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Metabolic Alkalosis due to Feeding Chicks in Breeding Adélie Penguins *Pygoscelis adeliae* under Natural Conditions

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ABSTRACT

Prolonged abnormal vomiting causes metabolic alkalosis. Many seabirds are known to feed their chicks by regurgitation. We hypothesized that metabolic alkalosis occurs in seabirds even under natural conditions during the breeding season. Adélie penguins *Pygoscelis adeliae* feed their chicks by regurgitating food for 50–60 d until the chicks fledge. In this study, the concentrations of Cl^-, HCO_3^-, Na^+, K^+, pH, and Pco_2 in the blood of breeding Adélie penguins were measured throughout the chick-rearing season. The pH of penguin venous blood shifted from 7.54 in the guarding period to 7.47 in the crèche period. Decreasing Cl^- and increasing HCO_3^- blood concentrations in parents were associated with increasing mass of their brood in the guarding period, the early phase of the rearing season, indicating that regurgitating to feed chicks causes loss of gastric acid and results in relative metabolic alkalosis. The inverse trend was observed during the crèche period, the latter phase of the rearing season, when parents spent more time at sea and have fewer opportunities for gastric acid loss. This was assumed to be the recovery phase. These results indicate that regurgitation might cause metabolic alkalosis in breeding Adélie penguins. To our knowledge, this is the first report to indicate that seabirds exhibit metabolic alkalosis due to regurgitation to feed chicks under natural conditions.

Introduction

The acid-base homeostasis in the body is maintained by adjustment of respiration and kidney metabolism. Under certain conditions, acid-base homeostasis disorders may appear. Alkalosis, a body condition with high pH, is divided into respiratory and metabolic alkalosis. The former is caused by the low blood partial pressure of CO_2 (Pco_2) associated with hyperventilation. In birds, it is well known that heat stress triggers panting, resulting in hypocapnia and respiratory alkalosis (Calder and Schmidt-Nielsen 1968; Murrish 1982). The latter is caused by an alteration of the blood bicarbonate ion concentration associated with metabolic disorder. The most common reason for metabolic alkalosis in patients is known to be abnormal vomiting (Ganong 2001*a*). Metabolic alkalosis owing to vomiting occurs as follows. Prolonged vomiting causes loss of hydrogen-rich stomach contents from the body. Because the acid-base homeostasis of extracellular fluid is balanced mainly by carbonate buffer, the reduction of hydrogen is compensated by conversion of carbonic acid to hydrogen and bicarbonate ions. In this process, the blood bicarbonate concentration and pH increase. Then the rate of ventilation decreases to compensate for the pH increment, resulting in elevation of the blood carbon dioxide concentration and a change in the balance of the carbonate buffer, which increases hydrogen and additional bicarbonate ion concentrations.

Many seabirds (e.g., penguins, shearwaters, albatrosses, petrels, pelicans, cormorants, herons, and gulls) are known to feed their chicks by regurgitation, although some seabirds feed their chicks by carrying food in their bill (terns and alcids). Regurgitating seabirds store prey in the digestive tract after foraging to deliver it to the nest. In the case of penguins, captured food is stored in the stomach (Croxall 1987). The gastric pH of king penguins ranges from 2 to 4 in nonfasting periods (Gauthier-Clerc et al. 2002; Thouzeau et al. 2003, 2004), indicating secretion of gastric acid in the stomach.

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The Adélie penguin *Pygoscelis adeliae* is one of the species in which the parents feed the chicks by regurgitating at the nest. Feeding continues until the chicks fledge at 50–60 d. During this period, the chick grows in mass from approximately 100 g as an egg to about 3.5 kg at the end of February (approximately 70% of the parent's body mass; Ainley 2002).

We hypothesized that metabolic alkalosis occurs in birds even under natural conditions, such as when the birds feed chicks by regurgitation. In this study, we examined effects of the regurgitation associated with feeding chicks on acid-base homeostasis in breeding Adélie penguins under natural conditions.

Material and Methods

Adélie penguins live in the Southern Ocean and breed along the Antarctic coast in austral summer. A breeding pair usually lays two eggs that are incubated for ca. 30 d. After the chicks hatch, parents alternate foraging and guarding chicks. The parents change roles every 1–3 d (guarding period). After a guarding period of ca. 22 d, both parents go to sea to forage and come back to the nest to feed chicks (crèche period).

The study was carried out on the Torinosu Cove (69°30′S, 39°34′E, December 2003–January 2004) and the Hukuro Cove (69°13′S, 39°39′E, December 2004–February 2005) colonies of Adélie penguins in Lützow-Holm Bay, Antarctica. We collected 37 blood samples in the Torinosu Cove colony and 93 samples in the Hukuro Cove colony of breeding Adélie penguins from 101 birds. Fifteen samples were collected from birds raising one chick, and 115 samples were collected from birds raising two chicks. Seventy-six birds were caught once, and 21 and four birds were caught twice and three times, respectively. For birds caught more than once, the interval between the blood collections was at least 10 d to alleviate capture stress. Blood collection from the parents was performed irregularly during the course of food delivery to chicks. Within one regurgitation phase, the blood status might change from the first regurgitation to the final regurgitation. Each blood value represented the status at a random time point in the oscillating blood status between each regurgitation phase. During blood collection, the chicks were kept in a box lined with a towel for protection against other penguins and the South Polar skua *Stercorarius maccormicki*. The birds were anaerobically bled from a foot vein using a heparinized syringe. The animals were restrained using a rubber sheet that covered the upper body and flippers to minimize disturbance of the physical condition. Because Le Maho et al. (1992) reported that handling stress induced changes in blood pH, we avoided making the birds excited and inducing hyperventilation. The blood was analyzed within 10 min after blood collection.

Concentrations of sodium, potassium, chloride, bicarbonate ions, pH, and P_{CO_2} were measured with a portable clinical analyzer (i-STAT, East Windsor, NJ) by introducing heparinized whole blood into a disposable cartridge (i-STAT EC8+ and CG4+; Larsen et al. 2002). The original values obtained by i-STAT were measured for a body temperature of 37°C. The body temperature of Adélie penguins averages 39°C (Boyd and Sladen 1967; Lenfant et al. 1969; Boyd and Sladen 1971; Ropert-Coudert et al. 2001). Because both pH and P_{CO_2} are affected by body temperature, the pH and P_{CO_2} values were corrected to 39°C for Adélie penguins. The formula for temperature correction was obtained from guidelines of the Clinical and Laboratory Standards Institute (2001). The formulas are

$$\text{pH corrected} = \text{pH}_{\text{at }37^\circ\text{C}} + [-0.0147 + 0.0065 \times (7.40 - \text{pH}_{\text{at }37^\circ\text{C}})] \times (t - 37),$$

$$P_{CO_2}\text{ corrected} = P_{CO_2\,\text{at }37^\circ\text{C}} \times 10^{0.019(t-37)},$$

where t is the body temperature.

The bicarbonate ion concentration was calculated on the i-STAT analyzer using the Henderson-Hasselbalch equation with pH and P_{CO_2}. This is dependent on the solubility of CO_2 in plasma. Although the i-STAT was designed for use on humans, it is reported that the calculation bias of the i-STAT analyzer was acceptable for chicken blood (Steinmetz et al. 2007). Therefore, it was assumed that the solubility of CO_2 in penguin blood is similar to that in humans.

The sampled birds were weighed and marked on their chest with black hair dye. The number of chicks and their mass were recorded. During the crèche period, each chick was marked with a numbered plastic flipper tag using a cable tie and weighed every 4–6 d. The tag was adjusted in size every 4–6 d according to the chick's growth. All tags were removed at the end of the study. The chick growth rate was estimated by the slope of the linear regression of chick mass on the date from early January to late January, since it is known that the growth rate is constant (Watanuki et al. 1993). All procedures were in compliance with the National Institute of Polar Research, Japan, Institutional Animal Care and Use Committee for Antarctic Research (project research Japanese Antarctic Research Expedition 45, 46) and the ethical guidelines of the Ministry of the Environment, Japan.

It was difficult in practice to estimate the cumulative amount of parental regurgitation. Because the chick growth rate and the size of the mass of the stomach contents of breeding adults on arrival at the nest are known to be constant within a breeding pair throughout the breeding season (Watanuki et al. 1993), the brood mass is proportionally related to the cumulative amount of parental regurgitation. Therefore, we used brood mass as a proxy index for the cumulative amount of parental regurgitation.

The bicarbonate ion concentration of nonbreeding penguins was calculated by the Henderson-Hasselbalch equation for humans based on pH and P_{CO_2} using the data from Murrish (1982). Blood data of a breeding penguin were grouped according to its brood's mass in kilograms. The effects of capture times on the blood parameters of a bird were examined by a linear model. For comparison of each blood parameter within groups, Tukey-Kramer's test was employed. Linear regression analysis was used to estimate the equation of the model for plasma bicarbonate concentration with plasma chloride con-

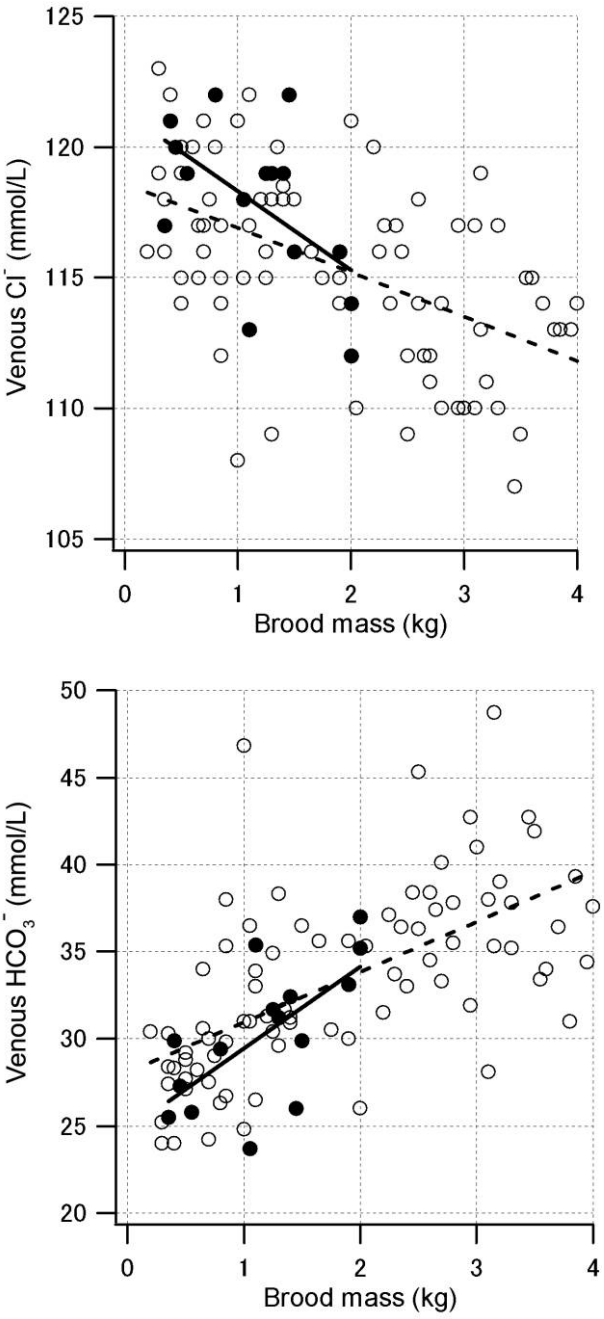

Figure 1. Relationships between brood masses and venous concentrations of chloride and bicarbonate ions of its parents in guarding period. The solid and dashed lines refer to data from parents of one- and two-chick broods, respectively.

centrations. Values are presented as means ± SD, with significance set at $P < 0.05$.

Results

No difference was observed between data obtained from the Torinosu Cove and Hukuro Cove colonies in our study, and the number of times a bird was captured did not affect blood parameters significantly. Therefore, we pooled these data for analysis. Each sampled penguin incubated eggs ($n = 16$) and guarded one ($n = 15$) or two chicks ($n = 99$). The mean mass of the parent birds was 4.66 ± 0.54 kg. It did not change significantly during the sampling season. The growth rate of each chick was 88 ± 33 g/d. The seasonally adjusted masses of chicks from one- and two-chick broods were not significantly different. The chicks weighed less than 2 kg in the guarding period. Figure 1 shows the trend of venous chloride and bicarbonate ion concentrations of breeding penguins, with the mass of its brood in the guarding period. Both chloride and bicarbonate ion concentrations were significantly changed with brood mass (linear model, $P < 0.05$). The number of chicks and interaction factors were not significant for the blood ion trend.

Because Adélie penguins usually lay two eggs, breeding adults guarding one chick had lost one of the two chicks during breeding. Therefore, parents raising one chick regurgitate more food cumulatively than the amount corresponding to the brood mass. To estimate the cumulative amount of parental regurgitation precisely, we excluded the data from penguins with one-chick broods in the later analyses of the relationship between parent blood status and chick mass (Figs. 2–5). One bird in the 6–7-kg brood mass stage revealed abnormally low pH

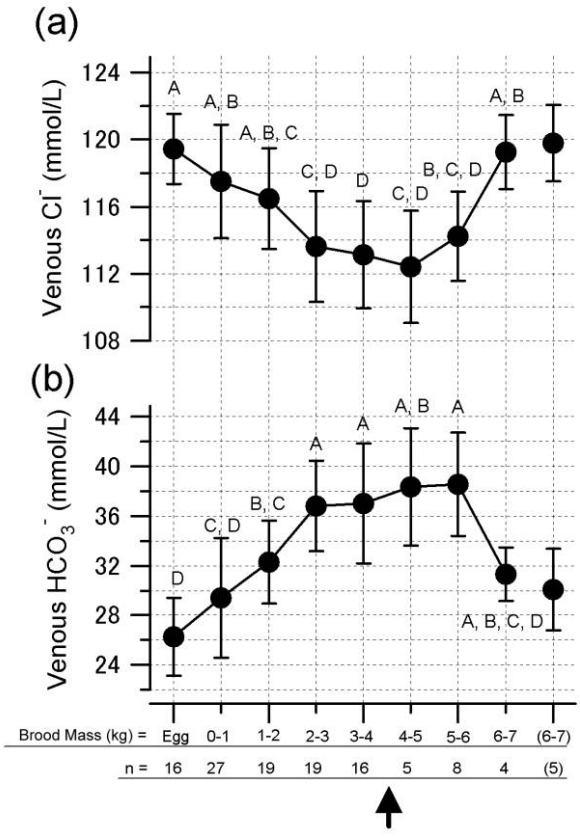

Figure 2. Mean ± SD venous concentrations of chloride and bicarbonate ions of breeding Adélie penguins with two-chick broods. Arrow indicates the line between guarding and crèche periods. Values of the 6–7-kg brood group in parentheses included data of a bird showing irregular values. Identical letters indicate no significant difference among means by Tukey-Kramer's test ($P < 0.05$).

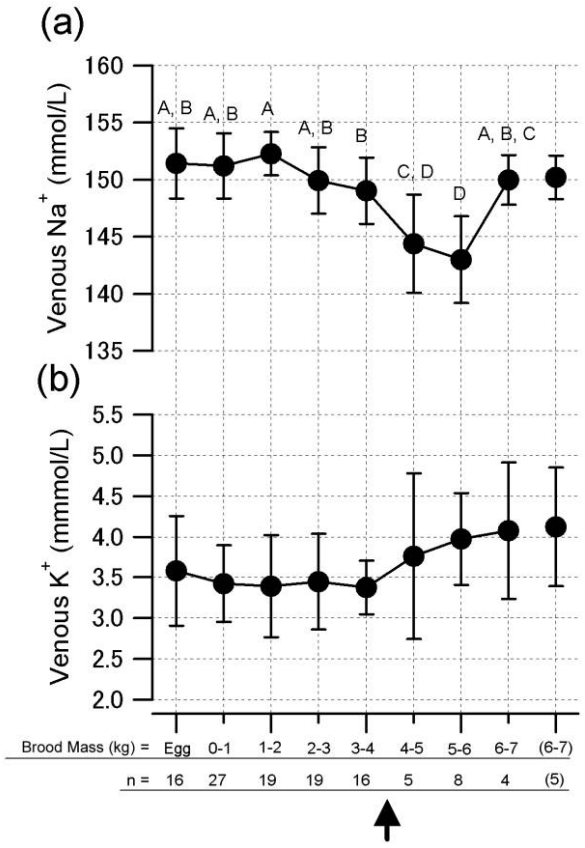

Figure 3. Mean ± SD venous concentrations of sodium and potassium ions of breeding Adélie penguins with two-chick broods. Arrow indicates the line between guarding and crèche periods. Values of the 6–7-kg brood group in parentheses included data of a bird showing irregular values. Identical letters indicate no significant difference among means by Tukey-Kramer's test ($P < 0.05$).

values and high P_{CO_2} values, although the reason is unknown. Therefore, we excluded the data of this bird from later analyses.

Biphasic trends were observed in venous concentrations of chloride, bicarbonate, and sodium ions (Figs. 2, 3). The venous chloride ion concentration of the parents decreased with increasing brood mass until the chicks reached 5 kg: from 119.4 ± 2.1 mmol/L in the egg stage to 112.3 ± 3.4 mmol/L in the 4–5-kg brood mass stage, which is the time of crèche formation. After that, venous chloride ion concentrations began to rise to 119.3 ± 2.2 mmol/L in the 6–7-kg brood mass stage (Fig. 2). An inverse trend was observed in the venous concentrations of bicarbonate ion. A mean concentration of 26.3 ± 3.2 mmol/L in the egg stage increased with brood mass to 38.5 ± 4.2 mmol/L as the maximum in the 5–6-kg brood mass stage, whereas the venous concentration of bicarbonate ion was 31.3 ± 2.2 mmol/L in the 6–7-kg brood mass stage, similar to the concentration in the 0–1-kg brood mass stage (Fig. 2). The venous sodium ion concentration did not change significantly from the egg stage to the 4-kg brood mass stage, but it dropped to 143.0 ± 3.8 mmol/L at the 5–6-kg brood mass stage, then recovered to 150.0 ± 2.2 mmol/L at the 6–7-kg brood mass stage (Fig. 3). Venous potassium ion concentrations did not alter significantly with changes in brood mass.

The venous blood pH of parents did not change significantly as the chicks gained mass, although the pH tended to decline with a brood mass of more than 4 kg; it ranged from 7.54 ± 0.10 at the 3–4-kg brood mass stage to 7.47 ± 0.04 at the 6–7-kg brood mass stage (Fig. 4). Venous blood P_{CO_2} gradually increased with the increase of brood mass in the early stage, from 34.14 ± 6.05 mm Hg in the egg stage to 47.01 ± 10.21 mm Hg in the 2–3-kg brood mass stage. After that, the level of venous blood P_{CO_2} was not significantly different throughout the season. As can be seen in the Davenport diagram of pH/bicarbonate (Fig. 5), the venous bicarbonate ion concentration continuously increased with brood mass from the egg stage to the 2–3-kg brood mass stage, indicating metabolic alkalosis. It remained stable as the brood mass increased from 3 to 6 kg and then suddenly dropped in the 6–7-kg brood mass stage.

There was a highly significant negative linear correlation between plasma bicarbonate and chloride concentrations ($r = -0.81$, $P < 0.0001$), with an almost equimolar loss of plasma chloride for each bicarbonate ion accumulated (Fig. 6).

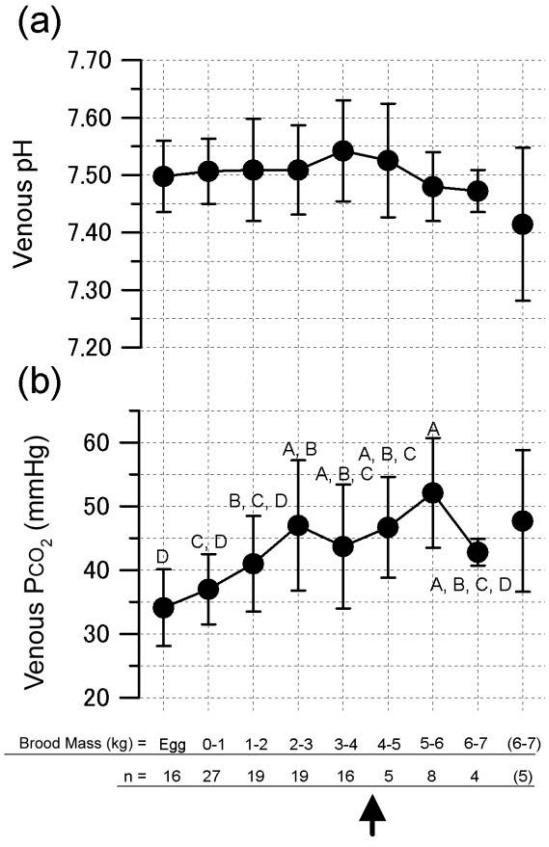

Figure 4. Mean ± SD venous pH and P_{CO_2} of breeding Adélie penguins with two-chick broods. Arrow indicates the line between guarding and crèche periods. Values of the 6–7-kg brood group in parentheses included data of a bird showing irregular values. Identical letters indicate no significant difference among means by Tukey-Kramer's test ($P < 0.05$).

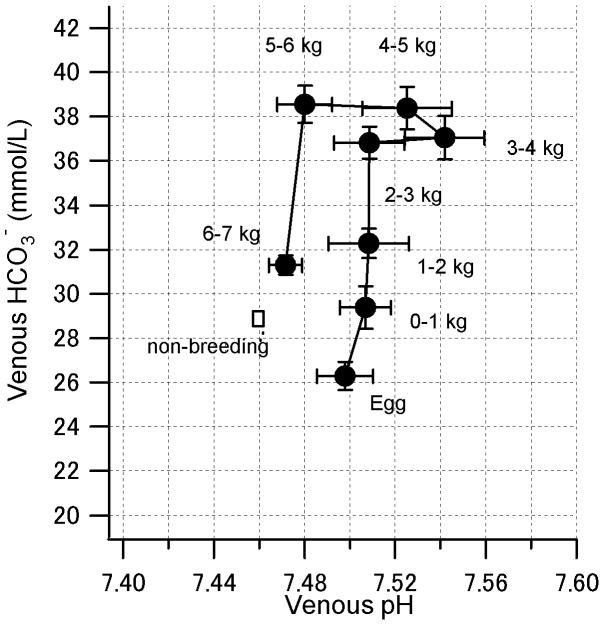

Figure 5. Davenport diagram of pH-HCO_3^- showing the blood acid-base status of breeding Adélie penguins with two-chick broods by brood mass stages from egg to 6–7 kg (*circles*) and that of nonbreeding penguins (*square*). Data of nonbreeding penguins are cited from Murrish (1982). Each symbol indicates mean ± 0.2 SD.

Discussion

Compared with other studies, the delay between blood collection and analysis in the present study was long (Murrish 1982). Thus, we cannot eliminate the possibility of influence of erythrocyte metabolic activity on blood parameters. However, the ambient temperature at our study site was around 0°C, and blood samples were kept in the shade until analysis. Therefore, we believe that the influence of erythrocyte metabolic activity would be minimal.

It is known that the gastric pH in the penguin is variable. It was reported that the stomach pH of the king penguin was close to 4 at the beginning of the incubation fast, gradually increased in a few days, and could be maintained at values as high as 6 during the incubating fast (Gauthier-Clerc et al. 2002; Thouzeau et al. 2004). They argued that high pH might contribute to preservation of stomach contents during a long-term fast. The physiological nature of the incubating fast in the king penguin may be very different from that of the Adélie penguin's fast in the guarding period. The fast duration of an incubating king penguin is around 3 wk, whereas that of the Adélie penguin in the guarding period is between 8 h and 3 d. Because adjusting the stomach pH took a few days in the king penguin, it does not seem reasonable that the Adélie penguin would adjust its gastric pH to high for preservation of the stomach contents for 1–3 d. Under the condition of nonfasting in the incubation period, the gastric pH of the king penguin, which arrives from the sea before the incubation fast, was 4.1 (stomach with food) and 2.9 (without food; Gauthier-Clerc et al. 2002), and that when departing for the sea after the incubation fast was 3.2 (with food) and 2.1 (without food). We expect that the pH of regurgitated stomach contents from breeding Adélie penguins would be similar to that of king penguins under the nonfasting condition.

Adélie penguin chicks do not forage at sea but obtain all food from the parents until fledging. At Cape Crozier (77°31′S, 169°23′E) in 1969–1970, the average maximum mass of chicks from one-chick broods reached 3.3 kg, and chicks from two-chick broods reached 3.1 kg (Ainley and Schlatter 1972). Although it is known that the adults raising two-chick broods go on foraging trips more frequently and deliver more food than parents of one-chick broods in *Pygoscelis* penguins, the relatively small difference in trip frequency (~15%) and amount of food delivered (~20%) cannot account for the double energy demands at two-chick nests (Lishman 1985; Meyer et al. 1997; Jansen et al. 2002). Thus, it seems likely that parents with larger broods must regurgitate a larger proportion of their stomach contents to their chicks in order to support the high growth rates of the brood by reducing the amount of stomach contents available for parent nutrition.

Blood chloride and bicarbonate ion concentrations were significantly changed by brood mass (Fig. 1). Because the brood mass of one chick was almost half that of chicks in two-chick broods at the same time point, the chronologic trends of these blood ions seemed to be associated with brood mass rather than individual chick mass. The results indicate that the change of blood status depended on the cumulative amount of parental regurgitation from the chicks hatched.

Compared with nonbreeding adults, adults at the egg stage showed higher pH and lower bicarbonate ion concentration

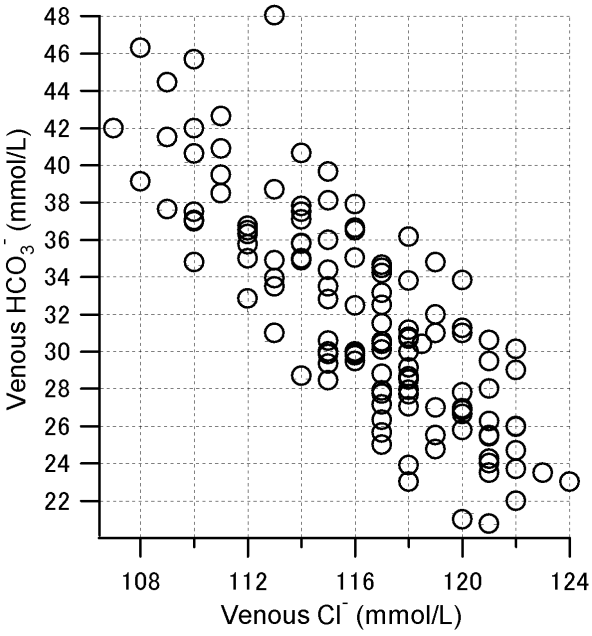

Figure 6. Relationship between venous ion concentrations of bicarbonate and chloride in breeding Adélie penguins ($r = -0.81$, $P < 0.001$). Regression equation is $[HCO_3^-] = 173.17 - 1.21[Cl^-]$.

(Fig. 5). Under high heat stress (26°–27°C), Adélie penguins begin panting, resulting in respiratory alkalosis, a high blood pH with a low bicarbonate ion concentration (Murrish 1982, 1983). At our study site, the ambient temperature was always less than 5°C. However, intense solar radiation occurred on the cloudless days of austral summer. The incubating adult does not leave the nest, which does not shade birds from the sun. This situation may cause heavy heat stress of incubating adults and cause respiratory alkalosis relative to the pH of nonbreeding penguins (Fig. 5). The venous blood pH of breeding Adélie penguins was higher than 7.50 from the 1-kg brood mass stage to the 5-kg brood mass stage, and then it decreased to around 7.47 at the 6–7-kg brood mass stage (Fig. 4). This indicated that breeding penguins were in relative alkalosis between the egg stage and the 5-kg brood mass stage at least.

The bicarbonate ion concentration increased at a similar rate from the egg stage to the 3-kg brood mass stage (Fig. 5). After that, the rate of increase slowed down. In our study, the mass of two-chick broods at crèche formation was 3.94 ± 0.72 kg, corresponding to the time when the rate of increase in the bicarbonate ion concentration in parents decelerated. Then, bicarbonate concentrations in parents decreased when the brood weighed 6–7 kg. According to Watanuki et al. (1993), stomach mass and the feeding rate for chicks of chick-rearing adults are constant throughout the breeding season, indicating that the volume of food regurgitated for chicks did not differ much. In contrast, parental feeding behavior for chicks is different between the guarding and the crèche period. In the guarding period, the adult stays to attend to its chick and keeps regurgitating, whereas the parent in the crèche period visits the colony for a short time and regurgitates intensively. If the condition of gastric pH of Adélie penguins is similar to that of king penguins under the condition of nonfasting, a stomach containing food will have a higher gastric pH than an empty stomach (Gauthier-Clerc et al. 2002; Thouzeau et al. 2004). Thus, adults would spend more time with an empty stomach in the guarding period than in the crèche period, resulting in a greater loss of gastric acid. In addition, biparental feeding in the crèche period allows each parent to regurgitate less.

These results indicate that breeding Adélie penguins exhibited a combination of two types of alkalosis, although the shift of blood pH was minor as a result of the adjustment by blood ion balance. When birds incubated an egg in the nest, respiratory alkalosis occurred (Murrish 1982). After the chick(s) hatched, metabolic alkalosis increased with brood mass growth through the guarding period. In the crèche period, parents stopped guarding the chicks, and alkalosis was likely decreased because the parents spent longer periods foraging at sea, which reduced opportunities for gastric acid loss (Watanuki et al. 1993; Kato et al. 2003). It is known that parents leave their chicks unattended when the chicks reach the stage of development at which they can thermoregulate (Sapin-Jaloustre and Bourliere 1951; Goldsmith and Sladen 1961; Goldsmith 1962), the chicks and parents recognize each another (Thompson and Emlen 1968), and chicks can find their way back to the nest site (Spurr 1975). According to our study, this time point also coincided with the period for adults exhibiting the heaviest metabolic alkalosis.

A relatively low blood sodium concentration was observed at the 4–6-kg brood mass stage (Fig. 3), and this was the most advanced stage of alkalosis. Although the major components are hydrogen and chloride ions, gastric acid also contains sodium ions (Ganong 2001*b*). The loss of gastric acid would cause lower concentrations of blood sodium. Both parents and chicks eat the same food in the guarding period, but the chloride concentration in the cloacal fluid of the parents is lower than that of chicks (Douglas 1968; Janes 1997). This may be caused by the high rate of absorption of chloride ions by parents. This is consistent with the lower blood chloride concentration of parents in the guarding period. Additionally, there was a strong negative correlation between the concentrations of blood chloride and bicarbonate ions (Fig. 6; $r = -0.81$). This would also indicate that bicarbonate ions compensated for the loss of chloride ions contained in gastric acid.

Breeding is one of the most important events in animal life. Mammals provide nutrition to their neonates by lactation. As a phenomenon of the physiological alterations associated with breeding activity, hypocalcemia in postparturient mammals is well known. It is a common disease in dairy cows and dogs, when the demand for calcium for milk production exceeds the body's ability to mobilize calcium reserves. This hypocalcemia can trigger collapse, muscle spasms, and eventually heart failure. The metabolic alkalosis in this study could be thought of as a bird-specific syndrome during the breeding season. It is worth noting that the variation in venous pH in breeding penguins was relatively small (7.45–7.50), although there was a continuous demand for regurgitation of the stomach contents for chicks. It may be a part of the physiological adaptation for breeding in this species. Many species of seabirds forage at sea and store food in their stomachs to feed chicks. Therefore, metabolic alkalosis in the breeding season may occur in a broad range of seabird species.

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