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Effect of Temperature, Duration of Storage and Sampling Procedure on Ammonia Concentration in Equine Blood Plasma

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Summary: The effect of storage duration at different storage temperatures on the plasma ammonia concentration of equine EDTA whole blood, EDTA plasma and heparin plasma samples was investigated. Further, the effect of jugular vein compression before and during blood sampling on the plasma ammonia values was evaluated. In EDTA whole blood kept at 4 °C there was no significant increase of ammonia content after 6 hours of storage, whereas the increase was already significant 3 hours after collection if the EDTA whole blood was kept at 20–22 °C. EDTA plasma samples stored at 20–22 °C, 4 °C and −20 °C showed mean ammonia formation rates of 26.8 µmol/l, 6.6 µmol/l and 0.03 µmol/l per day, respectively. There were no differences between ammonia values of EDTA and heparin plasma samples immediately after sampling and following 3 days of storage at 4 °C and 3 and 90 days of storage at −20 °C. Although significant, the absolute differences between the mean plasma ammonia contents of EDTA blood sampled with and without compression of the jugular vein were small (20.2 ± 4.1 µmol/l and 23.4 ± 4.3 µmol/l resp.).

Introduction

In human sports medicine there is great interest in the determination of plasma ammonia for evaluation of the adaptation to exercise and of the performance capacity of athlets (1–5). The same has been true for horses for several years (6–9). However, studies of the effect of equine blood specimen handling procedures on the ammonia concentration are scarce (10), although they are necessary for defining the conditions for the use of plasma ammonia determinations in blood samples collected from horses under field conditions. From work done on human blood samples it is known that handling procedures affect the ammonia values (11, 12). However, data gained for human blood samples may not be valid for horses due to species differences, as demonstrated by *Ogilvie* et al. (10) for cats and horses. Therefore, in this study the effect of storage time of equine EDTA whole blood, heparin and EDTA plasma at different temperatures on plasma ammonia concentrations was investigated. Further, the effect of vein compression before and while collecting the blood sample was also considered.

Materials and Methods

All of the trials used blood from clinically healthy, mature (5–10 years old) saddle-bred horses, which were not exercised intensively the day before blood sampling.

The blood was taken between 7:00–8:00 a. m. from the jugular vein using vacuum vials, without or with only slight vein compression; plasma was separated by centrifugation (15 minutes at 2000 min^{-1}) within one hour after sampling. Depending on the blood plasma specimen needed, the vials contained either sodium heparinate or EDTA (Becton Dickinson, No. 606457 and 606480).

The following influences on plasma ammonia concentration were investigated.

Storage of EDTA plasma

From six horses several vials of blood using EDTA as anticoagulant were taken. After centrifugation, aliquots of the plasma samples of each horse were stored at 20–22 °C, 4 °C and −20 °C. The ammonia concentration was determined immediately after centrifugation and after 1, 2, 3 and 7 days of storage at each temperature; additionally, it was determined on day 21 for samples kept at 4 °C and −20 °C.

Storage of heparin plasma and comparison with EDTA plasma

Five vials of blood containing heparin and five vials of blood containing EDTA as anticoagulant were obtained from one horse. Each vial was considered to be one sample and after