

## THE EFFECT OF TIME AT WHICH PLASMA SEPARATION OCCURS ON BIOCHEMICAL VALUES IN SMALL ISLAND FLYING FOXES (*PTEROPUS HYPOMELANUS*)

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**Abstract:** Heparinized blood samples from 15 adult small island flying foxes (*Pteropus hypomelanus*) were stored at 22°C for 0-, 6-, and 24-hr intervals prior to centrifugation and separation of plasma from erythrocytes. Mean plasma biochemical values of 16 analytes were determined from all samples. Mean values of blood urea nitrogen, creatinine, total protein, albumin, globulin, alkaline phosphatase, alanine transaminase, aspartate transaminase, cholesterol, calcium, sodium, and bilirubin did not change significantly over 24 hr at 22°C. Glucose was decreased at 6 and 24 hr. Potassium and phosphorus increased and chloride decreased, respectively, between 6 and 24 hr.

**Key words:** Hematology, plasma chemistry, storage, stability, *Pteropus*, bats.

### INTRODUCTION

It is generally recommended that serum or plasma be removed from contact with erythrocytes within 20–30 min of blood collection to avoid artifactual alterations in biochemical values.<sup>3</sup> Blood samples collected from free-ranging wild animals are prone to artifactual alterations in biochemical values because centrifugation and separation of plasma is frequently delayed. Consequently, knowledge of the stability of analytes under field conditions is crucial to correct interpretation of biochemical analysis. It has been shown that many biochemical analytes of domestic animals are stable (in serum or plasma) at 4°C for at least 24 hr,<sup>4</sup> some for up to 3 days.<sup>2,7</sup> However, there appear to be no similar published studies evaluating samples from bats or using blood maintained at warmer temperatures.

Prolonged plasma contact with metabolically active erythrocytes often results in lowered glucose and elevated phosphorus levels.<sup>4</sup> Plasma electrolyte levels within a sample may also be affected by altered erythrocyte membrane transport.<sup>1</sup> Further, prolonged blood storage before plasma separation predisposes to hemolysis, which also alters plasma biochemical values.<sup>5,6</sup> For example, analytes more concentrated in erythrocytes will artificially elevate plasma levels. Hemolysis will also interfere with

spectrophotometry and reflectance as well as chemical reaction assays.

The objective of the following study was to determine whether a delay in the time when plasma is separated from the red cells would significantly affect plasma biochemical values from blood maintained at room temperature for 6–24 hr.

### MATERIALS AND METHODS

Fifteen small island flying foxes (*Pteropus hypomelanus*) (eight female, seven male) were used. All animals were either captive-born or imported from their countries of origin at least 2 yr prior to the study. Only animals assessed as healthy based on physical examination and hematologic and plasma biochemical values within reference intervals for the collection were included in the study. All bats were housed in indoor/outdoor flight enclosures; fed a mixture of fruits, vegetables, commercial primate chow, and a vitamin supplement; exposed to a natural photoperiod; and given water ad libitum.

For blood collection, each bat was anesthetized with isoflurane (Aerrane, Anaquest, Liberty Corner, New Jersey 07938, USA) (5%, decreased to 2.5%) in oxygen (2 L/min) supplied through a mask attached to a nonbreathing system. A 3-ml, heparinized (sodium heparin 1,000 IU/ml) syringe and 25-gage needle were used to collect blood from the brachial vein on the medial aspect of the humerus. After collection, the needle was removed from the syringe and the sample was separated into three 1-ml aliquots. The baseline (0 hr) sample from each subject was centrifuged at 3,000 rpm for 5 min as soon as possible following collection (mean time = 13 min after collection) and the plasma was transferred to a 1-ml cryotube. The remaining samples were stored at room temperature (22°C) for approx-

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**Table 1.** Plasma biochemical values in the small island flying fox (*Pteropus hypomelanus*) ( $n = 15$ ): The effects of storage of whole blood at 22°C for 0, 6, and 24 hr before separation of plasma.

	0 hr		6 hr		24 hr	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Glucose (mg/dL)	126 $\pm$ 26	93–167	82 $\pm$ 34 <sup>a</sup>	25–138	29 $\pm$ 22 <sup>a,b</sup>	4–77
Phosphorus (mg/dL)	4.5 $\pm$ 0.7	2.6–5.8	4.3 $\pm$ 0.8	2.6–6.0	11.6 $\pm$ 3.5 <sup>a,b</sup>	5.6–20.4
Potassium (mEq/L)	3.9 $\pm$ 0.3	2.5–4.5	3.7 $\pm$ 0.6	2.4–5.7	5.2 $\pm$ 0.7 <sup>a,b</sup>	3.4–6.5
Chloride (mEq/L)	113 $\pm$ 2	109–117	112 $\pm$ 5	107–128	105 $\pm$ 3 <sup>a,b</sup>	99–109
Urea nitrogen (mg/dL)	5 $\pm$ 1	3–8	5 $\pm$ 2	3–9	6 $\pm$ 2	4–10
Creatinine (mg/dL)	0.7 $\pm$ 0.1	0.6–0.8	0.7 $\pm$ 0.1	0.6–0.8	0.8 $\pm$ 0.1	0.7–0.9
Total protein (g/dL)	7.6 $\pm$ 0.3	7.1–8.6	7.8 $\pm$ 0.4	7.2–8.6	7.9 $\pm$ 0.4	7.4–9.1
Albumin (g/dL)	3.6 $\pm$ 0.1	3.2–3.9	3.6 $\pm$ 0.2	2.7–4.2	3.6 $\pm$ 0.2	3.4–4.2
Globulin (g/dL)	4.0 $\pm$ 0.2	3.7–6.8	4.0 $\pm$ 0.2	3.7–6.9	4.2 $\pm$ 0.3	3.6–6.9
Alkaline phosphatase (U/L)	534 $\pm$ 144	362–912	550 $\pm$ 144	358–906	555 $\pm$ 140	377–925
Alanine transaminase (U/L)	8 $\pm$ 3	6–15	8 $\pm$ 3	5–15	9 $\pm$ 3	7–17
Aspartate transaminase (U/L)	30 $\pm$ 9	18–65	31 $\pm$ 18	18–90	30 $\pm$ 6	18–44
Cholesterol (mg/dL)	15 $\pm$ 10	3–25	14 $\pm$ 9	3–28	16 $\pm$ 10	3–29
Calcium (mg/dL)	8.5 $\pm$ 0.3	7.8–9.4	8.5 $\pm$ 0.5	7.1–9.3	8.6 $\pm$ 0.4	8.3–9.6
Sodium (mEq/L)	144 $\pm$ 2	144–159	148 $\pm$ 6	143–168	147 $\pm$ 3	141–152
Bilirubin (mg/dL)	0.10 $\pm$ 0.02	0.1–0.2	0.11 $\pm$ 0.03	0.1–0.2	0.13 $\pm$ 0.04	0.1–0.2

<sup>a</sup> Significantly ( $P < 0.05$ ) different from mean baseline at 0 hr.

<sup>b</sup> Significantly ( $P < 0.05$ ) different from mean values at 6 hr.

imately 6 (mean 5 hr, 48 min) and 24 hr (mean 23 hr, 45 min) before centrifugation and transfer of plasma as previously described. Plasma samples were stored at  $-70^{\circ}\text{C}$  for 4 days until transport to a commercial laboratory for biochemical analysis using an automated analyzer (Olympus AU5200). All samples were analyzed at the same time using the same analyzer.

A one-way analysis of variance for repeated measures was performed using a commercial statistical package (Minitab Inc., State College, Pennsylvania 16801-3008, USA) to compare means at each time point for every analyte and calculated ratio. A  $P < 0.05$  was accepted as statistically significant. For analytes in which a significant effect was demonstrated, a Tukey's pairwise comparison was performed to determine which time intervals were associated with significant changes in measured values.

## RESULTS

Mean plasma biochemical values are presented in Table 1. No evidence of hemolysis was noted in any of the samples. Blood urea nitrogen, creatinine, total protein, albumin, globulin, alkaline phosphatase, alanine transaminase, aspartate transaminase, cholesterol, calcium, sodium, and bilirubin were not significantly affected by 24 hr of storage at 22°C prior to centrifugation and removal of plasma for biochemical analysis. Mean glucose decreased over

both measured time intervals. Phosphorus remained stable for the first 6 hr of storage before separation of plasma but increased significantly between 6 and 24 hr. Similarly, potassium and chloride were stable for the first 6 hr of storage but increased and decreased, respectively, between 6 and 24 hr.

## DISCUSSION

Delaying centrifugation for 24 hr after blood collection produced a marked pseudohypoglycemia and mild hyperphosphatemia, hyperkalemia, and hypochloremia in the plasma of the flying foxes. However, the means of other biochemical variables were not significantly changed at 24 hr. The pseudohypoglycemia and hyperphosphatemia were most likely due to the continued metabolic activity of erythrocytes in the sample.<sup>4</sup> Cellular respiration and metabolism utilize glucose and adenosine triphosphate (ATP). As glucose levels decline, respiration and ATP production eventually cease. Available ATP is metabolized to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) and eventually to adenosines with the resultant accumulation of free phosphorous.<sup>1</sup> As ATP becomes depleted, active membrane transport, such as the sodium-potassium ATPase, is no longer functional. Electrolytes move passively along a concentration gradient toward equilibrium, with potassium moving extracellularly, causing a false elevation in plasma potassium. The decrease in chloride occurs

through a similar mechanism operating in reverse. This theory predicts a decrease in sodium associated with sample storage, which did not occur here. This may have been due in part to lower membrane permeability to sodium compared with other electrolytes.<sup>1</sup>

In conclusion, blood samples from small island flying foxes can be stored at 22°C for 6–24 hr before centrifugation without significant alteration of the mean concentration of most plasma biochemical analytes. This assumes the plasma sample contains no hemolysis.

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