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Respiratory and Acid-Base Physiology of the Purple Sea Urchin, *Strongylocentrotus purpuratus*, During Air Exposure: Presence and Function of a Facultative Lung

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Abstract. Upon exposure to air (emersion), the purple sea urchin *Strongylocentrotus purpuratus* releases an "emersion fluid" from its esophagus. Release of this fluid causes air to appear within the test (or calcareous theca), most likely inside the intestine. The air space is large, occupying 33.5% of the volume of the intrathecal space. The intestine containing air forms a facultative lung and contributes to the oxygenation of the perivisceral coelomic fluid (PCF) during emersion. During emersion, the mean partial pressure of oxygen (P_{O_2}) of the PCF declined from 56 to 24 torr (1 torr = 0.1333 kPa) after 2 h, remained relatively unchanged after 4 h, and rose to 39 torr after 8 h. The partial pressure of carbon dioxide (P_{CO_2}) rose from 2.6 to 3.8 torr after 2 h, remained unchanged after 4 h, and declined to 2.7 after 8 h. Due to the elevation of P_{CO_2} PCF pH declined from 7.41 to 7.17. PCF osmotic concentration, calcium ion concentration, chloride ion concentration, ammonium ion concentration, and protein concentration were unchanged by air exposure. Lactate levels in the PCF were undetectable. *S. purpuratus* was an osmoconformer and a chloride ion conformer at salinities down to 20.9 ppt. Below this salinity, the sea urchins died. The respiratory acidosis resulting from air exposure was uncompensated, supporting the hypothesis that compensation for a respiratory acidosis induced by air exposure does not occur in organisms that are unable to regulate ions in a dilute environment. We suggest that the facultative lung ensures a minimal P_{O_2} in the PCF, which may be especially important when the intrathecal space is full of ripe gonads, allowing the gonads to be more reliant on aerobic metabolism.

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Introduction

Intertidal organisms experience bouts of exposure to air, or emersion; these bouts vary in duration with the motility of the organism and the magnitude of the tide. Many intertidal organisms take up oxygen from the air during air exposure and thus sustain aerobic metabolism (deFur, 1988). Other organisms, poorly adapted for exchanging gases in an aerial medium, revert either wholly or in part to anaerobic metabolism (*e.g.*, deFur and McMahon, 1984; Spicer *et al.*, 1988) or reduce their metabolism (Burnett and McMahon, 1987).

The responses of intertidal invertebrates to periodic emersion have been well-studied in decapod crustaceans (see Burnett, 1988 for review) and bivalve molluscs (Widdows *et al.*, 1979; Booth *et al.*, 1984; Walsh *et al.*, 1984; Widdows and Shick, 1985; Shick *et al.*, 1986; Dwyer and Burnett, 1996). In every crustacean and mollusc studied, the hemolymph P_{O_2} increased substantially, generating a so-called respiratory acidosis. The molluscs partially compensated for this acidosis (Booth *et al.*, 1984; Dwyer and Burnett, 1996), and all but one of the crustaceans studied compensated nearly completely. The exceptional crustacean was the mud crab *Eurytium albidigitum*, which is also an osmoconformer (Burnett and McMahon, 1987). We hypothesize that the acid-base compensation observed in the crustaceans and the relatively weak compensation observed in

the molluscs is correlated with the strong regulation of hemolymph ionic concentration by the crustaceans and the weak regulation by the molluscs.

Burnett and McMahon (1987) speculated that the uncompensated acidosis in the crab *E. albidigitum* is responsible for the decline in oxygen uptake that occurs in this animal during air exposure. The mechanisms of compensation for acid-base disturbances are thought to involve the transport of ions such as sodium, chloride, and bicarbonate between the organism and its environment or between fluid compartments within the organism (Burnett and McMahon, 1987; Burnett, 1988). Since osmoregulation in crustaceans occurs primarily through the transport of sodium and chloride ions, it is suggested that osmoregulators (or more properly ion regulators) also possess the capacity to compensate for acid-base disturbances. An acidosis during air exposure that is left uncompensated could depress metabolism and decrease overall activity (Burnett and McMahon, 1987). These ideas suggest that good ion regulators have the best chance of compensating for an emersion-induced acidosis, and could thereby maintain aerobic metabolism and activity during emersion. Therefore, we hypothesize that osmoregulation is correlated in various intertidal animals with the capacity to compensate for a respiratory acidosis during emersion.

The purple sea urchin *Strongylocentrotus purpuratus* (Stimpson) inhabits the intertidal zone along the west coast of North America, where it may occur in great numbers. The water vascular system of echinoids constitutes their primary means for external gas exchange (Farmanfarmaian, 1966). The system terminates externally in tube feet, which make up the major surface for gas exchange with the ambient medium. When they are immersed in water, echinoids appear to be well adapted for exchanging oxygen and carbon dioxide through their tube feet (Fenner, 1973). The tube feet are waved in the water between the spines of the urchin; oxygen is taken up, and CO_2 is released from fluid that is circulated in the tube feet. This fluid then circulates to the ampullae (Fenner, 1973; Smith, 1978), where gases are exchanged with the perivisceral coelomic fluid. When the urchin is exposed to air, however, the tube feet withdraw almost completely and collapse; and the exchange of gases is greatly inhibited (Fenner, 1973).

A few studies have been done on sea urchins that were air exposed. The research of Webster and Giese (1975) and Johansen and Vadas (1967) touched briefly on the oxygen uptake and the P_{O_2} in the coelomic fluid in three species of *Strongylocentrotus*. Johansen and Vadas (1967) measured a coelomic fluid P_{O_2} substantially higher than that measured by Webster and Giese (1975), who showed that coelomic fluid P_{O_2} dropped rapidly and approached zero during emersion. More recently, Spicer *et al.* (1988) investigated acid-base status as a function of air exposure in two species of sea urchins. Their study revealed a mild respiratory acidosis that was completely compensated in both species. These studies plus the interesting findings of Bookbinder and Shick (1986), who demonstrated that the gonads of sea urchins are heavily dependent on anaerobic metabolism, led us to investigate the internal environment of the sea urchin during emersion. We aimed to determine whether a respiratory acidosis would occur, whether it would be compensated for, and whether these responses would be related to the ability of the sea urchin to osmoregulate.

We were especially intrigued by the large amount of fluid that sea urchins lose when they are air exposed. Since the tests of sea urchins are rigid, we reasoned that the fluid lost from within the test must be replaced by air. We thought that the air space appearing during emersion might be important to the urchin during a period of air exposure, serving essentially as a "facultative lung." Thus, we identified the source of the fluid lost during emersion, documented in detail the presence of an air space within the sea urchin, and gathered physiological data that would suggest a functional role for this facultative lung.

Materials and Methods

Collection

Purple sea urchins *Strongylocentrotus purpuratus* were collected by hand from tide pools along the southwestern coast of Charleston, Oregon, during the last two weeks of May 1990. The sea urchins were transported to the Oregon Institute of Marine Biology where they were kept on sea tables in flowing seawater (12°C and 32 ppt salinity). All animals were used within 3 weeks of collection.

Animals used to determine the source of emersion fluid were collected by hand in and around exposed rock jetties at South Mission Beach, San Diego, California, in June 1991. The animals were taken to the University of San Diego and held in recirculating seawater at 15°C, 34 ppt salinity (Instant Ocean salts).

Determination of the volume of fluid lost during emersion

For purposes of clarity and consistency, we use the term intrathecal space to designate the whole space contained within the calcareous theca or test (Hyman, 1955) of an urchin. This space encloses numerous compartments, including the perivisceral coelomic space and the gut. We noticed that, shortly after emersion, all urchins lost a relatively large amount of fluid from the intrathecal space; we call this emersion fluid (EF). Because the test is rigid, we surmised that the loss of this much fluid volume would create an air space somewhere within the theca. We used several different approaches to measure the volume of the EF and the resulting air space in the same urchins. First, the volume of fluid lost from each urchin during 8 h of air exposure was measured directly by placing the urchin over a funnel, collecting the fluid released, and then measuring its volume (Row B in Table 1; fluid volume is milliliters per

gram of sea urchin fresh weight, Row A). Second, individuals were weighed immediately upon air exposure (Row A in Table 1) and then emersed for 8 h. After this period of air exposure, the animals were again weighed, and the difference between the pre-emersion and post-emersion weights was taken as a measure of the EF lost during air exposure (using the density of water = 1 g ml^{-1}; Row C in Table 1). Third, several holes were drilled into the aboral surface to drain fluid from the intrathecal space of the emersed urchins. The holes were about 2 cm in diameter and located roughly 2–5 cm from the anal opening (depending on the diameter of the individual). The sea urchins were then inverted, the EF was drained, and its volume was measured in a graduated cylinder (Row E in Table 1). Once thoroughly drained, the urchins were submerged in seawater and allowed to fill completely. The seawater in the intrathecal space was subsequently drained and its volume measured (Row D in Table 1). The difference in the volume of seawater filling the intrathecal space and the volume of fluid remaining in the intrathecal space after emersion (Row D − Row E) is another measure of the air space (or fluid lost) during air exposure (Row F in Table 1).

Source of the emersion fluid (EF)

In separate experiments, we injected blue dextran (M.W. = 2,000,000) dissolved in seawater into each of two body compartments: the espohagus and the perivisceral coelomic cavity. We suspected these compartments of being the origin of the emersion fluid. We chose blue dextran because its color is easy to see in solution, and its large molecular weight makes it unlikely to be metabolized during the short duration (minutes) of these experiments, or to cross membranes between fluid compartments. In one experiment, we injected the blue dextran solution into the perivisceral coelomic cavity, air exposed the sea urchin, and collected the EF and examined it for the presence of blue dextran. In a second experiment, we injected the esophagus with the blue dextran solution, air exposed the animals, and collected the emersion fluid and examined it for the presence of blue dextran.

The concentration of blue dextran was 25 mg ml^{-1} in seawater (pH = 7.9), and 0.5 ml of this solution was injected into both compartments while the animal was submerged. The perivisceral coelomic cavity was injected through the aboral surface with a 1-ml syringe and 26-gauge needle. The sea urchin was placed in a holding tank, which contained seawater of 34 ppt salinity at 15°C. After being allowed to move about freely for 30 min, the animal was air exposed for up to 15 min at 15°C and the EF was collected. After air exposure, a 1-ml sample of perivisceral coelomic fluid was also drawn (as described below). The presence of blue dextran was confirmed by measuring the absorbance of the perivisceral coelomic fluid samples at 618 nm, the absorption maximum of blue dextran in seawater.

In the second set of experiments, individuals were injected with blue dextran into the esophagus with a 1-ml glass syringe connected to a short length of PE-50 tubing. While submerged, the animal was inverted and gently touched around the mouth, which induced the jaws to open. The thin tubing was then inserted about 1 cm into the esophagus, the dye slowly injected, and the tubing immediately withdrawn. After injection, there was no obvious leakage of fluid from the mouth, and the animals were either immediately air exposed (oral side down), or allowed to remain submerged for intervals between 1 and 10 min before being exposed to air. After air exposure, EF was collected at intervals for up to 10 min and evaluated for the presence of blue dextran, as described above.

Coelomic cavity pressure

Hydrostatic pressure within the intrathecal space was measured while the animals were submerged and, subsequently, during air exposure. For each trial, an individual was placed in well-aerated seawater (Instant Ocean salts) thermostatically set to 12°C. A hypodermic needle was inserted through the aboral surface extending into the main coelomic cavity of a submerged animal. The needle was connected *via* polyethylene tubing (PE 100) to a pressure transducer (Statham p23dB) coupled to a four-channel recorder (Beckman RA511). The pressure-measuring unit was calibrated statically. The animal was then emersed by draining water from the holding tank. The hydrostatic pressure in the perivisceral coelomic fluid was monitored continuously during the emersion period. All readings were in torr (1 torr = 0.1333 kPa).

Simultaneously the volume of EF lost by the sea urchin during emersion was measured. Fluid lost from the urchin after emersion was collected in a glass buret, open at the top and closed on the bottom with a three-way stopcock, that was connected *via* tubing to a second pressure transducer (Statham P23dB) with output to the recorder. The pressure of water in the buret increased with the volume and was calibrated prior to the experiment; thus we could simultaneously measure the volume of fluid released and the pressure in the perivisceral coelomic fluid.

Osmotic and ionic regulation at low salinities

We exposed *S. purpuratus* to salinities ranging from 17 to 32.5 ppt for 2 d; salinities were produced by diluting seawater with deionized water. Samples of perivisceral coelomic fluid (PCF) from individual urchins were withdrawn anaerobically with a 1-ml glass syringe equipped with a 22-gauge needle, which was inserted into the main coelomic cavity through the membrane surrounding the anus. These samples of coelomic fluid, as well as samples of ambient water, were assayed as follows: osmotic concentration was measured with a vapor pressure osmometer (Wescor Model 5100C); chloride ion concentration was measured with a

chloride titrator (Radiometer CMT10); and calcium ion concentration was measured with a calcium ion selective electrode (Radiometer ISE25Ca).

Measurement of respiratory variables during emersion

We exposed sea urchins to air by draining the sea tables of seawater. Plastic bags filled with ice were placed in the vicinity of the air-exposed animals to maintain them at a constant 12 ± 1°C ambient temperature. Care was taken to ensure adequate air spaces around the sea urchins.

Perivisceral coelomic fluid samples were drawn from animals after 0, 2, 4, and 8 h of air exposure. The samples were extracted as described above. Volumes ranging from 0.5–1 ml were typically drawn from each individual at each exposure period.

The pH of the coelomic fluid was measured with a microcapillary pH electrode (Radiometer BMS2). The total CO_2 content in the coelomic fluid (C_{CO_2}) was determined by the method of Cameron (1971). The partial pressure of carbon dioxide (P_{CO_2}) was determined with a P_{CO_2} electrode (Radiometer, Copenhagen) (deFur and McMahon, 1984), and that of oxygen was measured with an O_2 electrode (Radiometer, E5046). All electrodes (pH, P_{CO_2}, P_{O_2}) were maintained at 12 ± 0.1°C.

Measurements of chloride, calcium, ammonia, lactate, osmotic, and protein concentration during emersion

The coelomic fluid remaining after the respiratory variables had been sampled was assayed as follows: chloride and calcium ion concentrations were measured as described above; the ammonia content of each sample was assayed spectrophotometrically by the method of Solorzano (1969); lactic acid was converted to pyruvate with lactate dehydrogenase, and reduced NAD was measured spectrophotometrically (Sigma Technical Bulletin No. 826-UV); the remaining fluid was frozen and assayed later for protein concentration (a colorimetric assay; Sigma Technical Bulletin 541–2).

Determination of gonadal index

In May, 13 individual sea urchins from Oregon were removed from the water, blotted, and weighed immediately. After weighing, the sea urchins were carefully dissected; the gonads were removed, rinsed with cold seawater, blotted dry, and weighed. The gonadal index (percent wet weight of gonads per wet body weight) was then calculated. Animals used in all experiments were collected in late May or June. For comparison, we measured the gonadal index of sea urchins collected in Oregon in February 1997, a time when the gonads are larger and when *S. purpuratus* spawns along the Oregon coast (Gonor, 1973).

Statistical analysis

Many variables were analyzed for changes as a function of emersion time. Data were analyzed for normality and equal variance with SigmaStat 2.0 statistical software. Where data were normally distributed, and where variances were equal, a one-way analysis of variance was used to assess differences as a function of emersion time. Where significant differences were found, variables at different times of emersion were compared with the pre-emersion value, using Dunnett's method. When the data were not normally distributed, they were analyzed using a Kruskal-Wallis one-way analysis of variance on ranks. In this case, when differences were detected, pre-emersion values and values at an emersion time were compared by Dunn's method.

Results

Fluid lost during emersion

Since the test of the sea urchin is rigid, we surmised that the fluid lost during emersion must have been displaced by air. We measured the volume of this air (= fluid lost) in several independent ways. Direct measurement of the volume of fluid released (emersion fluid) when the urchin was air exposed resulted in an averaged weight-specific volume of 0.078 ml g^{-1} wet weight of urchin (Table 1, Row B). This was slightly, although not significantly (Wilcoxon signed rank test, $P = 0.064$), lower than the volume of 0.101 ml g^{-1} calculated from the weight difference between sea urchins weighed immediately after emersion and the same animals weighed after 8 h of air exposure (Table 1, Row C). We also independently measured the volume of the fluid in the intrathecal space (Table 1, Row D), as well as the volume of fluid contained within the intrathecal space after air exposure (Table 1, Row E). The difference between these two volumes approximates the intrathecal volume occupied by air (Table 1, Row F). This volume of air was 0.102 ml g^{-1} wet weight of urchin and takes up 33.5% of the intrathecal fluid volume. There is excellent agreement in the measurements of emersion fluid lost using the three techniques.

Our experiments with blue dextran revealed that the esophagus was the source of the EF. Blue dextran injected into the perivisceral coelom was not detected in the EF at any time. Blue dextran injected into the esophagus appeared in the EF within minutes of air exposure (Fig. 1). However, if the urchin was kept submerged after the injection, the longer it remained submerged prior to air exposure, the more dilute the blue dextran was in the EF that was finally released upon emersion (Fig. 1). Therefore, the dye in the esophagus is either rapidly absorbed by the walls of the esophagus, passed through the esophagus to the stomach, or diluted with ambient seawater.

Pressure in the perivisceral coelom of three different sea

Table 1

Volume of fluid released upon emersion and the size or volume of various parts or compartments of the sea urchin

Row	Part or component	Size or volume			
		Mean	Min	Max	SEM
A	Weight of sea urchin immediately upon emersion (g)	147.1	95.6	235.5	11.2
B	Emersion fluid (ml g^{-1}) (from volume of fluid collected)	0.078	0.054	0.107	0.004
C	Emersion fluid (ml g^{-1}) (from pre-emersion − post-emersion weight difference)	0.101	0.065	0.258	0.016
D	Intrathecal space fluid volume (ml g^{-1})	0.304	0.255	0.423	0.015
E	Post-emersion intrathecal space Fluid Volume (ml g^{-1})	0.202	0.136	0.314	0.015
F (D − E)	Air space or emersion fluid volume (ml g^{-1})	0.102	0.080	0.144	0.005
G	Gonad weight (g)	4.44	2.55	5.57	0.34
H (G/A − 100)	% Gonads	3.1	2.1	4.3	0.2

Volume is expressed per unit of pre-emersion urchin weight (Row A). Gonad weight and gonadal index are also given. All measurements were made on each of 10 animals.

urchins was slightly positive throughout submersion (pressure traces of one urchin are shown in Fig. 2) and showed some variation with the movement of the urchin. During emersion, the pressure within the perivisceral coelom fell to ambient and remained there throughout the exposure period (Fig. 2).

Osmotic and ionic regulation

Sea urchins did not survive 17 ppt salinity, the lowest level tested. At salinities of 20.9 ppt (568 mOsm kg^{-1}; 311 mmol Cl^{-} l^{-1}) and above, however, they were strict conformers with respect to osmotic, chloride, and calcium ion concentrations (Table 2).

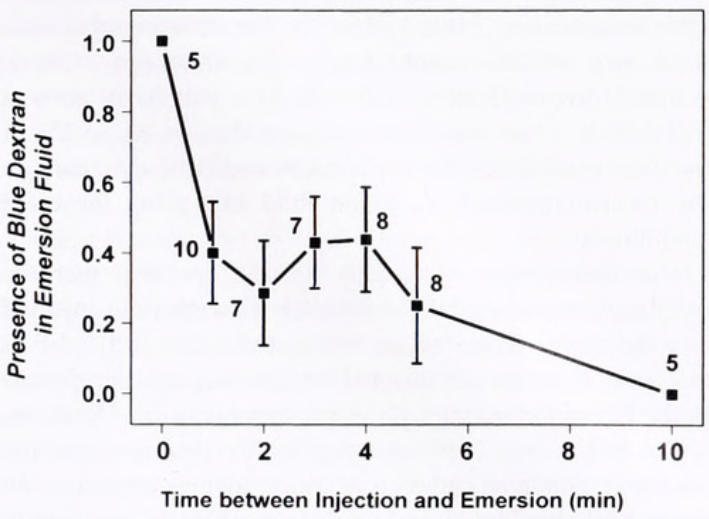

Figure 1. A solution of blue dextran injected into the esophagus of *Strongylocentrotus purpuratus* appears in the emersion fluid, but only if the time interval between injection and emersion is short. Blue dextran in the emersion fluid was scored as 0 (absent), 0.5 (present, but dilute), or 1 (present). Values are mean ± SEM. The number of observations is given next to each data point.

Respiratory and chemical variables

Monitoring of respiratory and chemical variables in the PCF during emersion revealed changes in some, but not all variables. During the first 2 h of air exposure, the P_{O_2} in the PCF fell significantly from 56 to 24 torr (Fig. 3); then from the 2nd to the 4th h, the P_{O_2} remained relatively unchanged, but still different from pre-emersion values. Finally, after 8 h, the P_{O_2} rose to 39 torr, still significantly different from pre-emersion values.

P_{CO_2} rose significantly within the first 2 h of air exposure from 2.6 to 3.8 torr (Fig. 3); it then remained significantly elevated after 4 h, and finally declined to 2.7 torr after 8 h, a value not significantly different from pre-emersion conditions. Coelomic fluid pH fell significantly during the 2nd and 4th h of emersion, but was no different from pre-emersion values after 8 h. Total carbon dioxide did not change significantly at any time as a result of air exposure.

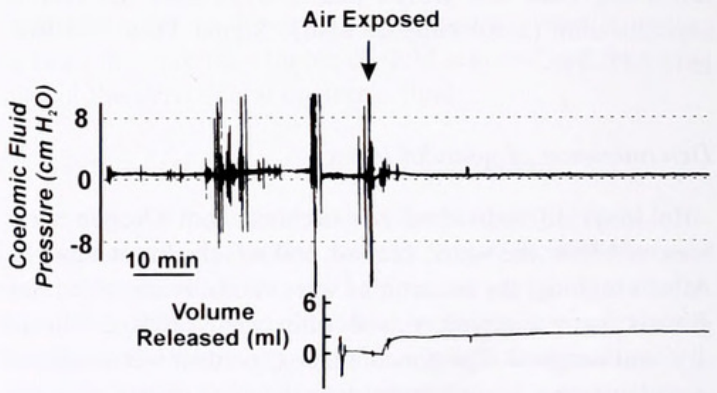

Figure 2. Perivisceral coelomic fluid pressure in *Strongylocentrotus purpuratus* before and during air exposure. The time of emersion is indicated by the arrow. The volume of emersion fluid released is measured simultaneously; note that the fluid is released within minutes of emersion. Oscillations in the pressure trace prior to air exposure are caused by the movement of the sea urchin.

Table 2

Osmotic, calcium ion, and chloride ion concentrations of the perivisceral coelomic fluid (PCF) in Strongylocentrotus purpuratus *as a function of ambient salinity values are mean ± standard error of the mean (SEM)*

Variable	Ambient salinity (ppt)		
	19.9	25.0	32.0
Osmolality (mOsm kg^{-1})			
medium (seawater)	594	839	921
PCF	568 ± 3.62	746 ± 1.44	943 ± 4.41
n	10	10	10
Calcium (mmol l^{-1})			
medium (seawater)	5.9	8.6	10.3
PCF	5.6 ± 0.08	7.9 ± 0.08	9.3 ± 0.15
n	9	10	9
Chloride (mmol l^{-1})			
medium (seawater)	326	448	506
PCF	311 ± 2.14	390 ± 0.94	499 ± 1.44
n	10	10	10

We were able to obtain gas samples from within the intestine of two urchins exposed to air for 9 h. The Po_2 values for the two samples were 122 and 125 torr, respectively.

The osmotic concentration in the coelomic fluid did not change during air exposure, nor did protein, Cl^-, Ca^{++}, or ammonia concentration (Table 3). There was never detectable lactate in the coelomic fluid.

Gonadal index

The gonads were small and occupied little of the intrathecal space. They constituted 3.11% of the wet body weight in animals collected in early June 1990 from the low intertidal of the Oregon coast (Table 1). This is much lower than the 8.04 gonadal index (SEM = 0.58; $n = 6$) that we measured on urchins collected from the same area in February 1997. These data are consistent with those of Gonor (1973), who reported gonadal indices of 8.4 in early March and 4.2 in June.

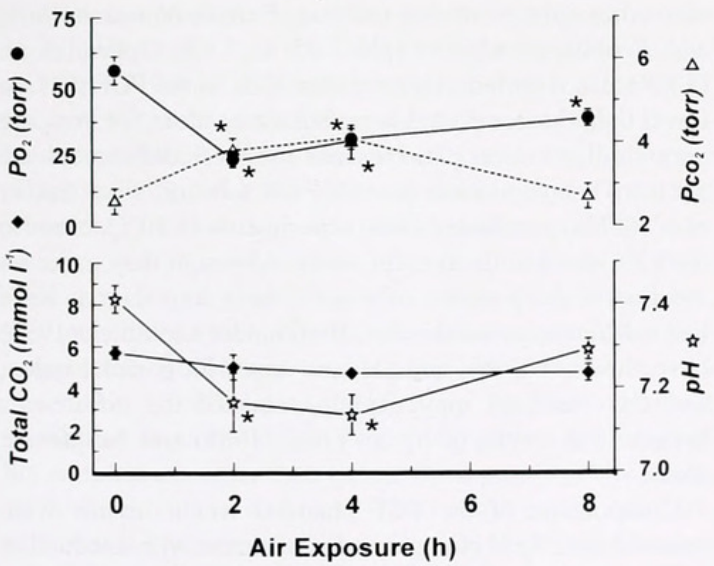

Figure 3. Respiratory and acid-base variables in the perivisceral coelomic fluid of *Strongylocentrotus purpuratus* as a function of the duration of air exposure. Values are mean ± SEM, $n = 10$. Significant differences ($P < 0.05$) from 0 h air exposure are indicated by *.

Discussion

The most striking finding of this study is that, when the sea urchin is emersed, air enters its gut, displacing 33.5% of the volume of the intrathecal space. We suggest that the intestine, containing air with a high oxygen capacitance, constitutes a facultative lung within the intrathecal space and oxygenates the perivisceral coelomic fluid.

Upon emersion, urchins rapidly release a large amount of emersion fluid (EF). This EF originates in the esophagus and perhaps a part of the stomach proximal to the esophagus, a conclusion we base on the appearance, once the urchin is emersed, of dextran blue previously injected into the esophagus (Fig. 1). We suggest that the muscles of the esophagus are not strong enough to hold its contained fluid when the urchin is emersed.

The long stomach and intestinal regions of the gut of regular echinoids are thin, delicate, and lightly muscled, and filled with a relatively constant volume of gut fluid and food (Buchanan, 1969). The fluid volume of the gut has been estimated to be between 6% and 14% of the total volume of the perivisceral coelomic cavity, depending on whether the animals were fed or starved (Buchanan, 1969). Buchanan's (1969) estimates are low because the fluid volume of the gut was measured after the urchin was dissected and the PCF had drained from around the gut; these conditions would have caused some EF to leak out of the gut from each end. The important point is that the gut contains a large amount

Table 3

Perivisceral coelomic fluid variables in Strongylocentrotus purpuratus *as a function of the duration of air exposure; no significant differences were found*

Variable	Duration of emersion (h)			
	0	2	4	8
Osmolality (mOsm kg^{-1})				
Mean	943.1	942.9	944.9	944.1
SEM	4.41	3.49	1.44	6.11
Calcium (mmol l^{-1})				
Mean	9.31	9.03	8.83	8.74
SEM	0.15	0.23	0.25	0.12
Chloride (mmol l^{-1})				
Mean	499.2	492.4	490.5	494.4
SEM	2.18	1.38	2.35	2.78
Ammonium (mmol l^{-1})				
Mean	0.038	0.046	0.041	0.056
SEM	0.02	0.03	0.03	0.03
Protein (mg ml^{-1})				
Mean	0.448	0.319	0.242	0.488
SEM	0.05	0.07	0.05	0.06

Number of observations (n) is 10 in all cases.

of EF that, when released upon emersion, is replaced by air. The fact that no negative hydrostatic pressures occur within the coelomic cavity when sea urchins lose this fluid (Fig. 2) suggests that air displaces the lost fluid easily. We submit that the air enters the anal opening and resides in the intestine with a volume occupying 33.5% of the volume of the perivisceral coelomic cavity (Table 1).

In echinoids the digestive tract is long and coiled and is the most likely place for this relatively large volume of air to reside. The stomach is the longest part of the digestive tract (Anderson, 1966), and makes almost a complete circuit of the body, hanging in five "bag-like festoons" from its attachments to the body wall. The intestine is next, turning and making a reverse circuit alongside the stomach before joining the rectum. A hemal system associated with the gut is particularly well developed in echinoids (Anderson, 1966) and would facilitate the diffusion of oxygen from the lumen of the gut into the PCF. One of us (A. Carroll) has observed that the posterior intestine of many specimens of emersed *Strongylocentrotus droebachiensis* is distended with air upon dissection, supporting our hypothesis that air enters the gut through the anus.

The function of this facultative lung is clearly respiratory. Gas sampled from the intestine of two sea urchins 9 h after emersion has a Po_2 of 122 and 125 torr, about 30 torr below the partial pressure of ambient air (155 torr). These results indicate that the oxygen taken into the sea urchin is being consumed by the animal. Oxygenated PCF may be especially important to *S. purpuratus* when the gonads occupy a significant mass within the urchin, and it may be adaptive in minimizing the dependence of the gonads on anaerobic metabolism. We return to this point below.

The Po_2 of the PCF of immersed *S. purpuratus* is similar to previous measurements in this species (Johansen and Vadas, 1967; Webster, 1975; Webster and Giese, 1975). Although Bookbinder and Shick (1986) measured a slightly higher gonadal index (about 4%) in *S. droebachiensis,* the PCF Po_2 and pH were nearly identical to those measured in the present study. The PCF pH of *S. purpuratus* was somewhat higher than values reported by Spicer *et al.* (1988) for two other species of sea urchins, *Psammechinus miliaris* and *Echinus esculentus* (pH 7.05 to 7.17). Spicer *et al.* (1988) also reported values of total CO_2 in the PCF that are lower than those reported here; but their values for Pco_2 are very similar to ours. The reasons for these differences are unclear. Temperature is probably not a factor, since Spicer *et al.* (1988) conducted their experiments at 10°C, close to the 12°C used in the present study. Although they make no mention of the presence of gonads, their animals may have had a different gonadal index. Bookbinder and Shick (1986) have shown that Po_2 and pH can vary with gonadal index, and this variation may explain some of the differences between the results of Spicer *et al.* (1988) and the present study.

Components of the PCF changed when urchins were emersed, and these changes were consistent with a reduction of gas exchange. PCF Po_2 declined while Pco_2 increased, and the rise in Pco_2 was accompanied by a decrease in pH. A similar decline in PCF Po_2 was measured by Johansen and Vadas (1967), with values of about 20 torr after 5 h of

air exposure. A decline of PCF P_{O_2} was also measured by Webster and Giese (1975), but their values declined to about zero after 3 h. These low values might well be attributed to the needle oxygen electrode used in these experiments. The electrode was inserted into the coelomic cavity but may have penetrated a gonad or some other tissue, producing a low reading. In fact, Bookbinder and Shick (1986) measured 0 torr of oxygen in the center of the ovary using an oxygen microelectrode.

Spicer *et al.* (1988) measured an increase in PCF P_{CO_2} during air exposure in the two species used in their study, and these pressure changes were not accompanied by a decrease in pH. This is in contrast to the results obtained in the present study, where PCF P_{CO_2} increased significantly and pH declined. The results of Spicer *et al.* (1988) indicate a rapid pH compensation for the P_{CO_2} increase in their urchins. They measured small but significant increases in PCF calcium and magnesium ion concentrations in both species, which may account for a part of this compensation. In *S. purpuratus,* we detected no significant changes in any of the ionic concentrations during emersion (Table 3), nor did we observe compensation to the respiratory acidosis. An interesting trend in the respiratory variables suggested that the urchins were exchanging gases more efficiently after 4 h than in the initial stages of emersion (Fig. 3). For example, PCF P_{O_2} after 8 h was elevated above the levels at 2 h, and P_{O_2} had returned to pre-emersion levels; and pH also showed a moderate increase. Another possible explanation for these changes is a reduction in oxygen demand. This hypothesis remains to be tested, but *S. purpuratus* is rarely exposed to air much longer than 4 h (N. B. Terwilliger, pers. obs.).

Bookbinder and Shick (1986) concluded that 76% to 92% of the overall metabolism of the ovaries is anaerobic at the physiological partial pressures of oxygen within the PCF. They reasoned that oxygen is consumed primarily in the superficial tissues and not deep in the ovary (Bookbinder and Shick, 1986). Gut tissues also metabolize anaerobically, but not to the extent of the ovaries. Interestingly, Bookbinder and Shick (1986) detected only very small amounts of lactate within the PCF, a result similar to our own; but when they incubated gonads under anoxic conditions, lactate production rose dramatically. On the basis of their measurements of gonad heat dissipation in a calorimeter, they concluded that the metabolism had a large anaerobic component (Bookbinder and Shick, 1986). However, this conclusion is called into question by the lack of lactate production at physiological P_{O_2}. The production of anaerobic endproducts other than lactate (Ellington and Lawrence, 1973; Bookbinder and Shick, 1986) could explain the lack of lactate accumulation at physiological P_{O_2}, but additional studies are needed to confirm this. However, the energy demands of the unfertilized egg in *S. purpuratus* are extremely small (Leong and Manahan, 1997); a low oxygen demand means that only the eggs deep within the gonad experience a very low concentration of oxygen. In any event, the diffusion of oxygen from the surface of the gonads to the deep tissues must be limited by the P_{O_2} in the PCF. Therefore, the higher the P_{O_2} in the PCF, the greater the amount of gonad that could remain aerobic. During emersion, a facultative lung full of air would serve to maintain aerobic metabolism, at least on the surface of the gonads.

Other tissues bathed by the PCF would also benefit from a well oxygenated medium. The gut of *S. droebachiensis* is less dependent on anaerobic metabolism than the ovaries (Bookbinder and Shick, 1986) and may benefit from direct contact with the air filling the gut. Webster and Giese (1975) reported that the isolated body wall of *S. purpuratus* has a high rate of oxygen uptake, suggesting that it consumes oxygen when oxygen is readily available.

The presence of air somewhere within the urchin explains why Webster and Giese (1975) were able to account for only 10% of the oxygen taken up by the internal tissues during air exposure. Their measurement of the rate of the depletion of oxygen in the PDF showed a rate slower than that expected if the intrathecal space were completely sealed from a supply of oxygen—the basis of their calculation. Thus, oxygen-rich air residing in the intestine—located within the intrathecal space—would prevent a rapid decline in oxygen in the PCF.

These results suggest that, in the sea urchin, the intestine participates in respiration. Others have also suggested that the digestive tract could serve as a respiratory surface in echinoids (Perrier, 1875; Cuénot, 1948, as cited by Farmanfarmaian, 1966). But Farmanfarmaian (1966) dismissed this possibility for a number of reasons that may still apply, provided sea urchins are immersed in seawater. Stott (1955) demonstrated clearly that water circulates through the mouth, esophagus, siphon, and intestine during periods when the animal is not feeding, and he suggested that this circulating water serves a respiratory function. In a similar manner, the air within the gut can play an important role in keeping the PCF oxygenated during emersion.

Finally, we return to one of the ideas that drove this study in the first place, that is, the proposed relationship between osmotic and ionic regulation, compensation for respiratory acidosis, and activity during emersion. Sea urchins are thought to be stenohaline (Binyon, 1966), and it is not surprising that in this study they did not tolerate salinities below 50% seawater. Our results (Table 2) are similar to those obtained by Giese and Farmanfarmaian (1963), who showed that *S. purpuratus* did not survive low salinity. Like the mud crab *Eurytium albidigitum* (Burnett and McMahon, 1987), which is also an osmoconformer, *S. purpuratus* undergoes a respiratory acidosis during emersion, and this acidosis, as in *E. albidigitum,* remains uncompensated after 8 h (Fig. 3).

Acid-base compensation occurred in the urchins studied by Spicer *et al.,* (1988); pH was maintained as P_{CO_2} rose.

The mechanism for this compensation was probably related to the small increase in calcium and magnesium in the PCF. However, this increase may be more related to a passive response of the calcified sea urchin test to the respiratory (*i.e.*, CO_2-induced) acidosis. Such responses have been documented in bivalves (Booth *et al.*, 1984; Dwyer and Burnett, 1996) and at least one crustacean (deFur and McMahon, 1984) in response to a respiratory acidosis induced by emersion, and they are thought to be passive (Booth and Mangum, 1978). The lack of any significant changes in the PCF variables during emersion in *S. purpuratus* (Table 3) suggests that no mechanisms, active or passive, are used to compensate for the acidosis.

These findings support our general hypothesis that intertidal organisms that remain active during emersion compensate for the respiratory acidosis induced by air exposure, and this compensation occurs only if the organism is also a good osmotic and ionic regulator. *S. purpuratus* is indeed not active during air exposure, and thus the hypothesis is supported. On the other hand, lack of activity during emersion of this species of sea urchin may reflect poor anatomical design for movement and respiration in air, rather than an inability to compensate for an emersion-related acidosis.

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