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

Takeshi Handa[1†] and Ken-ichi Yamamoto[1]

Abstract : Blood acid-base balance in the pearl oyster was examined by measuring blood pH, total CO_2 content (T_{CO2}), CO_2 partial pressure (P_{CO2}) and bicarbonate concentration ($[HCO_3^-]$). The blood pH, T_{CO2}, P_{CO2} and $[HCO_3^-]$ under normoxic condition at 28 ℃ 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 CO_2 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±0.2 mm (Mean±SE), shell height : 60.0±0.2 mm, shell width : 22.5±0.4 mm, and total wet weight : 24.5±0.5 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℃ in aerated seawater with added cultivated phytoplankton[3]. Twenty-four hours before collecting blood, the pearl oysters were transferred to the seawater which had already removed particles (>0.45 μm). All experiments were conducted in the seawater with a salinity 33 psu, water temperature 28℃, O_2 partial pressure 152 mmHg, pH 8.10, and total CO_2 content 1.8 mM.

Surgical procedures

The blood was collected from the anterior aorta using a polyethylene cannula (1.0 mm outer diameter, 20 cm length). The window (4 mm wide, 12 mm 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 15 minutes.

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 0.3 ml each time.

Blood gas analysis

The blood O_2 partial pressure (P_{O_2}, torr), pH and total CO_2 content (T_{CO_2}, mM) were immediately measured after each collection. P_{O_2} and pH were measured with a blood gas meter (BGM 200, Cameron Instruments) using O_2 and pH electrodes (E101, E301-351, Cameron Instrum ents). T_{CO_2} was measured with a total CO_2 analyzer (Capnicon 5, Cameron Instruments). Blood CO_2 partial pressure (P_{CO_2}, torr) and bicarbonate concentration ($[HCO_3^-]$, mM) were calculated by rearranging the Henderson-Hasselbalch equation[4]. In the equation, the CO_2 solubility coefficient (α_{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 CO_2 standard gases (CO_2 concentration 0.1-2.0%). The pH and total CO_2 content of the equilibrated samples were measured, and α_{CO_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 P_{O_2} and pH from 15 min to 180 min were 110-118 torr and 7.37-7.42, respectively (Figs. 1-2). P_{O_2} and pH at 5 min were significantly lower than those at 30 min or later (Figs. 1-2). The mean values of T_{CO_2}, P_{CO_2} and $[HCO_3^-]$ from 15 min to 180 min were 1.99-2.07 mM, 1.7-2.2 torr and 1.83-1.92 mM, respectively. T_{CO_2}, P_{CO_2} and $[HCO_3^-]$ at 5 min and 15 min were significantly higher than those at 30 min 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

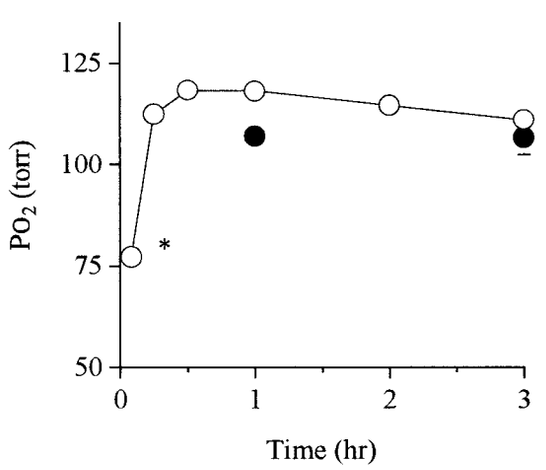

Fig. 1. Blood O_2 partial pressure (P_{O_2}) in the pearl oyster, *Pinctada fucata martensii*, at 28℃ under normoxic conditions. The values are shown means ± SE. Each value from the multiple and single collections is shown in open circles and closed circles, respectively. The asterisk indicates statistically significant difference from the other values (P<0.01).

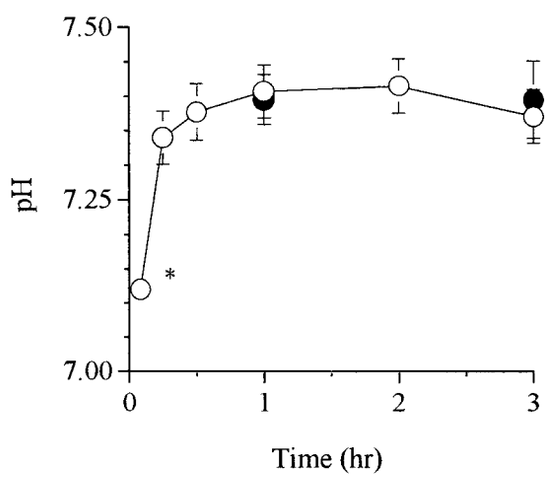

Fig. 2. Blood pH in the pearl oyster, *Pinctada fucata martensii*, at 28℃ under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

summarized in a pH-[HCO_3^-] 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 P_{O_2}, pH, T_{CO_2}, P_{CO_2} and [HCO_3^-] to 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

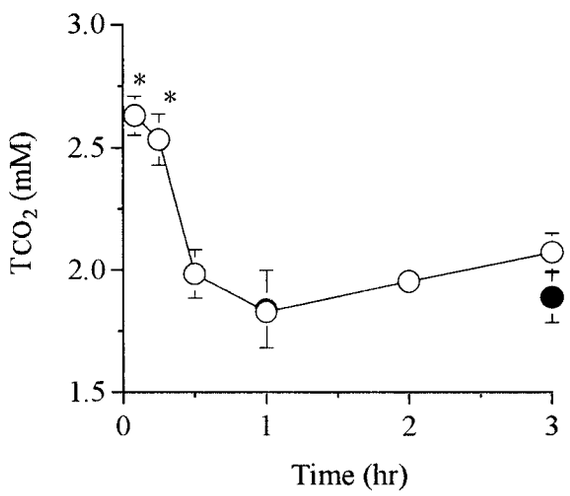

Fig. 3. Blood total CO_2 content (T_{CO_2}) in the pearl oyster, *Pinctada fucata martensii*, at 28℃ under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

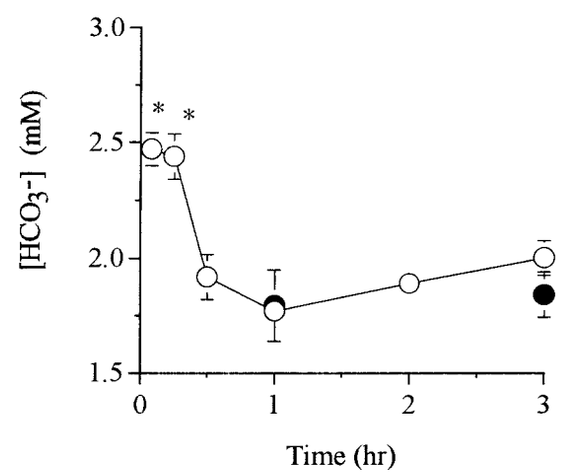

Fig. 5. Blood bicarbonate concentration ([HCO_3^-]) in the pearl oyster, *Pinctada fucata martensii*, at 28℃ under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

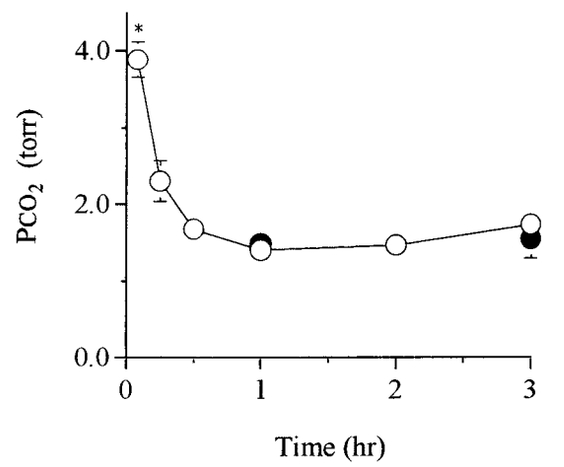

Fig. 4. Blood CO_2 partial pressure (P_{CO_2}) in the pearl oyster, *Pinctada fucata martensii*, at 28℃ under normoxic conditions. The values shown are means ± SE. The symbols and asterisk are the same as in Fig. 1.

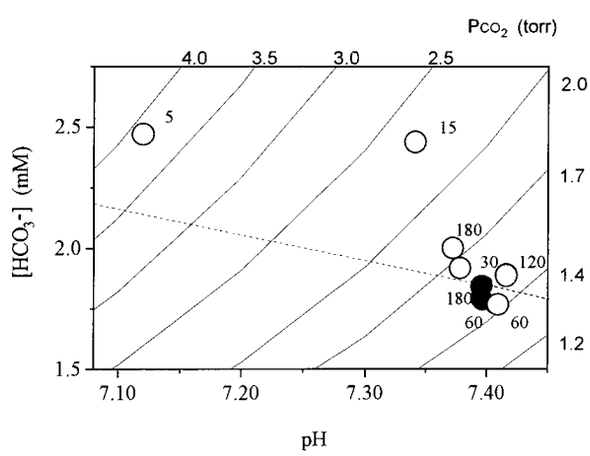

Fig. 6. Diagram summarizing the changes in blood pH, bicarbonate concentration ([HCO_3^-]), and CO_2 partial pressure (P_{CO_2}) in the pearl oyster, *Pinctada fucata martensii*, at 28℃ under normoxic conditions. The open circles are the mean values from the multiple blood collection. The closed circles are the mean values from the single blood collection. The numbers alongside each point show the elapsed time (min : minutes). The curved lines are P_{CO_2} isopleths. The dashed straight line is the non-bicarbonate buffer line.

little influenced after 30 min or later of the surgery.

The blood Po_2 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 decreases[5-8]. Therefore, the blood of pearl oysters in this study appeared to undergo temporary hypoxemia just after surgery.

The blood Pco_2 at 5 min was significantly higher than that at 15 min or later, and pH was lower. In body fluids of some bivalves, the discharge of CO_2 was inhibited and accumulated during air exposure[5-8]. The CO_2 in the blood of pearl oysters seemed to accumulate due to inhibition of its discharge during surgery. The accumulated CO_2 titrated toward acidity and lowered the blood pH, and the pearl oysters showed respiratory acidosis. According to the pH-[HCO_3^-] 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 [HCO_3^-] 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.

References

1) Yamamoto K, Adachi S, Koube H : Direct method of measuring the filtration volume in the pearl oyster, *Pinctada fucata martensii*. *J Nat Fish Univ*, **44**, 189-194 (1996)

2) Yamamoto K, Adachi S, Koube H : Effects of hypoxia on respiration in the pearl oyster, *Pinctada fucata martensii*. *Aquaculture Sci*, **47**, 539-544 (1999a).

3) Yamamoto K, Handa T, Nakamura M, Kitukawa K, Kita Y, Takimoto S, Nishikawa S : Effects of ozone-produced oxidants on respiration of the pearl oyster, *Pinctada fucata martensii*. *Aquaculture Sci*, **47**, 241-248 (1999b)

4) Davenport HW : The ABC of acid-base chemistry 6 th edition. University of Chicago Press, Chicago, 39-41 (1974)

5) Booth CE, McDonald DG, Walsh PJ : Acid-base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air-exposure on hemolymph acid-base status. *Mar Bio Lett*, **5**, 347-358 (1984)

6) Byrne RA, McMahon BR : Acid-base and ionic regulation, during and following emersion, in the freshwater bivalve, *Anodonta grandis simpsoniana* (Bivalvia : Unionidae). *Bio Bull*, **181**, 289-297 (1991)

7) Byrne RA, Shipman BN, Smatresk NJ, Dietz TH, McMahon RF : Acid-base balance during emergence in the freshwater bivalve *Corbicula fluminea*. *Physiol Zool*, **64**, 748-766 (1991)

8) Jokumsen A, Fyhn HJ : The influence of aerial exposure upon respiratory and osmotic properties of haemolymph from two intertidal mussels, *Mytilus edulis* L. and *Modiolus modiolus* L. *J Exp Mar Biol Ecol*, **61**, 189-203 (1982)

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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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1.8–2.1 mM，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1.7–2.0 mMを示した（水温28℃）。