre water)		
Mytilus			
Total phosphate	39·0	33·6	34·6
Anodonta			
Total phosphate	19·8	14·0	—

The concentrations of free amino acids in *Mytilus* and *Anodonta* were estimated by measuring the amount of carbon dioxide evolved on decarboxylation with ninhydrin. Aspartic acid, but not glutamic acid, decomposes to give 2 mmoles of carbon dioxide for 1 mmole of acid. Taurine, a sulphonic acid, is not estimated by this method. Taurine and aspartic acid were estimated separately by other means, and the total free amino acids calculated by adding taurine to the amount estimated by decarboxylation and subtracting the amount of aspartic acid (Table 10). Kelly (1904) estimated that taurine made up 5% of the dry weight of *Mytilus* muscle, corresponding to 100 mM./kg. water in the whole muscle. The chromatograms show that *Mytilus* muscle contains considerable quantities of glycine, alanine, glutamic acid and arginine and some histidine, tyrosine and threonine. *Anodonta* muscle contains glycine, arginine and glutamic acid. The concentration of amino acids in *Mytilus* blood is only 2·5 mM./kg. water, and is even lower, 0·5 mM./kg. water, in *Anodonta*. Duchâteau, Sarlet, Camien & Florkin (1952) estimated the quantities of free amino acids, excluding taurine, in *Mytilus* and *Anodonta* by bio-assay. They found that *Mytilus* muscle contained 166·2 mM./kg. total water and *Anodonta* muscle contained 9·5 mM./kg. total water; the part of the adductor assayed was not defined.

Hydroxycarboxylic acids occur in very low concentrations in lamellibranch muscle. Only 2·4 mM./kg. total water were recovered from *Mytilus* muscle.

Table 10. *Free amino acids in* Mytilus *and* Anodonta

(Each figure is the mean of six determinations.)

	mM./kg. total water				mM./kg. fibre water. Total sarcoplasm
	Carboxylic acid*	Taurine	Aspartic acid	Total whole muscle	
Mytilus					
Ventral adductor	155±3	91±4	8	238±5	295±6
Byssus retractor	126±4	75±3	8	193±5	273±6
Blood	2·5±1	—	—	—	—
Mytilus, 50% sea water					
Ventral adductor	93·5±4	66±4	6	153·5±5	183±6
Byssus retractor	73·4±4	57±3	6	124·4±5	166±6
Blood	5·4±2	—	—	—	—
Anodonta					
Ventral adductor	10·5±2	0	1	9·5±2	11·0±2
Blood	0·47±0·4	—	—	—	—
Anodonta, 18% sea water					
Ventral adductor	30±4	0	2	28±4	48±7

* By the method of Van Slyke, MacFadyen & Hamilton (1941).

The total osmotic pressure of the identified constituents

The sea water in which *Mytilus* had been living contained 573 mM./kg. water of chloride. This corresponds to 19·6‰ chlorinity, a freezing-point depression of 1·89° C. or an osmolar concentration of just over 1. The identified constituents of *Mytilus* muscle amount to less than 700 mM./kg. fibre water (Table 11). The totals given in Table 11 are themselves too high because in the resting muscle much of the arginine and phosphate, which are included separately in Table 11, are combined. On the other hand, no account is taken in Table 11 of calcium, magnesium or sulphate which may contribute to some extent to the osmotic pressure.

Table 11. *Major constituents of the osmotic pressure of* Mytilus *and* Anodonta *muscle*

(mM./kg. fibre water.)

	Mytilus		*Anodonta.* Fast adductor
	Fast adductor	Byssus retractor	
Na	79	95	5·3
K	152	134	21·3
Cl	94	152	2·4
PO_4	39	35	19·8
Amino acids	289	273	11·0
	653	689	59·8

The assayed osmotic constituents of *Anodonta* muscle amount to a total of 60 mM./kg. fibre water, but the muscle contains at least 8·4 and possibly as much as 10 mM. arginine phosphate/kg. fibre water in the resting state. The mean freezing-point depression of the blood of a large series of *Anodonta* amounted to

0·078° C. or 42 mM./kg. water (Potts, 1954). Some of the inorganic phosphate recorded in Table 9 may be in compounds not exerting an equivalent osmotic pressure. Calcium and manesium in *Anodonta* muscle (Table 8) must be largely combined with organic molecules.

CHANGES IN THE COMPOSITION OF THE MUSCLE IN RESPONSE TO A FALL IN THE CONCENTRATION OF THE BLOOD

Mytilus occurs in estuaries and can tolerate considerable changes in the composition of the blood. The sodium, potassium and chloride contents of muscles from *Mytilus* which had been living for at least 2 weeks in 50% sea water are given in Table 12.

Table 12. *Sodium, potassium and chloride content of some tissues of* Mytilus *living in* 50% *sea water*

(mM./kg. total water.)

	Na	K	Cl
Sea water	245	5·2	287
Blood	245	7·4 ± 0·26 (12)	286
Fast adductor	58·0 ± 6 (14)	91·4 ± 5 (14)	60·0 ± 4 (10)
Slow adductor	82·5 ± 5 (12)	76·6 ± 6 (12)	74·6 ± 4 (10)
Byssus retractor	79·8 ± 6 (12)	85·5 ± 4 (12)	92·5 ± 6 (10)

Mean ± standard error (no. of observations).

The percentage dry weight of the muscles is less than that of muscles from normal sea water (Table 3), and the inulin spaces have also been reduced (Table 6), so the individual muscle fibres must be osmotically swollen. From the figures in Tables 3 and 6 it follows that, when allowance is made for the solids in the blood, 100 g. of solids are associated with 247 g. of water in the fibres of the fast adductor of *Mytilus* from normal sea water, but are associated with 311 g. of water in animals from 50% sea water. Similarly, in the byssus retractor 100 g. of solids are associated with 266 g. of water in normal sea water and 319 g. in 50% sea water. In each case the distension is much less than the doubling of water content which would occur if the muscles behaved as simple osmometers. The fall in the potassium concentration of the muscles is a little greater than that caused by the osmotic uptake of water alone, but the fall in the sodium and chloride concentrations is very much greater. In the *Mytilus* muscles in normal sea water the average intracellular concentrations of sodium and chloride are 88 and 87 mM./kg. water respectively (Table 7). In 50% sea water these concentrations have fallen to 30 and 22 mM./kg. water.

The amino-acid content of the muscle also falls by an amount greater than that due to water uptake alone. In the normal ventral adductor the intracellular concentration of free amino acids is 295 ± 6 mM./kg. water (Table 10). In 50% sea water this falls to 183 ± 6 mM./kg. water. The uptake of water should have reduced the concentration to 234 mM./kg. water; the remainder of the reduction must be caused by the excretion, polymerization or metabolism of the amino acids. In the byssus retractor the intracellular concentration of free amino acids is 273 ± 6 mM./kg. water when the animals are in sea water, and only 166 ± 6 mM./kg. water when the

animals are in 50% sea water (Table 10). The osmotic swelling of the muscle fibres alone would reduce the concentration to 228 mM./kg. water. The concentration of free amino acids in the blood has risen to 5·4 mM./kg. water when the animal is in 50% sea water from 2·5 mM./kg. water in normal sea water. Some of the amino acids in the blood are probably derived from the muscles.

Analyses of *Anodonta* which had been living for 10 days in 18% sea water are recorded in Tables 3, 6, 7, 10 and 13. The percentage dry weights of the muscles

Table 13. *Sodium, potassium and chloride content of some tissues from* Anodonta *living in* 18% *sea water*

(mM./kg. total water.)

	Na	K	Cl
Sea water	87·0	1·92	102
Blood	84·0 ± 3 (6)	0·72 ± 0·1 (8)	75 ± 3 (6)
Fast adductor	64·6 ± 2·1 (6)	26·1 ± 0·4 (10)	48·1 ± 2·0 (6)
Slow adductor	65·8 ± 3·1 (6)	20·0 ± 0·4 (10)	59·2 ± 1·7 (6)
Ventricle	63·0 ± 4·2 (6)	27·7 ± 0·9 (10)	—

Mean ± standard error (no. of observations).

and the inulin spaces have increased in 18% sea water. In an *Anodonta* from tap water 100 g of dry matter in the ventral adductor are associated with 440 g. of intracellular water. In 18% sea water this has fallen to 187 g. of intracellular water. In the dorsal adductor the figures are 415 and 204 g. respectively. Exosmosis has removed over half the fibre water. Correspondingly, the intracellular concentrations of potassium have also doubled in 18% sea water (Table 7), but the intracellular concentrations of sodium and chloride have increased eight- or tenfold.

In both *Anodonta* and *Mytilus* adaptation to a changed osmolar concentration is brought about partly by water movement into or out of the muscle fibres, and partly by the increase or decrease of the total content of sodium, chloride and free amino acids in the muscles.

DISCUSSION

Sodium, potassium and chloride equilibria

In many of the muscles analysed the distribution of potassium and chloride across the fibre membrane appears to differ significantly from a Donnan equilibrium. For example, in the byssus retractor muscle of *Mytilus* from normal sea water the potassium concentration ratio between the inside and the outside of the fibres is 12·2 ± 0·4, while the chloride is 3·9 ± 0·3. Similarly, in *Anodonta* slow adductor the potassium ratio is 38 ± 3·0, while the chloride ratio is only 2·0 ± 0·35 (Table 14). In vertebrate striated muscle, in some invertebrate striated muscle and in many vertebrate and invertebrate nerves it is well established that the distribution of potassium and chloride approximates to a Donnan equilibrium (Boyle & Conway, 1941; Hodgkin, 1951; Shaw, 1955). The last two columns of figures in Table 14 therefore require careful consideration. The fast adductor of *Pecten*, the only

Table 14. *Concentration ratios of sodium, potassium and chloride ions inside and outside some lamellibranch muscle fibres*

	$\frac{Na_o}{Na_i}$	$\frac{K_i}{K_o}$	$\frac{Cl_o}{Cl_i}$
Pecten			
Fast adductor	11·4 ± 1·2	12·8 ± 0·5	16·8 ± 2·0
Slow adductor	6·0 ± 0·8	13·0 ± 0·6	6·9 ± 1·1
Mytilus			
Fast adductor	6·2 ± 1·0	12·2 ± 0·6	6·1 ± 1·1
Slow adductor	5·5 ± 0·9	11·8 ± 0·6	5·0 ± 0·9
Byssus retractor	5·2 ± 0·9	12·2 ± 0·4	3·9 ± 0·3
Ventricle	6·7 ± 2·5	12·6 ± 0·4	10·2 ± 7·2
Mytilus, 50% sea water			
Fast adductor	9·1 ± 2·5	14·3 ± 0·8	12·0 ± 1·3
Slow adductor	6·4 ± 1·6	13·0 ± 1·2	16·8 ± 12·8
Byssus retractor	10·2 ± 4·4	15·1 ± 10·7	10·2 ± 3·8
Anodonta			
Fast adductor	2·8 ± 0·36	47 ± 3	4·5 ± 1·3
Slow adductor	2·0 ± 0·31	38 ± 3	2·0 ± 0·35
Anodonta, 18% sea water			
Fast adductor	1·67 ± 0·18	61 ± 2·7	2·58 ± 0·4
Slow adductor	1·67 ± 0·25	63 ± 4·5	1·36 ± 1·5

muscle in the table comparable to vertebrate striated muscle or to *Carcinus* muscle in physical properties, has potassium and chloride concentration ratios close to a Donnan equilibrium. In the remaining muscles it is generally the slower muscles which differ most from the equilibrium conditions. Either the principles governing ionic equilibrium in lamellibranch smooth muscles differ from those in striated muscle, or some important factor has been neglected in calculating the results in Table 7.

In every case the apparent high concentration of chloride inside the fibres is associated with a high concentration of sodium. If the inulin space is not identical with the extracellular fluid, but is for some reason somewhat smaller, then the calculated concentrations of sodium and chloride inside the fibres will be too high. For example, if it is assumed that all the sodium in the byssus retractor muscle of *Mytilus* is extracellular, then the extracellular space would amount to 43% of the total water content, the intracellular chloride concentration would be reduced to 46 mM./kg. water and the intracellular potassium would be increased to 191 mM./kg. water which would be close to the conditions of a Donnan equilibrium. This could be the case if the muscle contains a third phase, neither blood nor sarcoplasm, inaccessible to inulin but containing large quantities of sodium and chloride. Such a third phase could be formed either by connective tissue between the fibres or by a specialized region of the fibres such as the sarcolemma. In the vertebrates connective tissue contains large quantities of sodium and chloride (Manery & Hastings, 1939). Olson (1938) described connective tissue between the fibres of *Thyone* muscle, which he correlated with the high chloride content of the muscle, but an histological examination of *Mytilus* byssus retractor by the present author,

using the same technique as Olsen (that is, fixing with Bouin and staining with Mallory's triple stain) revealed only negligible amounts of connective tissue.

Carey & Conway (1954) have described experiments on frog sartorii from which they conclude that most of the non-extracellular sodium is in the sarcolemma and only a small quantity is in the sarcoplasm. In order to account for the discrepancy between the observed and the equilibrium concentrations in *Mytilus* byssus retractor the sarcolemma would have to contain the very large quantity of 100 mM. Cl/kg. muscle. It is difficult to reconcile the potassium and chloride content of lamellibranch smooth muscle with Conway's model of a simple Donnan equilibrium.

In a recent review Robertson (1957) has discussed the distribution of potassium and chloride ions in the muscles of a number of invertebrates. On the basis of his own analysis of *Nephrops*, Shaw's analysis of *Carcinus* (1955) and Krogh's analysis of *Mytilus* and *Eriocheir* (1939) he concludes that only in *Carcinus* does the distribution approximate to a Donnan equilibrium. In the remaining animals the chloride concentration inside the fibres exceeds the equilibrium concentration.

Hodgkin (1958) has discussed ionic equilibria in systems in which the permeability to sodium is low compared with the permeability to potassium but not completely negligible, and in which a neutral pump operates absorbing one ion of potassium for each ion of sodium extruded. In these circumstances the equilibrium potential E is given by a modified Nernst equation

$$E=\frac{RT}{F}\ln\frac{[K_o]+b[Na_i]}{[K_i]+b[Na_o]},$$

where R is the universal gas constant, T is the absolute temperature, F is the faraday, K_o and Na_o; K_i and Na_i the concentrations of potassium and sodium outside and inside the system respectively and b is the permeability to sodium relative to the permeability to potassium. If the chloride ion is under no restraint other than the resting potential then

$$E=\frac{RT}{F}\ln\frac{[Cl_i]}{[Cl_o]},$$

and

$$\frac{[K_o]+b[Na_o]}{[K_i]+b[Na_i]}=\frac{[Cl_i]}{[Cl_o]}.$$

Values of b have been calculated for the analysed muscles from the data in Tables 1, 4, 5, 7, 12 and 13 on the assumption that these conditions apply in lamellibranch muscle (Table 15). b increases with decreasing speed of muscle. It does not differ significantly from zero in the fast adductor of *Pecten* but rises to $0{\cdot}059 \pm 0{\cdot}007$ in the byssus retractor of *Mytilus* and to $0{\cdot}71 \pm 0{\cdot}14$ in the slow adductor of *Anodonta*. In *Mytilus* from 50% sea water the standard errors have accumulated so much that the results are hardly significant, but the values of b calculated for *Anodonta* from 18% sea water are very close to those calculated for *Anodonta* from fresh water. This is not the only possible system consistent with the experimental results but as the potassium and chloride ions are not in a Donnan equilibrium any alternative explanation must involve the active transport of either potassium or chloride or both in addition to the transport of sodium.

Table 15. $b = \frac{\textit{permeability to potassium}}{\textit{permeability to sodium}}$ *in lamellibranch muscle, calculated from Tables* 9 *and* 10

Animal	Tissue	b
Pecten, 100% sea water	Fast adductor	-0.006 ± 0.003
	Slow adductor	0.023 ± 0.008
Mytilus, 100% sea water	Fast adductor	0.026 ± 0.009
	Slow adductor	0.036 ± 0.011
	Byssus retractor	0.059 ± 0.007
	Ventricle	0.006 ± 0.016
Mytilus, 50% sea water	Fast adductor	0.006 ± 0.010
	Slow adductor	-0.007 ± 0.012
	Byssus retractor	0.013 ± 0.014
Anodonta, fresh water	Fast adductor	0.32 ± 0.09
	Slow adductor	0.71 ± 0.14
Anodonta, 18% sea water	Fast adductor	0.26 ± 0.04
	Slow adductor	0.69 ± 0.09

SUMMARY

1. Measurements have been made of the inorganic ion and free amino acid content of a number of lamellibranch muscles. The volumes of extracellular fluid in the muscles have also been determined so that the intracellular concentrations can be calculated.

2. The fast portion of the adductor muscle of *Pecten* contains about 160 mM. K/kg. fibre water and only 43 mM. Na/kg. fibre water and 34 mM. Cl/kg. fibre water. The potassium and chloride are approximately in a Donnan equilibrium with the potassium and chloride in the blood.

3. In the slow portion of the adductor muscle of *Pecten* and in the two parts of the adductor and in the byssus retractor of *Mytilus*, the concentrations of potassium in the fibres are from 150 to 160 mM./kg fibre water, of sodium 73 to 95 mM./kg. fibre water and chloride 94 to 152 mM./kg. fibre water. The potassium and chloride in the fibres are not in a Donnan equilibrium with the potassium and chloride in the blood.

4. The fast and slow fibres of the adductor muscles of *Anodonta* contain 21 and 17 mM./kg. fibre water of potassium respectively, 5·3 and 7·2 mM./kg. fibre water of sodium, and 2·4 and 5·4 mM./kg. fibre water of chloride. The potassium and chloride in the fibres is not in a Donnan equilibrium with the potassium and chloride in the blood.

5. The fast fibres of *Mytilus* adductor contain 295 mM./kg. fibre water of free amino acids and 39 mM./kg. fibre water of acid-soluble phosphate compounds. The fast fibres of *Anodonta* adductor contain only 11 mM./kg. fibre water of amino acids and 19·8 mM./kg. fibre water of phosphate compounds.

6. *Mytilus* muscles fibres adapt to a reduced blood concentration, partly by an increase in water content and partly by a reduction in the sodium, chloride and free amino acid content.

7. *Anodonta* muscle fibres adapt to an increased blood concentration, partly by a reduction in the water content and partly by an increased sodium and chloride content.

8. The significance of these results is discussed. It is concluded that the ionic contents of the lamellibranch smooth muscles are consistent with equilibria systems in which the permeability to sodium is significant compared with the permeability to potassium and in which both a sodium and a potassium pump operate.

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