THE SODIUM FLUXES IN THE MUSCLE FIBRES OF A MARINE AND A FRESHWATER LAMELLIBRANCH

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INTRODUCTION

The body fluids of most animals are rich in sodium whilst the tissues are generally rich in potassium and poor in sodium. In those tissues which have been examined in detail, such as striated muscle, nerve or red blood cells, it has been found that the ionic contents of the cells are determined by the active extrusion of sodium and sometimes by the active accumulation of potassium, combined with a Donnan equilibrium of some of the ions. The active extrusion of sodium from a cell against an electrochemical potential requires energy. This energy can be calculated for any tissue from the electrochemical potentials of the sodium in the intracellular and extracellular fluids and from the rate of extrusion of sodium from the tissue.

The sodium concentrations in the blood of marine invertebrates are very much higher than in the blood of freshwater and terrestrial animals, and for this reason it seemed probable that the flux of sodium through the tissues of a marine invertebrate would be greater than the flux of sodium through the comparable tissues of a freshwater animal. The energy required to extrude the sodium from the cells might be considerably greater in a marine invertebrate than in a freshwater form as the energy required is a product of the flux of sodium and the electrochemical potentials of the sodium.

Tracer techniques have been used to measure the movement of sodium through amphibian and mammalian muscle (Keynes, 1954; Creese, 1954) and vertebrate and invertebrate nerve (Dainty & Krnjevic, 1955; Keynes, 1951, etc.), but no results are available for comparable tissues of a related marine and freshwater invertebrate. This paper records the results of experiments designed to measure the rate of exchange of sodium in the muscles of a marine lamellibranch, *Mytilus edulis*, in which the sodium concentration in the blood is similar to that of sea water, and of a freshwater lamellibranch, *Anodonta cygnaea*, in which the concentration of sodium in the blood is less than 3% of that in sea water.

MATERIALS

The rate of exchange of sodium between the cells and the blood was determined by equilibrating a piece of muscle with a saline containing ^{24}Na and then measuring the declining activity of the sodium in the muscle when the muscle was exposed to

a current of tracer-free saline. In order to determine the rate constant of the exchange of sodium in the intracellular phase it is necessary that the loss of ^{24}Na by diffusion from the extracellular phase should be very rapid, otherwise some of the labelled sodium leaving the cells may re-enter other cells instead of escaping from the tissue. The slower the rate of loss from the extracellular fraction the greater the error from this cause. If the rate of loss from the extracellular phase is too low it becomes impossible to distinguish between the intracellular and the extracellular phases.

A suitable tissue for the experiment must have two properties. It must be in the form of a thin sheet so that the rate of loss from the extracellular phase will be rapid, and it must survive well *in vitro* so that the ionic composition at the end of the experiment is similar to that of the fresh tissue, otherwise the results are of doubtful value. Preliminary experiments with *Mytilus byssus* retractor and with slices of the adductor muscles of *Mytilus* and *Anodonta* showed that these tissues were unsuitable as they lost a large part of their potassium during one hour's perfusion with saline. However, the ventricles of both *Anodonta* and *Mytilus* fulfilled the necessary requirements. They are easily isolated (Pilgrim, 1953) and consist of thin sheets of muscle less than 1 mm. thick even when contracted. They maintain an almost constant sodium and potassium content for several hours after isolation (Tables 2, 3), and according to Pilgrim will maintain mechanical activity for several days.

PROCEDURE

The ventricles were isolated by Pilgrim's method and suspended by a fine nylon thread in the active saline which was kept stirred at 15° C. After 2 hr. in the active solution the ventricles were removed, blotted carefully to remove surface fluid and immersed in a current of tracer-free saline in a pyrex tube 5 mm. in diameter. Preliminary experiments showed that the activity associated with the nylon thread was less than 1% of the total activity and was washed away in less than 1 min. The isolated ventricle showed some tendency to roll into a tube so it was essential to maintain a high rate of flow of saline to ensure an adequate washing of all the surfaces of the muscle. The normal rate of flow was 1·0 ml./sec. and tests with coloured solutions showed that the saline was completely replaced about every 15 sec.

Counting was by a G.M. 4 end-window counter and a scaler. Corrections were made for the dead time of the counter, the activity of the background and the decay of the ^{24}Na.

At the end of the experiment the ventricle was analysed for sodium and potassium. The muscle was weighed, dissolved in a drop or two of concentrated nitric acid, evaporated to dryness on a water bath and the residue dissolved in 5 or 10 ml. of distilled water. The sodium and potassium contents were measured by an EEL flame photometer. Experiments were carried out at both 5° and 15° C.

SOLUTIONS

Mytilus blood is similar in composition to sea water but contains more potassium to the extent of about 2 mM/kg. water (Potts, 1954). *Mytilus* ventricle was eluted with filtered sea water to which had been added 2 mM/l. of KCl and 1 mM/l. of

glucose. The final solution contained 480 mM Na/l., 12·1 mM K/l. and 560 mM Cl/l. The pH was in the range 7·8–8·1.

The radioactive saline containing ^{24}Na was prepared by dissolving 20 mg. of irradiated sodium carbonate, pile factor 10, in excess N-HCl and evaporating to dryness and then dissolving in 0·66 ml. of water containing 12·1 mM KCl/l. This produced a solution containing about 570 mM NaCl/l. and 12·1 mM KCl/l. which was then diluted to 10 ml. with the non-radioactive saline to produce a balanced salt solution. The pH of both solutions was always in the range 7·5–8·0.

Anodonta muscle was eluted with a saline containing 14 mM/l. NaCl, 0·5 mM/l. KCl, 5 mM/l. $CaCl_2$, 0·25 mM/l. Na_2HPO_4, and 1 mM/l. glucose. The pH was adjusted, with dilute NaOH, to 7·5. The solution resembles the average composition of *Anodonta* blood except that Cl^- has been substituted for HCO_3^-. *Anodonta* blood normally contains about 10 mM/l. of bicarbonate, but solutions containing so much bicarbonate are unstable and lose CO_2 to the atmosphere.

The radioactive saline was prepared by dissolving 20 mg. of irradiated sodium carbonate, pile factor 10, in 10 ml. of water and adding sufficient 0·1 N-HCl to bring the pH to 7·5. 1 ml. of 12 mM/l. KCl solution and 2 ml. of 65 mM/l. $CaCl_2$ solution were added and the solution was diluted to 26 ml. The final concentrations were: Na, 14·5 mM/l., K, 0·5 mM/l., and Ca, 5 mM/l. The composition closely resembled the average composition of *Anodonta* blood (Potts, 1954) but the solution slowly lost carbon dioxide and became more alkaline. HCl was added at intervals to keep the pH between 7·5 and 8·0.

EXTRACELLULAR SPACE AND INORGANIC COMPOSITION OF THE VENTRICLES

In a previous paper (Potts, 1958) details have been given of the inulin space, water content and inorganic composition of *Mytilus* and *Anodonta* ventricles. The water content of *Mytilus* ventricle is $80{\cdot}8 \pm 0{\cdot}6\%$ (w/w) and of *Anodonta* ventricle $87{\cdot}8 \pm 0{\cdot}8\%$. The inulin space of *Mytilus* ventricle is $26{\cdot}0 \pm 3{\cdot}4\%$ of the total water content and of *Anodonta* ventricle $30{\cdot}5 \pm 2{\cdot}5\%$. The intracellular concentrations of sodium, potassium and chloride in the two muscles are given in Table 1 together with the average composition of the extracellular fluids. The intracellular chloride content of *Anodonta* ventricle is unfortunately too small to be determined with accuracy.

DIAMETERS OF MUSCLE FIBRES

The average diameters of the muscle fibres of the ventricles of both *Mytilus* and *Anodonta* were determined so that the sodium fluxes through the fibre membranes could be calculated. The ventricles of both *Mytilus* and *Anodonta* are so thin that with good illumination the diameter of the individual fibres can be measured directly in fresh tissue.

The diameters of the muscle fibres were measured as follows. A ventricle was extended under a cover-slip and observed with a $\frac{1}{12}$ in. water-immersion objective.

Table 1. *The sodium, potassium and chloride content of the ventricle and blood of* Mytilus edulis *and of* Anodonta cygnaea (*from Potts*, 1958)

(mM/kg. water content)

	Na	K	Cl
Mytilus			
Whole ventricle	181 ± 10	120 ± 4	190 ± 9
Muscle fibres	73 ± 26	158 ± 4	56 ± 29
Blood	490	12·5 ± 0·2	573
Anodonta			
Whole ventricle	9·5 ± 0·2	10·5 ± 0·6	—
Muscle fibres	7·1 ± 0·7	14·9 ± 0·9	—
Blood	14·7 ± 1·3	0·45 ± 0·014	10·7 ± 1·0

The diameters of twenty adjacent fibres lying in one transect were then measured with a calibrated graduated eye-piece. This was repeated for four ventricles of *Mytilus* and four of *Anodonta*.

Errors may arise for the following reasons. When the fibres are crowded together some confusion may occur between the edges of the fibres. The sites chosen for the transects are necessarily ones where the fibres are well spaced and therefore perhaps not typical of the ventricles. The pressure of the cover-slip may extend the ventricle and therefore slightly reduce the diameters of the fibres. These errors are not likely to be very large and the method is preferable to fixing and staining the tissues which usually involves some shrinkage of the fibres.

The average diameter of the muscle fibres of *Mytilus* ventricle was $9{\cdot}5 \pm 0{\cdot}3\mu$ and of *Anodonta* ventricle $13{\cdot}5 \pm 0{\cdot}7\mu$.

THE CONCENTRATIONS OF SODIUM AND POTASSIUM IN THE TISSUES DURING THE COURSE OF THE EXPERIMENTS

The concentrations of sodium and potassium in the ventricles used in the experiments are given in Tables 2 and 3. In some of the experiments both the initial and final sodium and potassium concentrations of the ventricles were measured, but in most experiments the whole of the ventricle was used and so only the final concentrations could be determined. The sodium content of *Anodonta* blood is rather variable and some of the changes in the sodium content of the ventricle during the course of the experiment may be caused by differences between the sodium content of the blood and the eluting saline. The results in Tables 2 and 3 show clearly that the ionic contents of the ventricles at the end of the experiments were similar to those of fresh material. In particular there is practically no fall in the potassium content of either *Mytilus* or *Anodonta* ventricles. The sodium content of the eluting saline for *Mytilus* ventricle, 480 mM/l., was not identical with the sodium content of the blood of *Mytilus* quoted in Table 1, namely 490 mM/kg. water. Any effect this may have had on the intracellular concentration of sodium has been neglected in the subsequent calculations in which it has been assumed that the intracellular concentrations of the ions are those given in Table 1.

Table 2. *The sodium and potassium content of the ventricles of* Mytilus edulis *used in the experiments*

(mM/kg. water)

Mytilus (no.)	Before experiment		After experiment	
	Na	K	Na	K
1	194	128	230	145
2	261	119	193	155
3	132	122	199	125
4	—	—	279	105
5	—	—	185	150
6	—	—	268	125
7	—	—	203	133
8	—	—	192	137
Mean	195	123	219	134

Table 3. *The sodium and potassium content of the ventricles of* Anodonta cygnaea *used in the experiments*

(mM/kg. water)

Anodonta (no.)	Before experiment		After experiment	
	Na	K	Na	K
1	—	—	11·8	14·4
2	10·8	7·6	9·8	8·8
3	7·6	10·4	10·8	5·5
4	10·2	10·5	9·0	12·0
5	8·6	10·9	12·4	11·1
6	11·7	8·9	7·8	8·9
7	8·2	10·3	9·0	8·6
8	8·3	10·1	7·4	10·4
9	—	—	7·0	9·2
10	—	—	10·6	6·8
Mean	9·3	9·8	9·6	9·6

SODIUM FLUXES IN *MYTILUS* AND *ANODONTA* VENTRICLES

At 5° C. in all experiments the time course of the decay of the activity of the muscle, when eluted with a non-radioactive saline, approximates to the sum of two exponentials which may be represented by the expression $Ae^{-K_1 t}+Be^{-K_2 t}$. This is clearly seen when the results are plotted semi-logarithmically (Figs. 1, 3). The more rapidly declining part of the activity may be attributed to the sodium in the extracellular spaces, while the more slowly exchanging part may be attributed to the intracellular sodium (see Appendix). At 5° C. the distinction between the two parts is quite clear and after about 10 min. the activity in the extracellular sodium has become insignificant and the activity of the muscle declines as a simple exponential function of the time, $Be^{-K_2 t}$. At 15° C. (Fig. 2) the rate of exchange of sodium between the fibres and the extracellular fluid is considerably faster than

at 5° C. (Fig. 1), but the rate of diffusion from the extracellular spaces is not appreciably altered and the distinction between the two phases is not so clear. The rate of turnover of sodium inside the fibres of *Anodonta* muscle is rather faster than

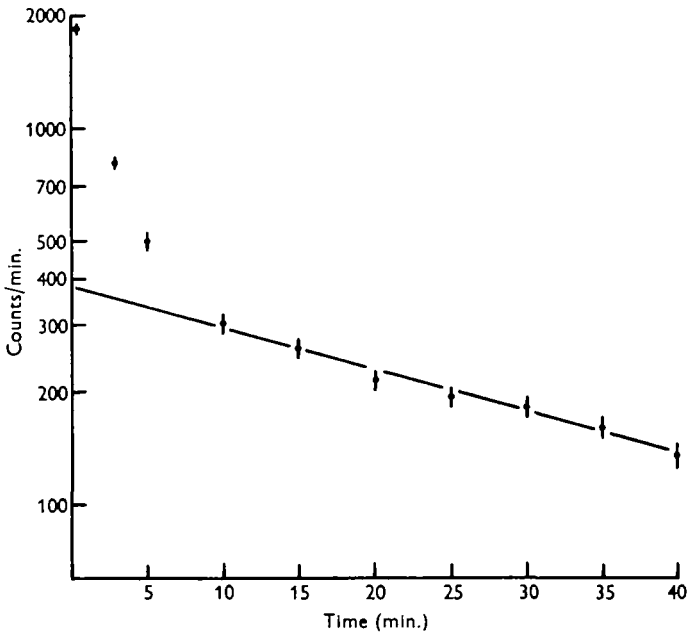

Fig. 1. The loss of ^{24}Na from *Mytilus* ventricle no. 2 when washed in inactive saline at 5° C. The straight line represents the loss from the fibres.

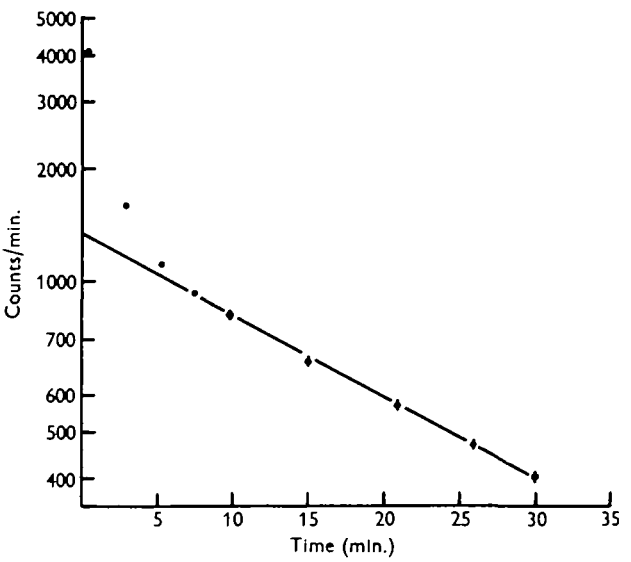

Fig. 2. The loss of ^{24}Na from *Mytilus* ventricle no. 10 when washed in inactive saline at 15° C. The straight line represents the loss from the fibres.

in *Mytilus* muscle and at 15° C. the extracellular and intracellular parts are not distinguishable, although at 5° C. they are still clear (Fig. 3). For this reason the sodium flux in *Anodonta* muscle at 15° C. could not be measured.

After the first ten minutes, in all experiments, the activity declines exponentially and so all the points lie on a straight line, $Be^{-K_2 t}$. By extrapolating this line back to $t = 0$, B can be obtained. The difference between the experimental curve and the straight line represents, to a first approximation, the diffusion of sodium from the extracellular fluid. The diffusion of a substance into, or out of, a thin sheet has

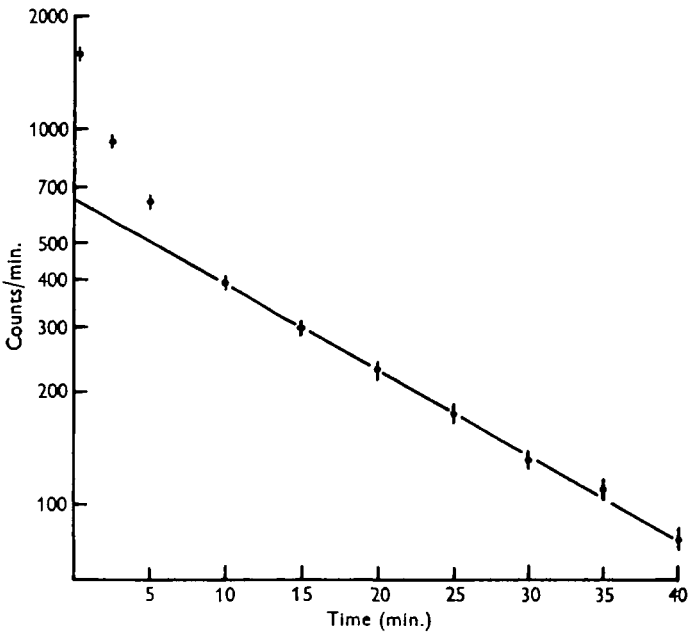

Fig. 3. The loss of ^{24}Na from *Anodonta* ventricle no. 4 when washed with inactive saline at 5° C. The straight line represents the loss from the fibres.

been investigated by Hill (1928). The rate of decline is most rapid at first but the fall becomes a simple exponential after about one-quarter of the substance has diffused out. In these experiments A and K_1 have been calculated on the assumption that the loss from the extracellular spaces is a simple exponential function of time since the sodium in the extracellular water declined so rapidly that the exact shape of the curve could not be determined.

The results of the experiments are summarized in terms of K_1, K_2, A and B in Table 4. In all cases the rate constant K_1 is several times larger than K_2. K_1 is almost independent of temperature while K_2 is lower at 5° C. than at 15° C.

CALCULATION OF SODIUM FLUXES IN MUSCLE FIBRES

The analysis of the efflux of sodium from a muscle containing both an extracellular and an intracellular phase is complicated because most of the fibres communicate not with the tracer-free saline but with the intracellular fluid which contains a

degree of radioactivity depending on K_1, K_2, A and t. Some of the ions leaving the fibres re-enter the fibres before they are swept away.

For this reason the apparent rate constant for the efflux of sodium from the fibres, K_2, will be less than the real rate constant k_2.

The mathematics of this system has been discussed by Harris & Burn (1949) and Keynes (1954) and Keynes has derived equations relating k_2 to K_2. Unfortunately these equations involve the thickness of the muscle and both *Mytilus* and *Anodonta* ventricles are so variable in thickness that it is convenient to rearrange the equations to eliminate the thickness of the muscle and some other quantities (see Appendix). k_2 may then be calculated from K_1, K_2, C_0, C_i and ϵ, where C_0 and C_i are the extracellular and intracellular concentrations of sodium and ϵ is the fraction by volume of the extracellular fluid.

The equations are only strictly applicable to a plane sheet of muscle and are not completely accurate even for that simple case, but the corrected values k_2 are probably to be preferred to the uncorrected K_2. The corrected values of the rate constant of the efflux from the intracellular fraction, k_2, are given in Table 4.

Using the corrected values of k_2, M, the flux of sodium through unit area of fibre surface can be calculated from the expression

$$M = k_2 \frac{V}{A} C_i \quad \text{(Keynes, 1954, eq. 1).}$$

Table 4. *Constants of the efflux of sodium from lamellibranch ventricles. For details see text*

No.	Temp.	A cts/min.	B cts/min.	$\frac{B}{A+B}$	K_1 hr.$^{-1}$	K_2 hr.$^{-1}$	k_2 hr.$^{-1}$
Mytilus	5° C.						
2		1476	380	0·204	11·1	1·50	1·69
3		1790	560	0·207	8·0	2·61	3·08
8		348	520	0·60	13·9	2·52	2·74
4		1830	1260	0·41	6·9	2·37	2·80
9		1260	930	0·42	5·3	1·64	1·91
Mean							2·44
5	15° C.	1780	1100	0·38	5·9	4·87	7·0
6		566	302	0·35	5·8	4·53	6·7
7		589	151	0·20	6·4	3·88	5·2
10		2620	1355	0·34	10·0	3·03	3·4
Mean				0·35			5·6
Anodonta	5° C.						
1		890	1510	0·63	8·3	3·80	6·9
2		1780	1160	0·39	6·9	2·66	4·4
4		930	650	0·41	6·4	3·08	5·5
6		510	1670	0·77	8·1	3·18	5·3
7		623	722	0·54	6·9	3·42	6·5
8		420	580	0·58	6·6	3·33	6·5
9		306	258	0·46	5·2	1·51	2·2
10		2250	3850	0·63	8·3	1·92	2·6
11		2900	3050	0·51	12·9	1·51	1·7
Mean				0·55			4·6

For an infinite cylinder $V/A = \frac{1}{2}r$, where r is the radius of the cylinder.

For *Mytilus* $r = 4{\cdot}75\mu$,

$C_i = 73$ mM/kg. water,

$k_2 = 2{\cdot}44$ hr.$^{-1}$ at 5° C.

$= 5{\cdot}6$ hr.$^{-1}$ at 15° C.

Hence $M = 12 \times 10^{-6}$ mM cm.$^{-2}$ sec.$^{-1}$ at 5° C.

$= 27 \times 10^{-6}$ mM cm.$^{-2}$ sec.$^{-1}$ at 15° C.

For *Anodonta*, $r = 6{\cdot}75\mu$,

$C_i = 7{\cdot}2$ mM/kg. water,

$k_2 = 4{\cdot}6$ hr.$^{-1}$ at 5° C.

Hence $M = 3{\cdot}1$ mM-cm.$^{-2}$ sec.$^{-1}$ at 5° C.

THE ENERGY REQUIRED FOR SODIUM EXTRUSION

The sodium removed from the muscle is secreted against both a concentration gradient, E_{Na}, and an electrical potential, E_v.

If it is assumed that the efflux of sodium is entirely an active extrusion, uncomplicated by an exchange diffusion, and if it is also assumed that the activity coefficient of the sodium inside the fibres is the same as the activity coefficient of the sodium outside the fibres, then the secretory work, W, is given by the expression

$$W = k_2 F(E_{Na} + E_v), \tag{1}$$

where

$$E_{Na} = \frac{RT}{F} \ln \left[\frac{Na_0}{Na_i}\right], \tag{2}$$

R is the universal gas constant, F the faraday, Na_i the intracellular concentration of sodium and Na_0 the extracellular sodium concentration.

E_v has not been measured in lamellibranch muscle but can be calculated approximately from the potassium and chloride concentration gradients across the sarcolemma. The distribution of potassium and chloride in *Mytilus* ventricle is close to a Donnan equilibrium in which $K_i/K_0 = Cl_0/Cl_i$ (Potts, 1958), and the resting potential may be calculated from the equation

$$E_v = \frac{RT}{F} \ln \left[\frac{K_i}{K_0}\right]. \tag{3}$$

Unfortunately it is not possible to measure accurately the intracellular concentration of chloride in *Anodonta* ventricle and there is some evidence (Potts, 1958) that the potassium-ion concentration gradient between the inside and the outside of some lamellibranch muscle fibres is greater than the concentration gradient of chloride ions. In these cases the electrical potential of the fibres may be less than the equilibrium potential of the potassium. However, in the absence of further information, it will be assumed that the potassium is in equilibrium with the resting potential.

After substituting in equation (1) for E_{Na} and E_v with the aid of equations (2) and (3)

$$W = k_2 Na_i RT\left(\ln\left[\frac{Na_0}{Na_i}\right] + \ln\left[\frac{K_i}{K_0}\right]\right).$$

For *Mytilus*, $Na_i = 73$ mM/kg. water,
$Na_0 = 480$ mM/kg. water,
$K_i = 150$ mM/kg. water,
$K_0 = 12{\cdot}1$ mM/kg. water,
$k_2 = 2{\cdot}44$ hr.$^{-1}$ at 5° C.
$k_2 = 5{\cdot}6$ hr.$^{-1}$ at 15° C.

Hence $W = 440$ cal./kg. fibre water/hr. at 5° C.
$= 1030$ cal./kg. fibre water/hr. at 15° C.

1 kg. of *Mytilus* ventricle contains 807 g. of water of which 26% is extracellular, so that 1 kg. of ventricle contains only 600 g. of fibre water. Hence

$W = 0{\cdot}265$ cal./g./hr. at 5° C.
$= 0{\cdot}62$ cal./g./hr. at 15° C.

For *Anodonta*, $Na_i = 7{\cdot}2$ mM/kg. water,
$Na_0 = 14{\cdot}0$ mM/kg. water,
$K_i = 14{\cdot}9$ mM/kg. water,
$K_0 = 0{\cdot}5$ mM/kg. water,
$k_2 = 4{\cdot}6$ hr.$^{-1}$.
Water content = 878 g./kg. muscle.
Extracellular space = 30·5% water content.

Hence $W = 0{\cdot}046$ cal./g./hr.

DISCUSSION

Most of the previous measurements of sodium fluxes have been made either on vertebrate tissues, in which the extracellular concentration of sodium is of the order of from 100 to 150 mM/l. or on *Sepia* axons in which surface/volume ratio of the cells is much smaller than in the lamellibranch muscle fibre, so those results are not exactly comparable with the results reported here. The sodium fluxes per unit area of the fibre surface of the lamellibranch muscles are of the same order as those reported for vertebrate muscles. At 5° C. the sodium flux through *Mytilus* ventricle fibre is about 12×10^{-6} mM/cm.^{2}/sec. and at 15° C. is about 27×10^{-6} mM/cm.^{2}/sec. Harris & Burn (1949) and Keynes (1954) reported sodium fluxes of 10 and $5{\cdot}4 \times 10^{-6}$ mM/cm.^{2}/sec. at 16° and 17° C. respectively, through the fibres of the frog sartorius. The difference between the frog and the marine lamellibranch may well arise from the much greater concentration of sodium in *Mytilus* blood. In *Anodonta* the sodium flux, $3{\cdot}1 \times 10^{-6}$ mM/cm.^{2}/sec. is less than in the frog. In the frog sartorius the rate constant k_2 for the exchange of sodium is much smaller

than in *Mytilus* but the fibres are much larger with a diameter of about 80μ. In the rat diaphragm the fibres are only about 20μ in diameter and k_2 is 3·75 hr.$^{-1}$ at 37° C. (Creese, 1954). In the rat diaphragm the sodium flux/unit area of fibre surface at 37° C. is as high as in *Mytilus* at 15° C., 27 mM/cm.^{2}/sec. The 20° C. temperature difference compensates for the fourfold difference in sodium concentrations in the external fluids. The only measurements of sodium fluxes in tissues for marine animals are of the giant axons of *Sepia* where the sodium flux through the surface of the axon is even larger than in *Mytilus* muscle and amounts to 40 mM/cm.^{2}/sec. during recovery from stimulation (Hodgkin & Keynes, 1954).

Although the sodium fluxes per unit surface area of lamellibranch muscles are comparable with those reported for other tissues, the theoretical energy required to maintain the flux is much larger in *Mytilus* than any previously reported. This is the result of the combination of a very narrow fibre, and hence a large surface volume ratio with a high concentration of sodium in the blood. The theoretical energy requirements of *Mytilus* muscle at 15° C. is 0·62 cal./g./hr. Keynes & Maisel (1954) calculated that in frog muscle only about 0·04 cal./g./hr. were required. Hodgkin & Keynes (1954) estimated that *Sepia* axons required about 0·08 cal./g./hr. In *Anodonta* muscle, where the ambient sodium concentration is much lower, only about 0·046 cal./g./hr. are required.

Levi & Ussing (1948) considered that the efflux of sodium from the frog sartorius was too large to represent an active process and suggested that part of it might be caused by an exchange diffusion requiring no energy. Hodgkin & Keynes (1955) have shown that in *Sepia* axons the sodium flux was not reduced even in the absence of external sodium under which conditions exchange diffusion would not occur. However, more recently Swan & Keynes (1956) have shown that the substitution of choline for sodium reduced the sodium efflux from frog muscle by more than half. In this case the energy requirement would be correspondingly reduced.

It is probable that in the lamellibranchs exchange diffusion is responsible for part of the efflux, but the apparent energy requirements of *Mytilus* ventricle are more than five times as great as those of *Anodonta* ventricle and exchange diffusion, if it occurs, is likely to take place in *Anodonta* ventricle as well. It is, therefore, very probable that the energy required for sodium extrusion is substantially greater in the marine species.

Measurements of the oxygen consumption of the two muscles, which will be reported in a later paper, show that in both animals the metabolic energy available is about twice as great as the apparent energy requirements for sodium extrusion, but the metabolic rate of the marine animal is several times greater than that of the freshwater animal.

This has a number of interesting implications in the field of osmotic regulation. It suggests, for example, that a freshwater animal may perform less ionic work than a marine animal; for although it has to perform a certain amount of ionic work at the body surface it may be saved a large amount of ionic work at the surface of each cell. Conversely the many marine animals which maintain a salt concentration

in the blood which is less than that of sea water, for example teleosts, selachians, lampreys, sturgeons, grapsoid crabs and many shrimps, may be more efficient than otherwise appears.

APPENDIX

DERIVATION OF k_2 FROM K_2

Keynes (1954) derived the following equations:

If U is the rate of loss of activity if all the fibres are exposed to non-radioactive saline and U' is the observed rate of loss in a plane sheet of muscle, then

$$\frac{U'}{U} = \frac{\lambda}{b}\tanh\frac{b}{\lambda} \quad \text{(Keynes, eq. 17)}$$

where b is half the thickness of the muscle and λ is the factor by which the distance any particle has to travel from the surface to any point inside is increased by obstacles, the muscle fibres. Also

$$\lambda^2 = \frac{\epsilon}{1-\epsilon}\frac{V}{A}\frac{C_0}{M}D' \quad \text{(Keynes, eq. 9)},$$

where ϵ is the fraction, by volume, occupied by the extracellular fluid, V/A is the volume/area ratio of the muscle fibres, C_0 is the molar concentration of extracellular sodium, M is the flux of sodium through the muscle fibre surface in mole/cm.^{2}/unit time and D' is the quantity of sodium diffusing through area $1/\epsilon$ of the muscle in unit time under unit concentration gradient.

λ and D' can be eliminated as follows.

If $t_{0.5}$ is the half time of washing out of radioactivity from the extracellular phase

$$t_{0.5} = \frac{0.28b^2}{D'} \quad \text{(Keynes, eq. 5)},$$

but

$$t_{0.5} = \frac{\ln 2}{K_1} = \frac{0.693}{K_1},$$

therefore

$$D' = \frac{0.28b^2K_1}{0.693} = 0.404b^2K_1.$$

Substituting for D' in Keynes, eq. 9

$$\frac{\lambda^2}{b^2} = 0.404\frac{\epsilon}{1-\epsilon}\frac{V}{A}\frac{C_0}{M}K_1.$$

But

$$M = k_2\frac{V}{A}C_i \quad \text{(Keynes, eq. 1)},$$

where C_i is the concentration of sodium inside the fibres and k_2 is the rate constant of exchange of sodium between the fibres and the extracellular fluid. Hence

$$\frac{\lambda^2}{b^2} = 0.404\frac{\epsilon}{1-\epsilon}\frac{C_0}{C_i}\frac{K_1}{k_2} \qquad \text{(X)}$$

and

$$\frac{U'}{U} = \frac{K_2}{k_2} = \frac{\lambda}{b}\tanh\frac{b}{\lambda}. \qquad \text{(Y)}$$

ϵ, C_0, C_i, K_1 and K_2 are known, hence by successive approximations k_2 can be determined.

In equation (X) above,

$$\frac{\epsilon}{1-\epsilon}\frac{C_0}{C_i} = \frac{\text{sodium in extracellular phase}}{\text{sodium in intracellular phase}} \simeq \frac{A}{B}.$$

In Table 4, k_2 has been calculated from values of ϵ, C_0 and C_i derived by chemical analysis. From the figures published by Potts (1958) $\epsilon C_0/(1-\epsilon)C_i = 1{\cdot}79$ for *Mytilus* and 0·75 for *Anodonta*. Only slightly different values of k_2 are obtained if A/B is used instead. Hence k_2 can be determined entirely from the experimental data.

The intracellular fraction of sodium in *Mytilus* ventricle is 30 %, calculated from the chemical data. When calculated as $B/(A+B)$ it is slightly higher, 35 %. For *Anodonta* the corresponding values are 52 and 55 %. The similarity of these values confirms the identity of the faster moving fraction with the extracellular fraction and the slower with the intracellular fraction.

SUMMARY

1. Measurements have been made, using ^{24}Na, of the efflux of sodium from the isolated ventricles of *Mytilus edulis* and *Anodonta cygnaea*.

2. In order to determine the efflux of sodium from the muscle fibres it is necessary to correct for the efflux of sodium from the extracellular space. It was not practicable to make such a correction to the results on *Anodonta* at 15° C.

3. The mean rate constants and effluxes of sodium from the muscle fibres are

Mytilus at 5° C., 2·44 hr.$^{-1}$; 12×10^{-6} mM/cm.^{2}/sec.
at 15° C., 5·6 hr.$^{-1}$; 27×10^{-6} mM/cm.^{2}/sec.

Anodonta at 5° C., 4·6 hr.$^{-1}$; $3{\cdot}1 \times 10^{-6}$ mM/cm.^{2}/sec.

4. The energy required for sodium extrusion, assuming it is entirely an active process, is:

Mytilus at 5° C., 0·26 cal./g./hr.
at 15° C., 0·62 cal./g./hr.

Anodonta at 5° C., 0·046 cal./g./hr.

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