Effects of sample handling on total carbon dioxide concentrations in canine and feline serum and blood

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Objective—To determine whether underfilling blood collection tubes leads to in vitro reduction in serum measured total CO_2 concentration ($[TCO_2]_m$) in canine and feline blood samples sufficient to create the impression of metabolic acidosis (pseudometabolic acidosis) or high anion gap.

Sample Population—Blood samples from healthy client-owned animals (16 dogs, 17 cats).

Procedure—Venous blood samples were collected in random order for determination of serum $[TCO_2]$ and blood gas tensions. Blood gas analysis was performed on iced, capped blood samples. In dogs, serum $[TCO_2]$ was measured in 1-, 3-, and 10-ml samples in 10-ml type-B tubes and in a 3-ml sample in 3-ml type-A tubes. In cats, serum $[TCO_2]$ was determined in 1-, 2-, and 3-ml samples in 3-ml type-A tubes and in a 3-ml sample in 10-ml type-B tubes.

Results—For dogs, serum $[TCO_2]$ in full-tube, 10-ml samples was a mean ± SD, 2.0 ± 1.1 mmol/L greater than that in 3-ml samples and 3.7 ± 1.3 mmol/L greater than the value in 1-ml samples; both differences were significant at $P < 0.0001$. The serum $[TCO_2]$ in full 3-ml samples was lower by 0.4 ± 0.6 mmol/L than the value in full-tube 10-ml samples (P=0.019). For cats, serum $[TCO_2]$ in full-tube, 3-ml samples was 0.5 ± 0.6 mmol/L greater than that in 2-ml samples (P = 0.004) and was 1.5 ± 0.8 mmol/L greater than the value in 1-ml samples ($P < 0.0001$). Serum $[TCO_2]$ in 3-ml samples of feline blood in 10-ml tubes was 0.8 ± 0.8 mmol/L lower than that in samples from full 3-ml tubes (P = 0.0007). In dogs and cats, $[TCO_2]$ in fully filled collection tubes was approximately 6 mmol/L higher when calculated from blood gas analysis data than when chemically determined in serum.

Conclusions and Clinical Relevance—Underfilling blood collection tubes results in a false decrease in serum $[TCO_2]$, which can contribute in part to descrepancies between blood gas and chemical analyses as estimates of plasma bicarbonate concentration. This, and other in vitro effects of sample handling and collection, may result in a false assessment of metabolic acidosis in dogs and cats. (*Am J Vet Res* 1997; 58:343–347)

Received for publication Jan 2, 1996.

Manuscript passed review Sept 16, 1996.

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Supported in part by the Morris Animal Foundation, Englewood, Colo.

This report represents a portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the PhD degree.

The authors thank Lori Koehler, Kathy Bird, Lisa Ulrich, Janice Parrow, Laurie Swanson, and Elaine Matthys for technical assistance, and Dr. Vickie King for statistical analytic assistance.

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Chemically determined serum total carbon dioxide concentration ($[TCO_2]$)[a] provides an estimate of blood bicarbonate concentration and often is used to assess the acid-base status of patients when blood gas analyses are unavailable. During an ongoing study of renal failure, we observed that $[TCO_2]$ in samples drawn within minutes of each other but determined by blood gas analysis versus chemical analysis differed nonsystematically. Other investigators reported that measured serum or plasma $[TCO_2]$ ($[TCO_2]_m$), determined by autoanalyzers, does not always correlate with calculated blood $[TCO_2]$ ($[TCO_2]_c$), as determined by gas analysis.[1,2] The cause of these observed discrepancies is multifactorial. Preanalytic differences in sample handling, such as exposure to air during processing, are important.[2,3] Other factors proposed as contributing to differences in $[TCO_2]_c$ include variability of the apparent dissociation constant (pK') and the solubility of CO_2, which contained in the Henderson-Hasselbalch equation used in the calculation.[2,4,b]

On the basis of a report by Herr and Swanson,[5] we hypothesized that the nonsystematic nature of the differences resulted from differing amounts of air present above the blood samples because of haphazard underfilling of blood collection tubes. In samples from human beings, underfilling of evacuated blood collection tubes promoted escape of CO_2 from serum into the air of vacuum above, which has a lower P_{CO_2} than blood, whether or not the tubes had been vented. This in vitro phenomenon resulted in $[TCO_2]_m$ values that underestimated true serum $[TCO_2]$.[5]

Because equipment for measurement of blood gas tensions is not routinely available to many veterinary clinicians, they must rely on serum $[TCO_2]_m$ rather than blood $[TCO_2]_c$ as an estimate of blood bicarbonate concentration ($[HCO_3^-]$). Thus, the degree to which serum $[TCO_2]_m$ is influenced by sample handling and its comparability with blood $[TCO_2]_c$ are important in routine assessment of acid-base status in animals. Underestimation of true $[TCO_2]$ can lead to erroneous diagnosis of metabolic acidosis, high anion gap (AG), or failure to diagnose metabolic alkalosis.

The purposes of the study reported here were to determine whether: underfilling of blood collection tubes leads to significant in vitro reduction in serum $[TCO_2]_m$ in canine and feline blood samples; the aforementioned sampling error lowers $[TCO_2]_m$ sufficiently to create the impression of metabolic acidosis (pseudometabolic acidosis) or high AG; the degree of needed correction can be estimated for a given amount of underfilling; duration between sample collection and analysis influences $[TCO_2]_m$ determinations of blood $[TCO_2]_c$ (blood gas analysis) and serum $[TCO_2]_m$ (chemical analysis) are in agreement; and samples collected and processed optimally, by use of