Serum or plasma samples not promptly separated from RBC may contain artifactually low glucose and high lactate concentrations due to the continued uptake and metabolism of glucose by RBC in vitro.\(^1\,\^2\) In blood samples from human beings, glucose concentration decreases in vitro at a rate of 0.36-0.56 mmol/L (6-10 mg/dL) per hour at 25°C.\(^1\,\^3\) Separation of serum from RBC within 15 to 30 minutes is considered necessary to prevent significant alterations in blood glucose concentration. Large and variable changes in lactate concentration can occur immediately after specimen collection, depending on temperature, pH, and glycolytic rate.\(^1\,\^4\) Sodium fluoride (NaF) inhibits several glycolytic enzymes by complexing with their cofactor, magnesium ion. Tubes containing NaF and potassium oxalate, the latter an anticoagulant, are recommended for the collection of human and animal blood samples to be analyzed for glucose and, particularly, lactate, to avoid artificial changes resulting from glycolysis.\(^1\,\^2\)

Serum or plasma samples not promptly separated from RBC may contain artifactually low glucose and high lactate concentrations due to the continued uptake and metabolism of glucose by RBC in vitro.\(^1\,\^2\) In blood samples from human beings, glucose concentration decreases in vitro at a rate of 0.36-0.56 mmol/L (6-10 mg/dL) per hour at 25°C.\(^1\,\^3\) Separation of serum from RBC within 15 to 30 minutes is considered necessary to prevent significant alterations in blood glucose concentration. Large and variable changes in lactate concentration can occur immediately after specimen collection, depending on temperature, pH, and glycolytic rate.\(^1\,\^4\) Sodium fluoride (NaF) inhibits several glycolytic enzymes by complexing with their cofactor, magnesium ion. Tubes containing NaF and potassium oxalate, the latter an anticoagulant, are recommended for the collection of human and animal blood samples to be analyzed for glucose and, particularly, lactate, to avoid artificial changes resulting from glycolysis.\(^1\,\^2\)

Serum or plasma samples not promptly separated from RBC may contain artifactually low glucose and high lactate concentrations due to the continued uptake and metabolism of glucose by RBC in vitro.\(^1\,\^2\) In blood samples from human beings, glucose concentration decreases in vitro at a rate of 0.36-0.56 mmol/L (6-10 mg/dL) per hour at 25°C.\(^1\,\^3\) Separation of serum from RBC within 15 to 30 minutes is considered necessary to prevent significant alterations in blood glucose concentration. Large and variable changes in lactate concentration can occur immediately after specimen collection, depending on temperature, pH, and glycolytic rate.\(^1\,\^4\) Sodium fluoride (NaF) inhibits several glycolytic enzymes by complexing with their cofactor, magnesium ion. Tubes containing NaF and potassium oxalate, the latter an anticoagulant, are recommended for the collection of human and animal blood samples to be analyzed for glucose and, particularly, lactate, to avoid artificial changes resulting from glycolysis.\(^1\,\^2\)

Sodium fluoride, potassium oxalate, or both, depending on their concentrations, may cause shrinkage and, to a lesser extent, lysis of RBC, which may reduce PCV by as much as 10%, and plasma constituents by 5%.\(^1\) Shrinkage and/or lysis of equine and phocid RBC in NaF/oxalate (NaF/Ox)-anticoagulated blood resulted in dilution of plasma by RBC cytosol and an artifactual decrease in plasma glucose concentration, compared to heparinized plasma.\(^5\,\^6\) The dilutional effect of NaF/Ox-induced RBC lysis would be expected to be greater in species whose RBC contained lower intracellular concentrations of glucose (or lactate) compared to plasma.

Feline RBC are poorly permeable to external glucose; their rate of glucose utilization is less than one-half that of dogs and human beings, but is higher than that of horses and cattle.\(^5\,\^9\) Because of low RBC glucose uptake or consumption rates, inhibition of glycolysis may be an unnecessary precaution when collecting feline blood samples for glucose and lactate determinations. Furthermore, low intracellular glucose concentrations in feline RBC may contribute to falsely decreased plasma glucose concentrations when RBC shrink or lyse. In critical care wards, in which concurrent PCV and lactate determinations frequently are indicated, substantial RBC shrinkage in NaF/Ox also may preclude accurate PCV determination on those samples. The impact of

Serum or plasma samples not promptly separated from RBC may contain artifactually low glucose and high lactate concentrations due to the continued uptake and metabolism of glucose by RBC in vitro.\(^1\,\^2\) In blood samples from human beings, glucose concentration decreases in vitro at a rate of 0.36-0.56 mmol/L (6-10 mg/dL) per hour at 25°C.\(^1\,\^3\) Separation of serum from RBC within 15 to 30 minutes is considered necessary to prevent significant alterations in blood glucose concentration. Large and variable changes in lactate concentration can occur immediately after specimen collection, depending on temperature, pH, and glycolytic rate.\(^1\,\^4\) Sodium fluoride (NaF) inhibits several glycolytic enzymes by complexing with their cofactor, magnesium ion. Tubes containing NaF and potassium oxalate, the latter an anticoagulant, are recommended for the collection of human and animal blood samples to be analyzed for glucose and, particularly, lactate, to avoid artificial changes resulting from glycolysis.\(^1\,\^2\)

Sodium fluoride, potassium oxalate, or both, depending on their concentrations, may cause shrinkage and, to a lesser extent, lysis of RBC, which may reduce PCV by as much as 10%, and plasma constituents by 5%.\(^1\) Shrinkage and/or lysis of equine and phocid RBC in NaF/oxalate (NaF/Ox)-anticoagulated blood resulted in dilution of plasma by RBC cytosol and an artifactual decrease in plasma glucose concentration, compared to heparinized plasma.\(^5\,\^6\) The dilutional effect of NaF/Ox-induced RBC lysis would be expected to be greater in species whose RBC contained lower intracellular concentrations of glucose (or lactate) compared to plasma.

Feline RBC are poorly permeable to external glucose; their rate of glucose utilization is less than one-half that of dogs and human beings, but is higher than that of horses and cattle.\(^5\,\^9\) Because of low RBC glucose uptake or consumption rates, inhibition of glycolysis may be an unnecessary precaution when collecting feline blood samples for glucose and lactate determinations. Furthermore, low intracellular glucose concentrations in feline RBC may contribute to falsely decreased plasma glucose concentrations when RBC shrink or lyse. In critical care wards, in which concurrent PCV and lactate determinations frequently are indicated, substantial RBC shrinkage in NaF/Ox also may preclude accurate PCV determination on those samples. The impact of
NaF/Ox preservative on feline RBC volume and glucose and lactate concentrations has not been determined.

Postcollection changes in blood glucose and lactate concentrations also could be affected by metabolic disorders that alter glucose uptake or glycolysis, such as diabetes mellitus and hyperthyroidism, 2 relatively common endocrinopathies in cats. Red blood cell glucose uptake and consumption are altered in humans and rats with diabetes, and thyroid hormone action causes increased lactate production.10-15 Feline RBC generate increased lactate under hyperglycemic conditions.16 Thus, metabolic alterations in cats with hyperthyroidism or diabetes mellitus could affect the degree of in vitro change in serum or plasma glucose and lactate concentrations.

It was the purpose of this study to compare the results of glucose and lactate analysis of routinely processed serum and NaF/Ox plasma samples from healthy control cats and cats with naturally occurring hyperthyroidism or diabetes mellitus. Results of these analyses were investigated further by evaluation of storage time and temperature on glucose and lactate concentrations in vitro, and the effect of NaF/Ox on RBC volume. The results of this study will help determine appropriate recommendations for collection methodology for accurate glucose and lactate measurement in cats, and may provide insights into feline RBC glycolytic alterations in metabolic diseases.

Materials and Methods

Clinical specimens

Blood samples were obtained from cats with diabetes mellitus (n = 30), hyperthyroidism (n = 27), and clinically healthy control cats (n = 19) as part of a previous study.17 Diabetic cats were identified on the basis of characteristic clinical signs and laboratory abnormalities, and included cats at the time of initial diagnosis, and those previously diagnosed as diabetic but presented for recheck examinations or for other clinical problems. Most clinical problems were related to the diabetes; 4 diabetic cats had unrelated problems including facial swelling, constipation, posterior paresis, and pleural effusion. Hyperthyroid cats were identified on the basis of characteristic clinical signs and laboratory abnormalities, including increased thyroxine concentration. Fasting blood samples were obtained from hyperthyroid cats prior to receiving radioiodine therapy. Control cats were identified on the basis of a physical examination, and results of a CBC and serum biochemical analysis.

Aliquots of blood obtained by jugular venipuncture were placed into serum clot tubes (red top; Becton Dickinson, Rutherford, NJ, USA) containing no additive, and 3.0-ml NaF/Ox tubes (grey top; Becton Dickinson), containing 7.5 mg NaF and 6.0 mg potassium oxalate. The NaF/Ox tubes were placed immediately on ice. Serum tubes were allowed to sit at room temperature (~25°C) for approximately 15 to 30 minutes until clot formation. Both NaF/Ox and serum tubes were then centrifuged at 2000×g for 10 minutes. Serum and plasma samples were separated and fast-frozen using acetone and dry ice. Samples were maintained at -20°C for up to 30 days prior to analysis. A few moderately lipemic samples were airfuged prior to analysis. Glucose was measured on an automated analyzer (Ciba-Corning, Oberlin, OH, USA) with a hexokinase method. Lactate (L-lactate) was measured spectrophotometrically using lactate dehydrogenase (Sigma Chemical Co., St. Louis, MO, USA). Results were expressed as mmol/L and mg/dL.

In vitro effect of storage time and temperature

Blood was obtained from 6 clinically healthy, specifically pathogen-free, adult cats of both sexes via jugular venipuncture, and placed into serum clot tubes, NaF/Ox tubes and tubes containing EDTA (Becton Dickinson). One serum sample and one NaF/Ox plasma sample were processed as described above. The EDTA samples, NaF/Ox samples, and remaining serum samples (on the clot) were placed at 25°C or 4°C. Serum and NaF/Ox samples were centrifuged after 1, 2, 4, or 8 hours, for spectrophotometric analysis (Spectronic Genesys 5, Fisher Scientific, Pittsburgh, PA, USA) of glucose and lactate concentrations (Sigma). Aliquots from EDTA and NaF/Ox tubes were used to determine PCV, by microhematocrit centrifugation (International Equipment Co., Boston, MA, USA).

Statistical analysis

Data were analyzed using paired Student’s t-tests for comparison of NaF/Ox and serum results, and one-way ANOVA for comparison of healthy, hyperthyroid and diabetic cats. Repeated measures ANOVA was used to evaluate changes in glucose and lactate concentrations over time for in vitro experiments. Correlation coefficients were obtained by least squares linear regression analysis. Results were considered significant when P < .05.

Results

Glucose concentrations were determined on paired samples from 19 control cats, 24 hyperthyroid cats and 25 diabetic cats. The mean glucose concentration in
NaF/Ox plasma was significantly lower \((P<.001)\) than mean serum glucose concentration (Table 1). There was significant linear correlation between paired plasma and serum values \((P<.001; r = 0.986; \text{Figure 1})\). Serum glucose concentration, NaF/Ox plasma glucose concentration, and the mean difference between these values were significantly \((P<.001)\) higher in diabetic cats than control cats. The difference in glucose concentrations between the two samples was directly related to serum glucose concentration \((P<.001; r = 0.611)\), such that hyperglycemic cats (serum glucose concentration \(\geq 11.1\) mmol/L \([200 \text{ mg/dL}]\)) had a significantly greater \((P<.0001)\) difference in glucose concentration than euglycemic cats. On a percentage basis, however, the mean difference between groups was not significant. Glucose concentrations in NaF/Ox plasma samples from hyperglycemic cats were \(13.9\% \pm 8.9\%\) lower than serum glucose concentrations; the difference in samples from euglycemic cats was \(10.3\% \pm 11.8\%\). Twenty hyperglycemic cats had diabetes mellitus; 2 were hyperthyroid. Several samples from diabetic cats showed mild to moderate lipemia.

Lactate concentration was determined on paired samples from 12 control cats, 22 hyperthyroid cats and 22 diabetic cats. Lactate concentration was significantly higher \((P<.05)\) in serum than in NaF/Ox plasma in all cats (Table 2). There was significant, linear correlation \((P<.0001; r = 0.757)\) between paired lactate values,
although more variation was noted, compared to glucose results (Figure 1). Serum lactate and NaF/Ox plasma lactate concentrations were significantly higher ($P < .008$) in hyperthyroid cats than control or diabetic cats, and the net change in concentration was slightly higher in hyperthyroid cats. Cats with hyperlactatemia ($> 2.5$ mmol/L [22.5 mg/dL] in NaF/Ox samples) had a significantly greater difference in lactate concentrations than normolactatemic cats ($P < .02$). On a percentage basis, however, the mean difference between groups was not significant. Lactate concentrations in serum samples from hyperglycemic cats were $32.2\% \pm 24.6\%$ higher than NaF/Ox plasma lactate concentrations; the difference in samples from euglycemic cats was $19.2\% \pm 35.1\%$. Fifteen cats with hyperlactatemia were hyperthyroid; 4 were diabetic, and one was a control cat that had struggled for several minutes and required extra restraint during sampling.

In vitro, a significant difference ($P < .001$) in glucose concentration of $0.7 \pm 0.2$ mmol/L (13 ± 4 mg/dL) was observed between serum (5.6 ± 0.3 mmol/L [101 ± 5 mg/dL]) and NaF/Ox plasma (4.9 ± 0.4 mmol/L [88 ± 7 mg/dL]), immediately (0 hour) after sample collection (Figure 2). This difference was of similar magnitude to that in routinely collected samples from clinically healthy, euglycemic cats. There was no further signifi-
cant change in glucose concentration in NaF/Ox plasma at 25°C or 4°C over the 8-hour period. Glucose decreased significantly (P < .006) in serum at 25°C, with a 15% (0.8 mmol/L [14 mg/dL]) decrease in the first hour, and a 10% decrease each subsequent hour. Glucose concentration did not change significantly in serum tubes stored at 4°C for up to 8 hours.

There was no significant difference in lactate concentration at 0 hour between NaF/Ox plasma and serum specimens (Figure 2). Lactate concentration increased significantly in serum at 25°C, with a 68% (0.98 mmol/L [8.8 mg/dL]) increase in the first hour, and an average 20% increase each subsequent hour. Lactate concentration in serum at 4°C increased slightly (but not significantly) in the first hour, from 1.45 ± 0.23 mmol/L (13.1 ± 2.1 mg/dL) to 1.98 ± 0.45 mmol/L (17.8 ± 4.1 mg/dL), and then remained stable. There was no change in lactate concentration in NaF/Ox plasma at either temperature for the duration of the incubation.

There was a significant difference in PCV of 7.0% ± 1.4% between NaF/Ox (31.4% ± 1.3%) and EDTA (38.4% ± 1.6%) plasma, immediately after sample collection (Figure 3). There was no change in PCV associated with storage temperature or time over the 8-hour incubation (data not shown). Mild to moderate hemolysis was visible in most NaF/Ox plasma samples, and in some serum samples.

**Discussion**

Use of NaF/Ox as an additive for feline blood samples effectively prevented significant elevations in plasma lactate concentration, but resulted in artifactual decreased plasma glucose concentration because of RBC shrinkage and probably lysis. Based on in vitro experiments, the difference between NaF/Ox plasma and serum glucose concentrations in blood routinely collected from healthy and ill cats could be explained almost entirely on the basis of NaF/Ox. Red blood cell shrinkage was confirmed by the lower PCV in NaF/Ox blood compared with EDTA-preserved blood. The decrease in PCV was observed immediately after placement of blood into NaF/Ox, similar to what was described for seals.5 The amount of RBC shrinkage in feline blood was comparable to or slightly greater than that described for humans, horses and phocids, and precludes use of NaF/Ox-preserved blood for accurate PCV determination in cats.15,16 Although we did not quantify free hemoglobin, qualitative observation of both serum and NaF/Ox plasma indicated hemolysis in many samples, which likely contributed to the decrease in PCV. It has been suggested that mild hemolysis caused by NaF/Ox may affect plasma glucose even in the absence of visible plasma discoloration.5 There was no apparent effect of RBC shrinkage or lysis on plasma lactate concentration.

The difference between NaF/Ox plasma and serum glucose concentrations was highest in diabetic cats, and proportionally related to the degree of hyperglycemia, although on a percentage basis the difference was only slightly higher than that observed in control or euglycemic cats. Diabetes mellitus may impact the rate of glycolysis and glucose uptake by RBC through effects on the glucose transporter (GLUT1), downregulation of insulin binding, changes in receptor affinity and decreased glucose-6-phosphate production.11-13,19,22 These metabolic alterations usually reduce glucose uptake and utilization, resulting in decreased intracellular glucose; however, some studies show increased uptake or glycolytic rate. While intracellular glucose concentration may be altered in RBC from diabetic cats, it was insufficient to have a notable effect on the percentage decrease in NaF/Ox plasma glucose concentration. It is also possible that RBC from diabetic cats are more susceptible to shrinkage or lysis, possibly as a result of hyperlipidemia or membrane abnormalities.

Because of the magnitude of decrease in glucose concentration in NaF/Ox plasma, serum was the specimen of choice for glucose determination. There was no significant change in glucose concentration in serum separated within 15-30 minutes or serum tubes stored at 4°C.

Compared with NaF/Ox plasma, serum lactate concentration in clinical specimens increased by 0.4 to 1.2 mmol/L (3.6 to 10.8 mg/dL) in the 15 to 30 minutes it took the blood to clot, attributable to ongoing RBC glycolysis. This difference was significant, and could be of clinical importance in the assessment of lactic acidosis, given the relatively narrow reference interval for lactate...
increased lactate concentration in hyperthyroid cats, although fasting would not be expected to affect RBC glycolytic rate. In addition, hyperthyroid cats may have had higher PCV than diabetic or control cats, with more total glycolytic activity. The control cat with hyperlactatemia had a difference between serum and plasma concentrations of only 0.5 mmol/L, comparable to the difference for normolactatemic cats. This suggested that hyperlactatemia in this cat did not reflect accelerated RBC glycolysis, but rather reflected release of lactate from skeletal muscle as a result of struggling.

Because of the magnitude and variability of increase in lactate concentration in routinely processed serum samples, NaF/Ox plasma was the specimen of choice for lactate determination in cats, since even serum separated within 15 to 30 minutes had changes in lactate concentration that could affect clinical diagnosis. Although the glucose consumption rate of feline RBC is lower than in all species studied except horses, cattle and pigs, the change in serum glucose concentration in 1 hour in vitro at 25°C (0.8 mmol/L) was slightly higher than that reported for human blood samples (0.4-0.6 mmol/L); whereas, the 10% decrease in each subsequent hour was similar to what occurs in human samples.1,3,5,6 Maintenance of serum samples on ice, or removal of serum immediately after clot formation would minimize the artifactual decrease in serum glucose concentration. The rate of glucose uptake and glycolysis at 4°C was slowed sufficiently to prevent changes in extracellular glucose concentration. Artifactual increases in lactate concentrations also were inhibited at 4°C after the first hour, presumably after intracellular glucose was metabolized.

In summary, NaF/Ox tubes were effective and necessary in preventing an artifactual increase in lactate concentration in feline blood; however, glucose concentration was underestimated in NaF/Ox plasma because of RBC shrinkage and dilution of plasma by RBC cytosol, which contains a lower concentration of glucose. Glucose determination should be performed on routinely processed serum separated from the clot within 15 to 30 minutes or stored at 4°C. Artifactual changes in both glucose and lactate may be affected by hyperglycemia and hyperlactatemia in cats with diabetes mellitus and hyperthyroidism.

Acknowledgements
The authors acknowledge the contributions of John Broussard, Mark Peterson and Robin Brigmon.

References


