Effects of duration of capture and sample handling on critical care blood analytes in free-ranging bottlenose dolphins

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Objective—To determine effects of duration of capture and sample-handling procedures on blood analytes in free-ranging bottlenose dolphins.

Design—Cross-sectional study.

Animals—154 free-ranging bottlenose dolphins of various ages and both sexes.

Procedures—Blood samples were drawn from each dolphin within 10 minutes of capture and before release and analyzed by use of a portable analyzer with a single-use 8-analyte disposable cartridge. Analyte values were compared according to duration between sample acquisition and analysis (time to run [TTR]) and duration between net encirclement and sample acquisition (time to bleed [TTB]).

Results—Neither TTB nor TTR significantly affected sodium or chloride concentration. Potassium concentration was not significantly affected by TTR, whereas the effect of TTB was significant. Glucose, total CO₂, HCO₃, Hct, and base excess of extracellular fluid values were significantly affected by TTR. Increased TTB resulted in significantly increased total CO₂, HCO₃, and base excess when TTR was kept within 10 minutes.

Conclusions and Clinical Relevance—The effect of TTB on certain acid-base and electrolyte values was readily measured in free-ranging bottlenose dolphins, and such values may provide a reference range for those variables. (J Am Vet Med Assoc 2006;229:1955–1961)

Bottlenose dolphins (Tursiops truncatus) are the most commonly stranded marine mammals on the east coast of the United States. Such dolphins often have multiple diseases of various etiologies. However, respiratory distress is a frequent finding in live stranded dolphins and may be the result of primary causes such as morbillivirus, parasitic pulmonary migration, thoracic trauma, and bacterial or fungal pneumonia.

Secondary causes may include cardiogenic shock, heart failure, and airway obstruction. It is imperative for the responding veterinarian to quickly assess the ventilation and physical condition of the dolphin so that appropriate management can be pursued. This information may also be vital for assessing the potential for successful rehabilitation. In many ways, an increasing interest in bottlenose dolphins has driven a desire to understand the potential effects that handling may have on these dolphins. It is logically assumed that the sudden influence of gravity on aquatic mammals held out of the water may lead to gradual detrimental changes in their ventilation capacity. However, scant definitive data exist to establish a link between ventilation impairment and time out of water in cetacean species.

A portable clinical analyzer has been used in several species with success, including humans, dogs, horses, and northern elephant seals. This system functions adequately even in nonconventional settings such as helicopter transport, outdoor endurance competitions, rugged backcountry settings, and a marine mammal rehabilitation facility even when used by untrained staff. The analyzer is also helpful when monitoring various disease states as well as acid-base and electrolyte imbalances.

To evaluate use of the analyzer for critical care assessment of free-ranging bottlenose dolphins, the goals of the study reported here were to evaluate the effect of delayed sample analysis, which is often unavoidable under field conditions; to determine whether the duration between net encirclement and sample acquisition affected acid-base and electrolyte balance; and to determine a preliminary reference range for certain blood analytes.

Materials and Methods

Study sites—The study sites were located in the Indian River Lagoon, Fla, and in the estuarine waters of Charleston, SC, including the Stono River Estuary as well as Charleston Harbor and its main riverine tributaries (ie, the Ashley, Cooper, and Wando Rivers).

Dolphins—One hundred fifty-four free-ranging bottlenose dolphins were captured, examined, sampled, marked, and safely released in June (Indian River Lagoon) and August (Charleston) of 2003 and 2004. Dolphins were entrapped

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with a net, and blood samples were collected from dolphins in the water after the dolphins were restrained by experienced handlers. Only samples from apparently healthy nonjuvenile dolphins of both sexes were included in this study. Additionally, all dolphin-capture protocols were approved by the Harbor Branch Oceanographic Institution Institutional Animal Care and Use Committee.

Samples—Two blood samples per dolphin were drawn from the periarterial venous rete in the flukes. The goal was to obtain at least one (postcapture) sample within 10 minutes of initial capture. The dolphin was then moved for the remainder of the clinical evaluation and sampling to a boat of sufficient size to hold the dolphin, dolphin handlers, monitoring personnel, and veterinary staff on an open deck surface. This processing boat was equipped with sufficient closed-cell foam padding to support the dolphin and staff, water buckets with sponges to maintain the dolphin in a moistened state, and an occlusive canvas shade covering to provide protection from direct sunlight. After the on-boat evaluation, dolphins were returned to the water and a second (prerelease) sample was obtained just prior to the dolphin’s release.

The blood-sampling site was prepared with an aseptic surgical scrub (2% chlorhexidine gluconate) and an alcohol-soaked gauze pad. A 19-gauge, 1.9-cm butterfly catheter with an attachment for an evacuated tube was used to collect blood samples.  

A 0.5- to 1.0-mL sample was collected into a 1.0-mL self-venting blood gas collection syringe with lyophilized heparin directly from the butterfly catheter at the last step in the blood-sampling process. Hematologic variables were analyzed by use of a portable analyzer with a single-use 8-analyte disposable cartridge. Manufacturer’s guidelines for the collection system and operational processes were followed. Three to 4 drops of heparin anticoagulated blood was inserted into a single cartridge. Analytes measured were glucose, BUN, sodium, potassium, chloride, pH, and Hct. The TCO2, anion gap, hemoglobin, Pco2, HCO3, and BEcalc are variables calculated by use of formulas for humans. The analyzer has only been calibrated for human erythrocytes; further analyses of Hct and hemoglobin values were not performed. Instead, Hct values were used to evaluate errors associated with sample processing only.

Times of day recorded for each dolphin included time of initial encirclement, time blood samples were taken, and time the samples were analyzed with the analyzer. For pH and Pco2 analysis, the analyzer should be used for processing blood samples within 10 minutes; for all other variables, the analyzer should be used for processing blood samples within 30 minutes. Duration between the sample being drawn and analyzed was referred to as the TTB. Duration from the dolphin’s initial encirclement to blood sampling was referred to as TTB. The TTB was treated as a continuous timeline from the initial capture of the dolphin to either the postcapture blood sampling or the prerelease blood sampling. In this respect, the effect of time was measured from a common point of origin for both blood samples.

Statistical analysis—An LME model was used to investigate the effect of TTB and TTR on each variable. Each individual dolphin was treated as a random effect, and postcapture and prerelease measurements were treated as replicates; TTB and TTR were treated as continuous covariates. Because of the number of variables, the overall experimentwise error was controlled by use of a Bonferroni-adjusted critical value for assessing significance (α = 0.005). After fitting the LME for each variable, residuals were examined for outliers by use of normal probability plots. If the effect of a variable (TTB or TTR) was not significant, that variable was removed from the model, and the analysis was repeated with only the single remaining covariate.

Intervals for each variable in the initial postcapture blood samples were estimated according to recommendations of the International Federation of Clinical Chemistry. When the LME indicated that TTR was an important factor for a given variable, only samples processed in ≤10 minutes were included for that variable. A nonparametric bootstrap procedure was used to estimate the interpercentile reference limits (2.5th and 97.5th percentiles) and their associated 90% confidence interval, as described. Briefly, the original (n = 114) observations were resampled with replacement to obtain n values, and percentile estimates were computed. The resampling procedure was repeated for 1,000 iterations, and the mean percentile values and 90% confidence limits were computed from the 1,000 estimates. Prior to the estimation of the reference intervals, outliers were identified by use of the method described by Horn et al. Because of skewness, glucose values were log transformed prior to the determination of outliers.

Results

Effect of TTR and TTB—Only dolphins for which postcapture and prerelease samples were obtained were included in the analysis (n = 114). In addition, samples for which the TTB or TTR were not recorded were omitted from the LME analysis, which resulted in 100 samples being analyzed. For none of the parameters measured was substantial deviation from model assumptions observed. Median TTB was 33 minutes (range, 16 to 146 minutes) for initial postcapture blood sampling and 117 minutes (range, 41 to 239 minutes) for prerelease blood sampling or the prerelease blood sampling. In this respect, the effect of time was measured from a common point of origin for both blood samples.

Table 1—Results from an LME model of the effect of TTB and TTR on blood analytes in free-ranging bottlenose dolphins.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Direction of effect</th>
<th>TTB Coefficient</th>
<th>TTB P value*</th>
<th>Direction of effect</th>
<th>TTR Coefficient</th>
<th>TTR P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>↑</td>
<td>0.282</td>
<td>&lt;0.001</td>
<td>↓</td>
<td>-0.288</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BUN</td>
<td>NS</td>
<td>0.589</td>
<td>&lt;0.001</td>
<td>↑</td>
<td>0.135</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sodium</td>
<td>NS</td>
<td>0.031</td>
<td>NS</td>
<td>↑</td>
<td>0.016</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium</td>
<td>↑</td>
<td>-0.001</td>
<td>NS</td>
<td>↑</td>
<td>0.029</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chloride</td>
<td>NS</td>
<td>0.062</td>
<td>NS</td>
<td>↓</td>
<td>0.042</td>
<td>NS</td>
</tr>
<tr>
<td>TCO2</td>
<td>↑</td>
<td>0.027</td>
<td>&lt;0.001</td>
<td>↓</td>
<td>-0.097</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCO3</td>
<td>↑</td>
<td>0.026</td>
<td>&lt;0.001</td>
<td>↓</td>
<td>-0.090</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH</td>
<td>NS</td>
<td>0.089</td>
<td>NS</td>
<td>↓</td>
<td>0.091</td>
<td>NS</td>
</tr>
<tr>
<td>BEcalc</td>
<td>↓</td>
<td>-0.030</td>
<td>&lt;0.001</td>
<td>↑</td>
<td>-0.101</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values of P < 0.005 indicate a significant effect. NS = Not significant.
lease blood sampling. Median TTR was 10 minutes (range, < 1 to 148 minutes) for initial postcapture blood sampling and 7 minutes (range, < 1 to 75 minutes) for prerelease blood sampling.

The LME analysis indicated that neither TTB nor TTR significantly affected values for sodium or chloride (Table 1). Similarly, TTR did not significantly affect potassium \((P > 0.005)\). The effect of TTB was significant \((P = 0.001)\) for potassium, but the regression coefficient was extremely small \((-0.0012)\), indicating that the magnitude of the effect would not be biologically important. Increasing TTB and TTR significantly \((P = 0.001)\) affected glucose, \(T_{\text{CO}_2}\), \(HCO_3^-\), \(Hct\), and \(BE_{\text{ECF}}\) values. Glucose, \(T_{\text{CO}_2}\), \(HCO_3^-\), and \(BE_{\text{ECF}}\) were all significantly increased with increasing TTB and significantly decreased with increasing TTR. A significant decrease in \(Hct\) was detected with increasing TTB, whereas \(Hct\) significantly increased with increasing TTR. No effect of increasing T TB was observed for BUN, and a significant \((P = 0.001)\) increase in BUN was detected with increasing TTR.

Initial postcapture values were graphed against the values for the same dolphin’s subsequent prerelease values to determine changes attributable to increasing TTB within individuals (Figure 1). A 45° line was drawn on the graphs to better illustrate patterns of agreement. With the exception of a few outlying data points, BUN values were located along the 45° line, indicating little effect of handling time on BUN values. In contrast, data points for glucose were primarily above the line, indicating an increase in most individuals’ glucose values between samples obtained early versus later. An increase in \(T_{\text{CO}_2}\) was also detected between postcapture and subsequent prerelease samples, although the increase was detected more commonly in individuals with lower values in the initial sample. A similar pattern was detected for other blood gases. The differences in \(Hct\) values between postcapture and prerelease blood samples were more difficult to interpret because of the strong and simultaneous effect of TTR. When both blood samples were processed in 10 minutes or less (TTR \(\leq 10\)), the \(Hct\) values were in fairly good agreement. However, if TTR for either of the samples was extended, the \(Hct\) values were erroneously increased, and correlation between the 2 samples was substantially diminished.

Given the effect of TTR on blood gas measurements, analysis was performed to examine the potential impact of not meeting the recommended processing times of \(\leq 10\) minutes for pH and \(P_{\text{CO}_2}\) and \(\leq 30\) minutes for all other variables.24 To further investigate the effect of processing time, samples were allocated into 3 TTR groups: samples tested \(\leq 10\) minutes after collection, samples tested \(> 10\) to \(\leq 20\) minutes, and samples tested \(> 20\) minutes after collection (Figure 2). A 2-factor ANOVA for \(T_{\text{CO}_2}\) using blood-sampling group (postcapture vs prerelease) and TTR group (as described) as factors was performed. Results indicated that TTR group significantly affected
CO₂ values (F test, P < 0.001). Comparison between the first versus the second groups revealed that the effect was significant (Tukey honestly significant difference test, P = 0.017). Analysis was repeated for other blood gases and yielded similar results. Because of the observed effect of TTR on glucose, BUN, CO₂, HCO₃, pH, and BEₑcf, the data set used for estimation of ranges for these variables was limited to those samples that were tested ≤ 10 minutes after blood collection (n = 128; Table 2).

Discussion

Electrolytes were not appreciably affected by TTB or TTR. This pattern was not surprising for sodium and chloride because both electrolytes are in highest concentration in serum, but are maintained in a fairly tight range and would not be expected to be immediately influenced by short-term stress from capture and handling. In fact, only major physiologic phenomena such as vomiting, diarrhea, dehydration, and renal failure appear to cause clinically observable changes in electrolyte values. Although the effect of TTB on potassium values was significant, the model coefficient was small, suggesting that the effect was not biologically important. For example, even a 1-hour increase in TTB would be expected to cause only a 0.07 mEq/L decrease in potassium value. Previous studies reveal that capture and transport stress lead to increased serum glucose concentrations in brushtail possum, coral trout, green sea turtles with green turtle fibropapillomatosis, snapper, wild bighorn sheep, wild beluga whales, and wild grizzly bears. In addition, stress-induced catecholamine-associated increases of serum potassium concentrations in snapper, wild bighorn sheep, wild beluga whales, and grizzly bears are described. However, in each instance, the increase was reported to be transient. Increased serum sodium and chloride concentrations are also reported in association with capture stress in snapper and wild grizzly bears and grizzly bears also have increased BUN concentration. The analytes TCO₂, HCO₃, and BEₑcf increased significantly during handling, (ie, between the initial blood sample and the subsequent

Table 2—Proposed reference ranges (2.5th to 97.5th percentile values [90% confidence intervals]) for various analytes in heparinized blood of bottlenose dolphins (n = 50 to 111 dolphins) and published values derived from serum samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No.</th>
<th>Minimum (mg/dL)</th>
<th>Maximum (mg/dL)</th>
<th>Published range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>53</td>
<td>78.22 (76.00–81.33)</td>
<td>122.3 (116.7–132.0)</td>
<td>62–139</td>
</tr>
<tr>
<td>BUN</td>
<td>55</td>
<td>47.0 (45.0–52.4)</td>
<td>82.3 (79.3–86.0)</td>
<td>45–72</td>
</tr>
<tr>
<td>Sodium</td>
<td>111</td>
<td>143 (142–145)</td>
<td>158 (157–160)</td>
<td>151–158</td>
</tr>
<tr>
<td>Potassium</td>
<td>104</td>
<td>3.2 (3.2–3.3)</td>
<td>4.2 (4.1–4.3)</td>
<td>3.2–4.4</td>
</tr>
<tr>
<td>Chloride</td>
<td>112</td>
<td>110.4 (109.0–112.2)</td>
<td>124.8 (124.0–125.4)</td>
<td>108–118</td>
</tr>
<tr>
<td>TCO₂</td>
<td>54</td>
<td>21.5 (21.0–23.0)</td>
<td>37.9 (35.3–39.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Anion gap</td>
<td>54</td>
<td>–7 (–9 to –3)</td>
<td>24 (20–26)</td>
<td>NA</td>
</tr>
<tr>
<td>pH</td>
<td>108</td>
<td>7.17 (7.15–7.21)</td>
<td>7.43 (7.42–7.44)</td>
<td>NA</td>
</tr>
<tr>
<td>P₅₅</td>
<td>55</td>
<td>39.2 (38.2–41.8)</td>
<td>64.0 (61.5–68.9)</td>
<td>NA</td>
</tr>
<tr>
<td>HCO₃</td>
<td>54</td>
<td>20.0 (19.0–22.0)</td>
<td>36.0 (33.6–37.0)</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = Not available.
sample obtained just prior to release), which may be a direct physiologic response to decreased CO₂ displacement during respiration over time. Although dolphin-handling protocols required that adequate respirations were maintained throughout the evaluation process that was performed out of the water, this finding was not surprising because the effect of gravity on these aquatic mammals is likely to be greater with increased time the dolphin remains out of the water. In all likelihood, there was a gradually increasing ventilation-perfusion mismatch because respirations remained constant, less perfused portions of the lungs received most of the inhaled gas, and the more highly perfused dependent portions of the lungs were unable to adequately exchange gases. However, validation of human-based formulas for use in marine mammals was not undertaken in this study and could play a role in the discrepancies between the blood gas measurements and the measurement of pH. It is surprising that blood pH was not appreciably altered during TT and TB. This countereffect phenomenon may represent a higher than expected buffering capacity in dolphins that stabilizes blood pH during periods of extended breath holding. Of concern is the possibility that when the respiratory rate is not adequately maintained by dolphin care staff, the ventilation-perfusion mismatch will increase dangerously and overwhelm this buffering system. Helpful remedial or preventative measures include decreasing the time out of water, regularly repositioning the dolphin, or intervening medically by providing a respiratory stimulant such as doxapram hydrochloride. Other causes of ventilation-perfusion mismatch, however, must be ruled out prior to medical intervention. These causes include, but are not limited to, respiratory disease, cardiac disease, cardiopulmonary shock, respiratory tract obstruction, severe thoracic trauma, or CNS-mediated respiratory depression. These causes may be ruled out through the combined use of a physical examination and prudent use of the analyzer used in the study reported here.

Delay in processing samples (ie, TTR) was in many instances greater than the goal of ≤ 10 minutes. Unfortunately, the realities of a study in the field compromised the timeline of sample processing on several occasions. Results indicated that a failure to process samples in ≤ 10 minutes for pH and PaCO₂ analysis and 30 minutes for all other variables can effectively mask changes in blood gas values. The literature indicates that allowing blood to stand (without exposure to air) before testing allows PaCO₂ to increase and pH to decrease because of metabolic processes, which will cause HCO₃⁻ and TCO₂ to be overestimated. Also, glucose values decrease in blood samples over time. If heparinized blood is allowed to stand before testing, potassium values will first decrease slightly, then increase over time. The effects of increased TTR and increased TT in opposite directions; values increased with increased TT and decreased with increased TTR. Further investigations are required to determine which effects predominate and realistic time frames for blood collection.

For several variables measured with the analyzer reported here, there are published serum reference values. Among these, several interesting and important findings were observed. Sodium values obtained with the present analyzer were lower than those published for free-ranging bottlenose dolphins. This suggested that the 7 to 9 mmol/L discrepancy in sodium values between the 2 techniques represented incomparable results. However, results of a previous study in dogs and horses indicated that there was a poor correlation between the analyzer used in our study and automated chemistry analyzers, although only heparinized blood was used in both machines. Regardless, caution should definitely be taken when comparing sodium values obtained via different instruments. As with any analyst, it is advisable to interpret laboratory values in the context of instrument- and sample-specific reference ranges.

Potassium values obtained by use of the analyzer were comparable to those previously reported for free-ranging bottlenose dolphins. The values obtained in our study were 0.1 to 0.3 mmol/L lower than that in the previous study. This was consistent with findings in humans, in which rupture of platelets during coagulation can increase serum potassium concentrations by 0.1 to 0.7 mmol/L.

The chloride values obtained in the study reported here were consistently higher than those of published values for free-ranging bottlenose dolphins. The substantial overestimation in blood chloride concentration that resulted from use of the analyzer in this study was consistent with findings of previous studies in other species. Discrepancies in chloride values have been attributed to the narrow range of values for chloride, sensitivity of chloride ion-selective electrode systems to the effects of protein, and increased concentrations of BUN. In a study of humans in hemodialysis units, mild increases in BUN concentration resulted in large (> 6 mmol/L) increases in chloride values obtained with the analyzer used in our study. As a result, values obtained with published literature were small, apparently consistent, and unlikely to be of clinical importance.

The glucose values obtained in this study were well within the published serum ranges for free-ranging bottlenose dolphins (Table 2). The published serum range extends further on the lower end, likely as a result of technique differences. An important difference between techniques was that the heparinized blood samples were processed within 10 minutes, whereas the serum samples were obtained by first allowing coagulation to occur over a 20- to 40-minute period, prior to harvesting the serum. This longer period, during which serum is exposed to the glycolytic activity of blood cells, can result in falsely lowered serum glucose concentrations.
An important source of potential variation, the duration between sample collection and processing (TTR), was evaluated in this study. Time to run was a major confounder, with strong potential to affect blood variables for a given dolphin. Given this potential source of error, every effort should be made to adhere to the manufacturer's recommendation of sample processing within 10 minutes of obtaining the sample. When TTR was included as a covariate in the statistical model to control for delay in processing of some samples, a clear effect on blood analytes was observed in association with the duration of dolphin handling. Nevertheless, results provided preliminary range values for the examined variables in bottlenose dolphins, as determined by use of the instruments and methodology used here.

The point-of-care blood analyzer used in this study was easily portable and dependable and offered an additional diagnostic modality for use by marine mammal practitioners. Its use may substantially improve real-time monitoring of cetaceans in captive care facilities or field standing conditions.

References

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