

手術後のアコヤガイPinctada fucata martensiiにおける血液酸塩基平衡

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The blood acid-base balance in the pearl oyster, *Pinctada fucata martensii*, after the surgery

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Abstract : Blood acid-base balance in the pearl oyster was examined by measuring blood pH, total CO₂ content (T_{CO2}), CO₂ partial pressure (Pco₂) and bicarbonate concentration ([HCO₃⁻]). The blood pH, Tco₂, Pco₂ and [HCO₃⁻] under normoxic condition at 28 °C were 7.35–7.42, 1.8–2.1 mM, 1.4–1.8 torr and 1.7–2.0 mM, respectively, although temporary respiratory acidosis was observed just after the surgery for blood collection.

Key words : Pearl oyster, Pinctada fucata martensii, Blood acid-base balance, Cannulation, Surgery

Introduction

The pearl oyster, *Pinctada fucata martensii*, contributes to pearl fisheries, and is an important species in Japan. The process of pearl production is similar to the growth of shell valves, and is directly related to metabolism. The metabolism of the pearl oyster has been studied in terms of regulation of ventilation volume, oxygen uptake and oxygen utilization^{1,2)}. However, there are few reports on the blood gas properties from the viewpoint of the CO² dynamic phase and acid-base balance. Research into the blood acid-base status would contribute to efficient calcification for pearl formation.

Therefore, we examined blood O_2 partial pressure, pH, total CO_2 content, CO_2 partial pressure and bicarbonate concentration (blood acid-base balance) under normoxic condition. In the pearl oyster, most blood drawing methods cannot be applied to examine the blood gas properties. We developed a blood drawing method using a cannula, and examined the blood gas properties.

Materials and Methods

Experimental animals and conditions

The experiments used 50 pearl oysters (shell length :

 $57.0 \pm 0.2 \text{ mm}$ (Mean ±SE), shell height : $60.0 \pm 0.2 \text{ mm}$, shell width : $22.5 \pm 0.4 \text{ mm}$, and total wet weight : $24.5 \pm 0.5 \text{ g}$). The animals were obtained from a marine farm in Tsushima, Nagasaki prefecture. After cleaning the shell valves, they were reared for one month at $28 \degree$ in aerated seawater with added cultivated phytoplankton³⁾. Twenty-four hours before collecting blood, the pearl oysters were transferred to the seawater which had already removed particles (> 0.45μ m). All experiments were conducted in the seawater with a salinity 33psu, water temperature $28\degree$, O₂ partial pressure 152mmHg, pH 8.10, and total CO₂ content 1.8mM.

Surgical procedures

The blood was collected from the anterior aorta using a polyethylene cannula (1.0mm outer diameter, 20cm length). The window (4mm wide, 12mm length) was made at the umbo of the left shell valve, and the cannula was inserted into the anterior aorta with a stylet. The window was closed with denture adhesive and superglue. The cannulated animal was placed in the seawater. This surgical operation took 15minutes.

Blood collection

Multiple collections of blood through the cannula were

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carried out at 5, 15, 30, 60, 120 and 180 min after the surgery (n=20). A single collection of blood through the cannula was carried out at 60 min (n=15) and 180 min (n=15) after the surgery. The blood collection volume was 0.3ml each time.

Blood gas analysis

The blood O₂ partial pressure (Po₂, torr), pH and total CO2 content (Tco2, mM) were immediately measured after each collection. Po2 and pH were measured with a blood gas meter (BGM200, Cameron Instruments) using O2 and pH electrodes (E101, E301-351, Cameron Instrum ents). Tco2 was measured with a total CO2 analyzer (Capnicon 5, Cameron Instruments). Blood CO₂ partial pressure (Pco2, torr) and bicarbonate concentration ([HCO3⁻], mM) were calculated by rearranging the Henderson-Hasselbalch equation⁴⁾. In the equation, the CO_2 solubility coefficient ($a co_2$) and apparent dissociation constant of carbonic acid (pKapp) of the pearl oysters were analyzed using blood collected 1 hour after surgery, and the blood samples were equilibrated with the CO2 standard gases (CO₂ concentration 0.1-2.0%). The pH and total CO2 content of the equilibrated samples were

measured, and $a \cos^2$ and pKapp were determined.

Statistical analysis

Repeated analysis of variance was used for changes to test of the blood properties with time course. Post hoc testing was performed using Scheffe's multiple comparison analysis. The unpaired t-test was used to compare the properties of the blood collected by the different methods.

Results

The mean values of Po₂ and pH from 15min to 180min were 110–118 torr and 7.37–7.42, respectively (Figs. 1–2). Po₂ and pH at 5 min were significantly lower than those at 30min or later (Figs. 1–2). The mean values of Tco₂, Pco₂ and [HCO₃⁻] from 15min to 180min were 1.99–2.07 mM, 1.7-2.2 torr and 1.83-1.92 mM, respectively. Tco₂, Pco₂ and [HCO₃⁻] at 5min and 15min were significantly higher than those at 30min or later (Figs. 3–5). There was no significant difference in the blood properties with the multiple collections and the single collection (Fig. 1–5). The change in the acid-base status in pearl oysters was

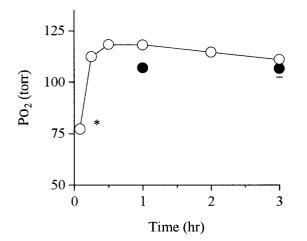


Fig. 1. Blood O² partial pressure (Po²) in the pearl oyster, *Pinctada fucata martensii*, at 28°C under normoxic conditions. The values are shown means ± SE. Each value from the multiple and single collections is shown in open circles and closed circles, respectively. The asterisk indicates statistically significant difference from the other values (P<0.01).</p>

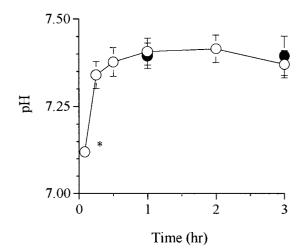


Fig. 2. Blood pH in the pearl oyster, *Pinctada fucata martensii*, at 28°C under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

summarized in a pH-[HCO₃⁻] diagram (Fig. 6). The mean values at 5 and 15 min were above the non-bicarbonate buffer line, although the values at 30 min or later concentrated near that line.

Discussion

We examined blood Po2, pH, Tco2, Pco2 and $[\rm HCO3^{-}]$ to

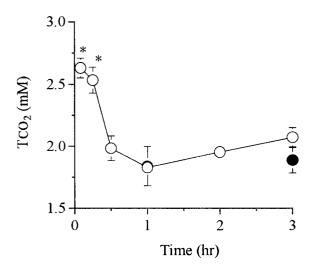


Fig. 3. Blood total CO₂ content (Tco₂) in the pearl oyster, *Pinctada fucata martensii*, at 28°C under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

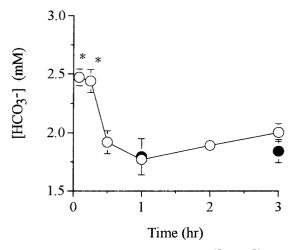
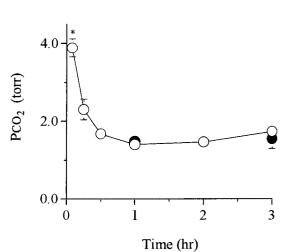


Fig. 5. Blood bicarbonate concentration ([HCO3⁻]) in the pearl oyster, *Pinctada fucata martensii*, at 28°C under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.



evaluate the blood acid-base balance after surgery. The

blood properties just after surgery changed significantly,

but were stable after 30 min. The blood properties in the

multiple collections were not significantly different from those in the single collection. These facts indicated that

the blood properties in this experimental condition are

Fig. 4. Blood CO₂ partial pressure (P_{CO2}) in the pearl oyster, *Pinctada fucata martensii*, at 28 °C under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

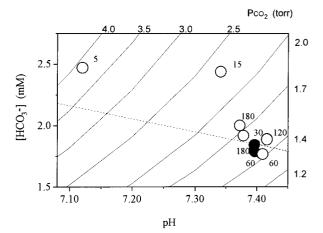


Fig. 6. Diagram summarizing the changes in blood pH, bicarbonate concentration ([HCO3⁻]), and CO2 partial pressure (Pco2) in the pearl oyster, *Pinctada fucata martensii*, at 28°C under normoxic conditions. The open circles are the mean values from the multiple blood collection. The closed circles are the mean values from the single blood collection. The numbers alongside each point show the elapsed time (min : minutes). The curved lines are Pco2 isopleths. The dashed straight line is the non-bicarbonate buffer line.

little influenced after 30 min or later of the surgery.

The blood Po₂ in pearl oysters just after surgery (at 5 min) was significantly lower than that at 15 min or later because the animals were exposed to the air and closed their shell valves during surgery. When marine blue mussels and fresh water clams close their shell valves or are exposed to the air, the oxygen partial pressure of body fluids rapidly decreases⁵⁻⁸⁾. Therefore, the blood of pearl oysters in this study appeared to undergo temporary hypoxemia just after surgery.

The blood Pco₂ at 5 min was significantly higher than that at 15min or later, and pH was lower. In body fluids of some bivalves, the discharge of CO2 was inhibited and accumulated during air exposure⁵⁻⁸⁾. The CO₂ in the blood of pearl oysters seemed to accumulate due to inhibition of its discharge during surgery. The accumulated CO2 titrated toward acidity and lowered the blood pH, and the pearl oysters showed respiratory acidosis. According to the pH-[HCO3-] diagram, the mean values at 5 min and 15 min were located above the non-bicarbonate buffer line. The value at 5 min was close to the line, and that at 15 min was apart. Furthermore, the mean values at 30 min or later concentrated the line. From these facts, the increased [HCO₃⁻] compensated for the respiratory acidosis, and made the pH gradually increase within 30 minutes. These results correspond with the time in which the effect on hypoxemia disappears. The temporary hypoxemia and respiratory acidosis disappeared within 30 min after the surgery, and the oxygen and acid-base status were stable afterwards under the normoxic condition. These results suggested that the blood collection at 30 min or later after surgery is useful for the research of respiratory physiology in the pearl oyster because the blood properties are stable.

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アコヤガイ血液の酸塩基平衡を明らかにするため、血液のpH,全炭酸含量、二酸化炭素分圧および重炭酸イオン濃度を調べた。アコヤガイ血液は採血の為に施した手術直後に一時的な呼吸性アシドーシスを示したが、手術から30分経過すると血液性状は安定し、血液pHは7.35-7.42、全炭酸含量は1.8-2.1mM,二酸化炭素分圧は1.4-1.8 torr および重炭酸イオン濃度は1.7-2.0mMを示した(水温28℃)。