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Acid-base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion

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Abstract

The acid-base status of two sea urchins, *Psammechinus miliaris* (Gmelin) and *Echinus esculentus* (L.) during experimental emersion has been investigated. Sea urchins were collected from the Firth of Clyde between August and September 1987. The carbon dioxide capacity of the coelomic fluid of *P. miliaris* was greater than that of *E. esculentus*, although both were low and only marginally greater than that of sea water. The pH of the coelomic fluid was also low (7.05 to 7.17) and was influenced mainly by the internal partial pressure of CO_2 (P_{CO_2}). Acid-base disturbance in the coelomic fluid of both species during emersion, although minimal, was more pronounced in *E. esculentus* than in *P. miliaris* and was due primarily to an increase in the internal P_{CO_2}, although there was an increase in the concentration of L-lactate in the coelomic fluid of *E. esculentus*. The coelomic fluid of both species was in a state of perfectly compensated respiratory acidosis. An increase in the concentration of divalent ions (Ca^{2+} and Mg^{2+}) may be related to the dissolution of the test as a source of carbonate buffer.

Introduction

The ability to maintain respiratory gas exchange in air during the period of low tide is a common feature of animals inhabiting the marine littoral zone. Where high humidities prevail, e.g. beneath boulders or amongst littoral algae on rocky shores, gas exchange may continue throughout the period of emersion even in the absence of specific morphological or physiological adaptations of the respiratory system (Newell 1979). Although lack of oxygen is rarely a problem in air, emersed animals may be faced with greater difficulties in the removal of carbon dioxide, which has potentially serious consequences for the acid-base balance of an animal.

For example, in littoral Crustacea (which have probably received most attention) such as the shore crab *Carcinus maenas*, emersion is accompanied by an initial respiratory acidosis due to an increase in the partial pressure of CO_2 (P_{CO_2}) in the blood, which is in turn compensated by an increase in the concentration of bicarbonate ions (Truchot 1975). A similar emersion-related increase in the bicarbonate concentration of the blood was also found for the littoral amphipod *Orchestia gammarellus* (Spicer and Taylor 1987). In the red rock crab *Cancer productus*, the acidosis that occurred during emersion was of both metabolic and respiratory origin, although the mechanism of compensation was similar to that found for *Carcinus maenas* (DeFur and McMahon 1984). In the lugworm *Arenicola marina*, however, a different response was recorded during periods of emersion. In the blood of this species an uncompensated acidosis was incurred, the magnitude of which increased during the period of low tide (Toulmond 1973).

Although only limited data are available for other littoral invertebrates, studies of the acid-base balance of other aquatic animals when respiring in air confirm that, in general, the blood, whilst having an elevated P_{CO_2}, is in a state of compensated respiratory acidosis compared to the blood of animals respiring in water (Truchot 1981).

The respiratory physiology of littoral echinoderms during emersion has received very little attention compared with other invertebrate phyla represented in the littoral zone, such as the Crustacea and the Mollusca (Newell 1973, 1979). The studies of Johansen and Vadas (1967) on the effect of aerial exposure on the rate of oxygen uptake and on the partial pressure of oxygen in the coelomic fluid ($P_{cf}O_2$) of three *Strongylocentrotus* species (Echinoidea) and that of Murphy and Jones (1987) on the effects of temperature on the aerial and aquatic rates of oxygen consumption of the starfish *Asterina gibbosa* are two notable exceptions. There is, to our

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knowledge, no information available on the acid-base balance of any echinoderm species during a period of emersion.

Sarch (1931) studied a number of echinoderm species and found that the coelomic fluid exhibited an appreciable buffering capacity which he attributed to the bicarbonate/carbonic acid buffer system. A number of early studies examined the CO_2 content of echinoderm body fluids (see Hyman 1955 for references) but, because they often omitted to record other vital information such as pH, their results are of little comparative value. The acid-base balance of echinoderm body fluids has scarcely been investigated since the advent of modern analytical techniques (Shick 1983).

The present study sets out to compare the CO_2-combining properties of the coelomic fluid of two echinoids, *Psammechinus miliaris* and *Echinus esculentus*, and to examine changes in acid-base balance during prolonged emersion. *P. miliaris* is a small (< 50 mm diam), essentially littoral echinoid which is found beneath rocks and among algae on coasts around the British Isles (Mortensen 1927). The common sea urchin *E. esculentus* is a sublittoral species, which is common on nearly all rocky shores and comes inshore during the spring to breed. At this time it is often found exposed low in the littoral zone (Reid 1935).

Materials and methods

Collection and maintenance of sea urchins

Psammechinus miliaris (Gmelin) were collected by hand from littoral pools and from beneath rocks, at and below the level of high water of neap tides, on a rocky promontory to the west of Kames Bay, on the Isle of Cumbrae, Firth of Clyde, Scotland, during August and September 1987. *Echinus esculentus* (L.) were collected during dredging trips made by the University Marine Biological Station's research vessel "Aora" from a depth of 40 m in the Fairlie Channel, to the east of the Isle of Cumbrae during the same period.

After collection, the sea urchins were transported back to the Department of Zoology, University of Glasgow, in large plastic containers (40 litres capacity) of sea water. On arrival they were kept in a number of large tanks (< 40 individuals per tank) in a recirculating seawater aquarium (salinity = 32‰) held at 10 °C ± 1 C°. The sea urchins were used in experiments within three days of capture.

Construction of CO_2 equilibrium curves

Coelomic fluid for the construction of CO_2 equilibrium curves was extracted from freshly caught individuals using a 2 ml syringe, the needle of which was inserted directly into the main coelomic cavity via the peristome on the oral surface. The contents of the syringe were then kept on ice until required (< 10 min). The total CO_2 content ($C_{cf}CO_2$) of oxygenated (P_{O_2} = 155 torr) coelomic fluid was determined using 100 μl aliquots of pooled sample. The samples were tonometered in a Radiometer BMS II, maintained at 10 °C, against a range of CO_2 tensions (0.5 to 6.0 torr) supplied by precision gas-mixing pumps (Wostoff, Bochum, FRG). The coelomic fluid was tonometered for at least 20 min to allow complete equilibration to occur before replicate 20 μl samples were removed for the measurement of $C_{cf}CO_2$ using the method of Cameron (1971). The pH of the coelomic fluid (pH_{cf}) at each CO_2 tension was determined on 40 μl samples using the microcapillary electrode of the BMS II which was connected to a PHM 73 pH meter (Radiometer, Copenhagen, Denmark). The functional pK'_1 values for the coelomic fluid of *Psammechinus miliaris* and *Echinus esculentus* were calculated over the pH range 6.7 to 7.3 using the Henderson-Hasselbalch equation in the form

$$pK'_1 = pH - \log((C_{cf}CO_2 - \alpha P_{CO_2})/\alpha P_{CO_2}), \quad (1)$$

where α is the solubility coefficient of CO_2 in sea water (0.0468 mmol l^{-1} $torr^{-1}$ at 10 °C) (Harvey 1955).

Burton (1987) has recently stated that the appropriate values of the constants in the Henderson-Hasselbalch equation may be unclear, especially (as in this case) when a species is being considered for the first time. He also suggests that if the equation is to be applied accurately, the definition of every term must be in context and the value of pK'_1 must match the definitions of all the other terms. For example, it is known that the value of the "constant" pK'_1 can vary with pH (Burton 1987). Unless the effect of pH on pK'_1 is taken into account, serious errors could occur when calculating other parameters. To overcome this problem during the present study, values for pK'_1 were determined *in vitro* over a wide pH_{cf} range (see previous paragraph) and the appropriate values for inclusion in the equation for the calculation of *in vivo* $[HCO_3^-]_{cf}$ (see "Measurement of pH C_{CO_2} and P_{CO_2} of coelomic fluid") were interpolated from a graph demonstrating the relationship between pH_{cf} and pK'_1 *in vitro* (Fig. 2).

In vitro non-bicarbonate buffer lines were then constructed for oxygenated samples of pooled coelomic fluid at 10 °C from the data obtained as outlined in first paragraph of this section. The concentration of bicarbonate ions in the coelomic fluid ($[HCO_3^-]_{cf}$) was calculated using the equation

$$[HCO_3^-]_{cf} = C_{cf}CO_2 - \alpha P_{CO_2}. \quad (2)$$

Emersion experiment

The effect of emersion on acid-base balance in the coelomic fluid of both *Psammechinus miliaris* and *Echinus esculentus*, was studied by carrying out the following experiment. Ten individuals of either species were placed in each of four 10-litre plastic aquaria containing 8 litres of filtered sea water (32‰ S), maintained at 10 °C in two

thermostatically-controlled water baths. The sea water was constantly aerated by means of an air stone placed in each tank.

After 12 h, the water contained in two of the tanks was siphoned off and the inside carefully dried using blotting paper. The third tank containing sea urchins still fully immersed in aerated sea water was used as a control. The top of each tank was then covered with tissue paper that had been saturated in sea water. This ensured the maintenance of a high relative humidity within the tanks containing emersed sea urchins. Five individuals of each species were removed at 2, 4, 7, 12, 18 and 24 h intervals after emersion. Control individuals were sampled only at 0 and 24 h. The coelomic fluid (1 to 2 ml) was extracted anaerobically from individual sea urchins using a 2 ml syringe, the needle of which (23 g) was inserted through the test into the main coelomic cavity.

Measurement of pH, C_{CO_2} and P_{CO_2} of coelomic fluid

The *in vivo* pH of the coelomic fluid was obtained by drawing samples of the coelomic fluid (40 μl) directly into the microcapillary pH electrode of a Radiometer BMS II which was connected to a PHM 73 pH meter (Radiometer, Copenhagen). The total CO_2 content of the coelomic fluid ($C_{cf}CO_2$) was determined on duplicate 10 μl samples using the method of Cameron (1971). The *in vivo* P_{CO_2} of the coelomic fluid ($P_{cf}CO_2$) was determined by injecting 50 to 100 μl samples into a thermostated cell (at 10 °C) containing a CO_2 electrode (Radiometer, Copenhagen) which was also connected to a PHM 73 pH meter (Radiometer, Copenhagen).

The *in vivo* $[HCO_3^-]_{cf}$ for both *Psammechinus miliaris* and *Echinus esculentus* was calculated from the following form of the Henderson-Hasselbalch equation:

$$[HCO_3^-]_{cf} = \text{antilog}\,(pH_{cf} - pK'_1) \cdot (\alpha \cdot P_{cf}CO_2). \quad (3)$$

In an attempt to determine whether or not the coelomic fluid was in complete equilibrium and thus to validate the use of the Henderson-Hasselbalch equation to calculate *in vivo* $[HCO_3^-]_{cf}$, calculated values of $P_{cf}CO_2$ were compared with values of $P_{cf}CO_2$ obtained by direct measurement.

Ionic and biochemical analysis of coelomic fluid

The remaining individual samples of coelomic fluid were centrifuged at 10 000×*g* for 10 min to remove any cells before the protein concentration and the ionic composition of the coelomic fluid were measured. The concentration of protein in the coelomic fluid was determined using a Coomassie Blue technique (Reid and Northcote 1981). The concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} ions in the coelomic fluid were determined, following dilution with deionized water, using an atomic absorption spectrophotometer (Pye Unicam SP 90). Lanthanum chloride was added to each of the dilutions used in the estimation of Ca^{2+} ions. The concentration of Cl^- ions was determined using a chloride titrator (Jencons).

The concentrations of L-lactate and other organic acids present in the coelomic fluid were estimated using a high-performance liquid-chromatography (HPLC) system (Gilson, France) coupled to a Bio-Rad Aminex HPX-87H organic acid analysis column (300×7.8 mm). Samples (50 μl) of coelomic fluid were deproteinized by the addition of 50 μl perchloric acid (0.6 *M*). The resultant suspension was then centrifuged at 10 000×*g* for 10 min to remove the precipitated protein, after which the supernatant was neutralised using 10 μl K_2CO_3 (2 *M*). The sample was again centrifuged at 10 000×*g* for a further 10 min in order to remove the precipitated potassium perchlorate. The supernatant was decanted and frozen in liquid nitrogen prior to being stored at −20 °C until required.

Samples (20 μl) were introduced into the column using a 25 μl Hamilton micro-syringe. The column was eluted with H_2SO_4 (25 m*M*) at a rate of 1 ml min^{-1}. All the organic acids of interest were eluted within a 16 min period and detected by a spectrophotometer at a wavelength of 210 nm. The results were recorded on a chart recorder (Tekman TE 200/1, Tekman Ltd, England). The following standards were run in order to establish the identity of the unknown organic acids: oxaloacetate, malate, pyruvate, succinate, lactate, acetate, fumarate and propionate.

The concentration of L-lactate in the coelomic fluid was also estimated using the enzymatic method of Gutmann and Wahlefeld (1974) with the following modifications. Samples (100 μl) of the coelomic fluid were centrifuged at 10 000×*g* for 10 min to remove cellular material and the concentration of L-lactate was measured in duplicate 40 μl samples. EDTA (12 m*M*) was added to the reaction mixture to remove any free copper ions which may interfere with the end point of the reaction (Engel and Jones 1978). Because the concentrations of L-lactate in the coelomic fluid were very low, it was necessary to carry out these determinations using a spectrophotofluorimeter (Baird-Atomic EP 101) which provided greater sensitivity when working at very low metabolite concentrations.

Results

CO_2 transport

Carbon-dioxide equilibrium curves obtained for the coelomic fluid of both *Psammechinus miliaris* and *Echinus esculentus* at 10 °C are presented in Fig. 1 together with a CO_2 equilibrium curve constructed for sea water (32‰ S). Although the capacity of CO_2 in the coelomic fluid of *P. miliaris* was slightly greater than that in *E. esculentus*, the values for capacity were very low. For example, in *E. esculentus* the CO_2 equilibrium curve of oxygenated coelomic fluid did not differ significantly from sea water at

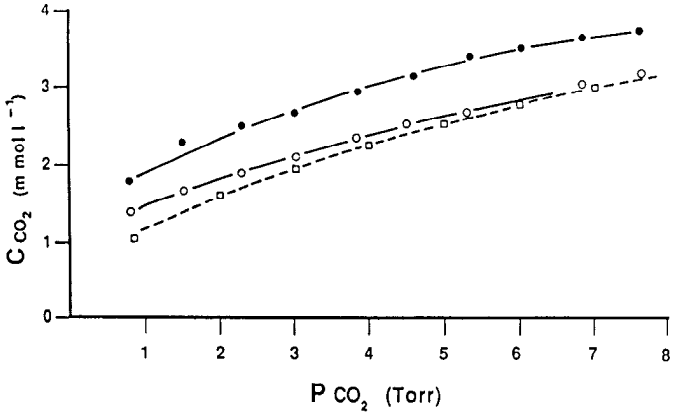

Fig. 1. *Psammechinus miliaris* (•) and *Echinus esculentus* (○). In vitro carbon-dioxide equilibrium curves for oxygenated coelomic fluid of sea urchins and of sea water (□) measured at 10 °C. C_{CO_2}: total CO_2 content; P_{CO_2}: partial pressure of CO_2. Values are means of two determinations

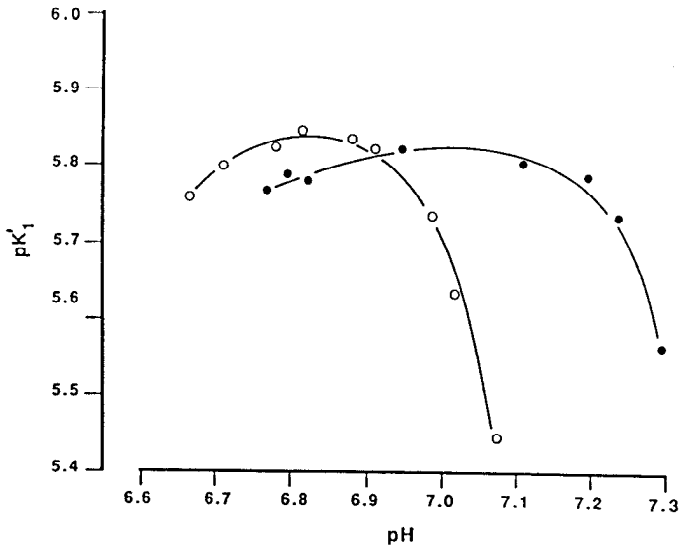

Fig. 2. *Psammechinus miliaris* (•) and *Echinus esculentus* (○). Relationship between equilibrium constant pK'_1 and pH of oxygenated coelomic fluid in vitro at 10 °C

high $P_{cf}CO_2$ values. The equations to describe the non-bicarbonate buffer lines for the coelomic fluid of both *P. miliaris* and *E. esculentus* were $[HCO_3^-] = 27.87 - 3.60\ pH_{cf}$ and $[HCO_3^-] = 28.06 - 3.80\ pH_{cf}$, respectively. The calculated non-bicarbonate buffer values for the coelomic fluid of *P. miliaris* and *E. esculentus* were similar at −3.6 to −3.8 mmol (pH unit)$^{-1}$, respectively. Both values were slightly greater (but not significant at $P > 0.05$) than that measured for the air-equilibrated sea water used in this study.

The pK'_1 values calculated for the coelomic fluid of both *Psammechinus miliaris* and *Echinus esculentus* varied markedly over the pH range 6.6 to 7.3 (Fig. 2). The maximum pK'_1 values were 5.81 (at pH = 7.0) and 5.85 (at pH = 6.85) for *P. miliaris* and *E. esculentus*, respectively. At higher pH_{cf}, the value of pK'_1 decreased significantly. Functional pK'_1 values used to calculate values of $[HCO_3^-]_{cf}$ were interpolated from these graphs (see Fig. 2).

Experimental emersion

Table 1 presents the pH_{cf}, $C_{cf}CO_2$ and $P_{cf}CO_2$ values for *Psammechinus miliaris* and *Echinus esculentus* during emersion. A comparison of the pH_{cf} of control individuals maintained in normoxic sea water showed that there was a significant difference ($P < 0.05$) in the pH_{cf} of *P. miliaris* and *E. esculentus*. There was no significant change in the pH_{cf} of *P. miliaris* or *E. esculentus* over the 24 h emersion period.

There was a small but significant increase in the $P_{cf}CO_2$ of both species during emersion. There was no significant difference ($P > 0.05$) between the measured values of $P_{cf}CO_2$ and those calculated using the Henderson-Hasselbalch equation.

Values of $[HCO_3^-]_{cf}$ *in vivo* were calculated using the values of $P_{cf}CO_2$ and pH_{cf} from Table 1 for both species at different times during the period of emersion, and have

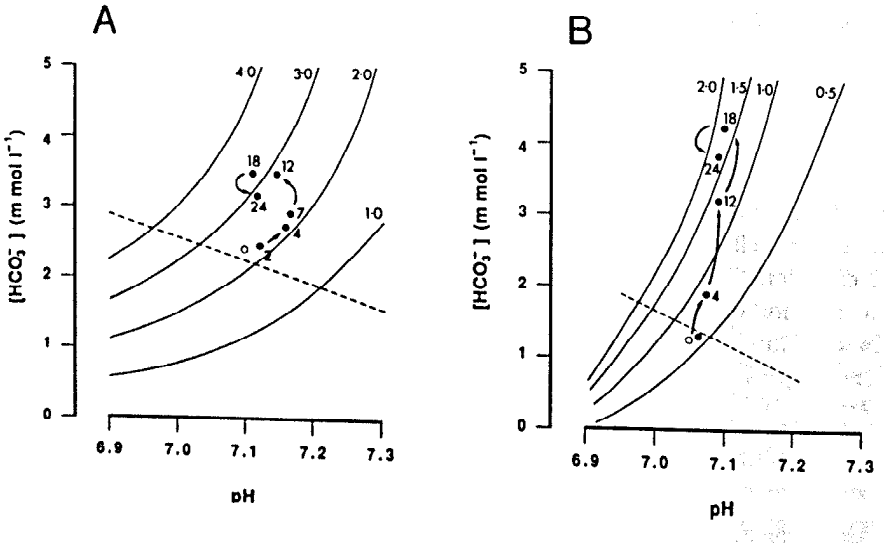

Fig. 3. *Psammechinus miliaris* (A) and *Echinus esculentus* (B). Graphic representation (after Davenport 1974) of relationship between concentration of bicarbonate ions ($[HCO_3^-]_{cf}$) and pH (pH_{cf}) under conditions of emersion (10 °C). Numbers beside data points (•) represent duration of exposure (h), arrows linking data points show direction of change; calculated P_{CO_2} (torr) isopleths are also included. Oblique line represents mean buffer lines for oxygenated coelomic fluid at 10 °C

Table 1. *Psammechinus miliaris* and *Echinus esculentus*. Changes in pH_{cf}, $C_{cf}CO_2$ and $P_{cf}CO_2$ during experimental emersion. Values are means ± SD of five determinations

Immersion period	*P. miliaris*			*E. esculentus*		
	pH_{cf}	$C_{cf}CO_2$ (mmol l^{-1})	$P_{cf}CO_2$ (torr)	pH_{cf}	$C_{cf}CO_2$ (mmol l^{-1})	$P_{cf}CO_2$ (torr)
0 h	7.10±0.03	2.50±0.22	2.2±0.3	7.05±0.03	1.20±0.08	0.9±0.2
2 h	7.10±0.07	2.59±0.38	2.4±0.4	7.05±0.05	1.23±0.14	1.0±0.3
4 h	7.16±0.06	2.95±0.61	2.2±0.4	7.06±0.07	2.08±0.07	1.1±0.2
7 h	7.16±0.09	2.84±0.36	3.4±0.4	7.06±0.07	1.98±0.38	1.1±0.3
12 h	7.14±0.10	3.61±0.75	3.2±0.2	7.09±0.04	3.05±0.17	1.4±0.3
18 h	7.11±0.04	3.38±0.11	3.2±0.4	7.10±0.06	4.51±1.26	1.6±0.4
24 h	7.12±0.03	3.32±0.87	3.2±0.3	7.10±0.05	4.40±0.18	1.7±0.2
24 h (control)	7.10±0.04	2.53±0.31	2.2±0.3	7.06±0.03	1.20±0.09	1.0±0.3

Table 2. *Psammechinus miliaris* and *Echinus esculentus*. Changes in concentrations of major ions (mmol l^{-1}) in coelomic fluid during experimental emersion. Values are means ± SD of five determinations

Immersion period	$[Na^+]$	$[Cl^-]$	$[K^+]$	$[Mg^{2+}]$	$[Ca^{2+}]$
P. miliaris					
0 h	403.9±13.2	504.6±13.5	10.99±0.58	52.82± 0.68	8.17±0.25
2 h	430.4±13.4	518.1±11.1	11.24±0.56	55.37± 0.86	8.24±0.14
4 h	434.7±32.5	532.2±30.5	12.24±1.79	63.12±10.33	9.00±0.70
7 h	425.2±17.8	561.6±31.4	13.20±0.79	66.74± 4.21	9.28±0.75
12 h	421.4±30.1	558.8±35.4	13.61±1.87	57.10±12.90	9.55±1.39
18 h	420.0±47.1	544.5±37.2	12.77±1.31	61.95±11.30	8.65±0.81
24 h	426.8±37.9	574.7±50.3	13.00±1.46	66.72±10.71	8.86±0.56
24 h (control)	405.2±12.5	508.3±15.3	10.84±0.60	52.17± 0.88	8.15±0.22
E. esculentus					
0 h	417.2±11.2	527.5±16.1	11.24±0.84	51.45±3.12	8.26±0.25
2 h	414.4± 3.7	491.3±23.4	11.48±1.19	57.25±1.77	8.05±0.31
4 h	408.7± 6.0	514.8±18.1	11.43±1.46	58.63±4.47	8.23±0.26
7 h	428.1±36.3	508.5±10.8	9.81±1.17	64.80±0.99	8.59±0.12
12 h	431.5±16.6	512.6±12.3	12.26±1.02	64.81±1.34	9.28±0.79
18 h	413.4±20.2	540.1±11.0	10.95±0.49	61.69±3.21	9.58±1.29
24 h	455.8±51.1	548.8±34.7	11.78±1.22	68.95±9.69	9.48±1.55
24 h (control)	408.9± 9.3	522.4±12.3	11.43±0.94	52.55±2.99	8.09±0.21

been plotted against the corresponding values of pH_{cf} in the form of "Davenport" diagrams (Davenport 1974) (Fig. 3). Although there was an increase in $[HCO_3^-]_{cf}$ in *Psammechinus miliaris*, this increase was not significant ($P>0.05$) until 12 h after emersion (Fig. 3A). In contrast to the small increase in $[HCO_3^-]_{cf}$ in *P. miliaris*, the $[HCO_3^-]_{cf}$ of the coelomic fluid of *Echinus esculentus* increased significantly ($P<0.01$) during the period of emersion from an initial value of 1.16±0.08 to 4.45± 0.17 mmol l^{-1} after 24 h emersion (Fig. 3B).

The changes in the ionic composition of the coelomic fluid during the period of emersion are given in Table 2. There was no significant difference between the two species in the initial concentrations of the ions, with the exception of Cl^- and Mg^{2+} which were significantly greater ($P<0.01$) in *Psammechinus miliaris* than in *Echinus esculentus*. During emersion, although there was no significant difference in the concentrations of Na^+ or K^+ ions in the coelomic fluid of either species, there were significant increases ($P<0.05$) in the concentrations of Mg^{2+} and Ca^{2+} ions. After 24 h emersion, there was also a significant increase ($P<0.01$) in the concentration of Cl^- ions in the coelomic fluid of *P. miliaris* but not of *E. esculentus*.

The concentration of protein in the coelomic fluid of both *Psammechinus miliaris* and *Echinus esculentus* was very low, with values ranging between 0.23–0.41 mg ml^{-1} and 0.17–0.32 mg ml^{-1}, respectively. These values did not alter significantly ($P>0.05$) upon emersion.

HPLC was used to identify the organic acids present in the coelomic fluid: L-lactate/succinate, acetate and fumarate. Oxaloacetate, malate, pyruvate and propionate were not detected. Since the peaks obtained were extremely small, quantitative analysis was difficult. The traces presented in Fig. 4 are those obtained for the coelomic fluid of both *Psammechinus miliaris* and *Echinus esculentus*.

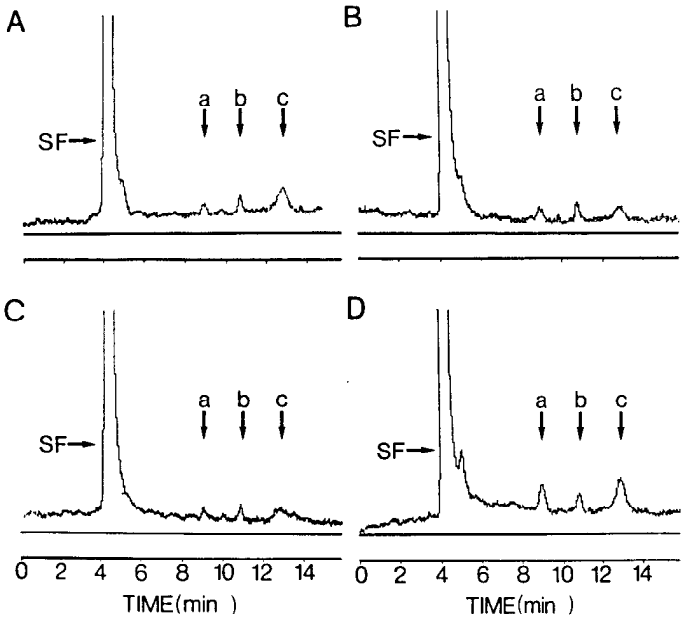

Fig. 4. *Psammechinus miliaris* and *Echinus esculentus*. Representative traces of organic acids present in equal volumes of coelomic fluid after 12 h experimental emersion, obtained using ion-exchange high-performance liquid chromatography. (A) *P. miliaris* control; (B) *E. esculentus* control; (C) *P. miliaris* after 12 h emersion; (D) *E. esculentus* after 12 h emersion. Organic acid peaks are indicated by vertical arrows: a, lactate/succinate; b, acetate; c, fumerate. SF: solvent front

Table 3. *Psammechinus miliaris* and *Echinus esculentus*. Changes in concentrations of [L-lactate]$_{cf}$ (mmol l^{-1}) during experimental emersion. Values are means ± SD of five determinations (with exception of *, where $n = 1$)

Immersion period	*P. miliaris*	*E. esculentus*
0 h	0.061 ± 0.010	0.078 ± 0.008
2 h	0.034 ± 0.004	0.092 ± 0.014
4 h	0.067 ± 0.010	0.115 ± 0.011
7 h	0.073 ± 0.004	0.152 ± 0.012
12 h	0.052 ± 0.010	0.167 ± 0.053
18 h	0.062 ± 0.010	0.142 *
24 h	0.061 ± 0.013	0.145 ± 0.013
24 h (control)	0.061 ± 0.011	0.082 ± 0.006

The upper traces are for control individuals (immersed in normoxic water) and the lower ones for individuals which had been emersed for 12 h. Three distinct peaks are present in each of the traces. These represent L-lactate/succinate, acetate and fumarate, with retention times of 8.8, 10.8 and 12.6 min, respectively. Unfortunately, because of the very similar retention times of lactate and succinate, this technique was not able to differentiate between the lactate and succinate peaks.

There was an increase in the lactate/succinate peak in the coelomic fluid of *Echinus esculentus* after 12 h emersion, but not in *Psammechinus miliaris*. This correlated well with the enzymatic estimation of L-lactate (see below). By calculating the predicted area under the curve which would correspond to the data obtained from the enzymatic analysis of lactate, it can be deduced that 70 to 80% of the observed peak can be accounted for by L-lactate. It would appear, therefore, that if succinate was present it was only in small amounts. The concentration of fumarate in the coelomic fluid of both species increased after 24 h emersion. The fumarate peak appears large relative to the other two peaks, but this is due to the extremely high absorbance of fumarate at 210 nm. The acutal concentrations of fumarate recorded were very low, i.e., < 0.0004 mmol l^{-1}.

The changes in the concentrations of L-lactate in the coelomic fluid ([L-lactate]$_{cf}$) of *Psammechinus miliaris* and *Echinus esculentus* during experimental emersion (measured enzymatically) are presented in Table 3. There was no significant difference between the two species in the concentration of L-lactate in the coelomic fluid of the control individuals kept in normoxic sea water. During the first 12 h of emersion, there was no significant increase ($P > 0.05$) in [L-lactate]$_{cf}$ of *P. miliaris*, but in *E. esculentus* [L-lactate]$_{cf}$ increased significantly ($P < 0.01$) from a value of 0.078 to 0.167 mmol l^{-1} during the first 12 h exposure, but did not increase further during the next 12 h exposure.

Discussion

Buffering in coelomic fluid

In vertebrates, the presence of a high buffering capacity in the blood is achieved via bicarbonate, phosphates, plasma proteins and haemoglobin (Davenport 1974) but in the blood or coelomic fluid of invertebrates, a high buffering capacity, when present, is due almost entirely to the presence of proteins (Truchot 1981). The CO_2 capacity of the coelomic fluid of both *Psammechinus miliaris* and *Echinus esculentus* was only marginally greater than that of sea water. This low CO_2 capacity can be attributed primarily to the very low protein concentration in the coelomic fluid of these two species which were similar to the value of 0.3 mg ml^{-1} quoted by Robertson (1939) for *E. esculentus*. It would appear, therefore, that a low protein concentration is characteristic of echinoderm body fluids in general (Binyon 1972).

The reason for the greater CO_2 capacity of the coelomic fluid of *Psammechinus miliaris* compared with that of *Echinus esculentus* is unknown and is unlikely to be due to the slightly higher protein content of the coelomic fluid of *P. miliaris*. Whilst it is true that CO_2 capacity would also be affected by the presence of lipids in the coelomic fluid (since CO_2 is more soluble in lipid than in water), the limited information currently available (see Fagerlund 1969) would seem to indicate that the concentrations normally present in the coelomic fluid are too low to make any significant contribution to CO_2 transport.

A number of authors have noted that the CO_2 content of the coelomic fluid of echinoderms, although low compared to similar values for other aquatic animals (Ca-

meron 1979), was always higher than that of the surrounding sea water (see Hyman 1955 for references). This elevated $C_{cf}CO_2$ was considered to be responsible for the reduction in pH_{cf} (by 0.5 to 1.5 pH units compared with sea water) that has been frequently recorded in echinoderms. The fact that the pH_{cf} of echinoderms is lower than the pH of the body fluids of other marine invertebrates has also been recognised for some time (Mangum and Shick 1972, Howell et al 1973, Houlihan and Duthie 1981) and is confirmed in this present study. Farmanfarmaian (1966) attributed low pH_{cf} to the retention of respiratory CO_2, and some recent authors have suggested that it may also be due to the presence of non-volatile organic acids (Mangum 1973, Brown and Shick 1979, Houlihan and Duthie 1981). In both *Psammechinus miliaris* and *Echinus esculentus*, which have mean pH_{cf} values of 7.10 and 7.05, respectively, it would appear that the pH of the coelomic fluid is influenced mainly by the $P_{cf}CO_2$ (even though $P_{cf}CO_2 < 3$ torr in both species).

Emersion and CO_2

When *Psammechinus miliaris* and *Echinus esculentus* were emersed, although there was no significant difference in pH_{cf}, there was a small but significant increase in both $C_{cf}CO_2$ and $P_{cf}CO_2$ after 24 h. This increase was much more apparent in *E. esculentus* than in *P. miliaris*. The proportionally greater increase in $[HCO_3^-]_{cf}$ in *E. esculentus* compared with *P. miliaris* may be related to the increase in $P_{cf}CO_2$ in *E. esculentus* which showed an apparent increase of 100% compared with 50% in *P. miliaris*.

It would appear that any expected, emersion-related, respiratory acidosis in the coelomic fluid is buffered by an increase in $[HCO_3^-]_{cf}$ with the result that both species are in a state of perfectly compensated acidosis when exposed to air. This is very similar to the situation that exists for the body fluids of other water-breathers respiring in air (Rahn 1966, Howell et al. 1973, Reeves 1977, Truchot 1981). The time scale is different, however, in that the respiratory acidosis would appear to be compensated almost immediately in the echinoderm species studied, whereas in other animals there is a hypo-ventilatory-induced hypercapnia which depresses the pH of the body fluids before there is any significant compensatory increase in $[HCO_3^-]_{cf}$ (Truchot 1981). For example, in the shore crab *Carcinus maenas*, compensation was not complete until at least 12 h after experimental emersion (Truchot 1975). The reasons for "immediate" compensation in the coelomic fluid of *Psammechinus miliaris* and *Echinus esculentus* may be that the increase in $P_{cf}CO_2$ noted upon emersion takes place much more gradually than in other animals. This may be correlated with an emersion-related decrease in and/or the presence of an already very low metabolic rate and therefore low rate of CO_2 production (Johansen and Vadas 1967).

Emersion and organic acids

Anaerobiosis in echinoderms was originally thought to follow the classic glycolytic pathway, with L-lactate as the only end-product (Doezema 1969, Ellington and Lawrence 1973, Ellington 1976, Bianconcini et al. 1980. More recently, however, other metabolic pathways which are present in other invertebrate groups have been proposed. Ryabushko et al. (1980) suggested the occurrence of the glucose-6-phosphate/succinate pathway, similar to that found in some molluscs. This suggestion was further strengthened by the work of Ellington and Lawrence (1973), who found large ratios of malate/lactate dehydrogenase activities in the gut tissues of several species of echinoids.

Upon emersion there was an increase in the concentrations of L-lactate, succinate and fumarate in the coelomic fluid of *Echinus esculentus* and in the concentration of fumarate in *Psammechinus miliaris*. The concentrations of fumarate were, however, extremely low. L-lactate appears to be the major end-product accumulating in the coelomic fluid of *E. esculentus* during emersion, accounting for 70 to 80% of the L-lactate/succinate peak obtained by HPLC. The actual increase, however, is very small, indicating that in both species aerobic metabolism is still responsible for meeting most metabolic demands throughout the emersion period and/or that the metabolic rate has been suppressed. It is unlikely, therefore, that the increase in $[HCO_3^-]_{cf}$ is related to a need to buffer any increase in the concentrations of non-volatile organic acids observed in the coelomic fluid upon emersion.

Emersion and ionic composition

The source of increased $[HCO_3^-]_{cf}$ during compensation is at present unknown. It cannot be attributed solely to water loss from the sea urchins during the period of experimental emersion, since the concentrations of all the major ions did not increase proportionally. It is possible, however, that the variation encountered, particularly in the first 7 h of the emersion period (in *Psammechinus miliaris* at least) may be due to a desiccation-induced, differential regulation of the ionic content of the coelomic fluid. The concentrations of all the major ions in the coelomic fluid of *P. miliaris* and *Echinus esculentus* were similar to those found by Robertson (1939) for the coelomic fluid of *E. esculentus* and did not vary significantly ($P > 0.05$) over the experimental period, with the exception of the divalent ions Mg^{2+} and Ca_2^+ (and Cl^- ions in the coelomic fluid of *P. miliaris*). One possible explanation as to the origin of $[HCO_3^-]_{cf}$ which would result in an increase in the concentrations of magnesium and calcium ions in the coelomic fluid of both species in response to emersion is that the test is being used as a source of $CaCO_3$ or other carbonate buffers. The role of exoskeletal carbonate buffers in acid-base regulation has been suggested for both crustacean and molluscan species

(Dugal 1939, Henry et al. 1981). Binyon (1972), however, was unable to find any increase in the concentration of calcium ions in the coelomic fluid of seastars stranded by the tide. An emersion-related respiratory acidosis in the coelomic fluid of *P. miliaris* and *E. esculentus* may increase dissolution of the test, providing a source of carbonate buffer, and so enable a more rapid compensation than would be accomplished by metabolic compensation alone.

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