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EFFECTS OF HANDLING STRESS ON PLASMA ENZYMES IN HARP SEALS, *Phoca groenlandica*

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Abstract: Three harp seal pups, *Phoca groenlandica,* were captured on the ice of the Gulf of St. Lawrence, and subjected to 3 h of transportation and handling stress. The activities of creatine kinase (CK), aspartate aminotransferase (AspAT), aldolase, alanine aminotransferase, gamma glutamyl transpeptidase, and leucine aminopeptidase were determined in serial blood samples collected for 4 d following the stress episode. Marked elevation of plasma CK activity was observed 3 h after capture. Values returned to normal in 12 h in two seals, and by 24 h in the third. Slight elevations in AspAT were also noted; the remaining enzymes were unaffected. Plasma CK is recommended as a sensitive indicator of handling stress in seals.

INTRODUCTION

Stress is an important component of capture, restraint and confinement of wild animals. Its observable effects range from degenerative changes in muscle, i.e. capture myopathy, to acute and otherwise unexplainable death.[1] One aspect of stress often overlooked is its role as a mortality factor in wild populations. Recent studies on ringed seals, *Phoca hispida,* have shown that stress can affect survival of seals faced with potentially threatening situations such as exposure to oil, or to seasonal or annual oscillations in food production.[3,11] The ability to detect and quantify stress in these animals can therefore be critical.

There are many methods by which stress can be studied; most utilize blood as the sampling medium. Stressed seals show adrenal-mediated changes in hematologic parameters such as packed cell volume, and total and differential leukocyte counts.[12] Uric acid, hydrocortisone, potassium,[14] and especially plasma sodium,[9,11] have also been shown to be influenced by stress in seals.

Plasma enzyme levels are useful as an index of tissue destruction associated with physical and exertional stress in terrestrial,[1,15] and aquatic species.[10] This study was undertaken to determine which of 6 plasma enzymes are affected by handling-stress in harp seal pups, *Phoca groenlandica.* The enzymes creatine kinase (CK), aspartate aminotransferase (AspAT) and aldolase (ALD) were chosen because of their known location in muscle.[4,21] The other enzymes, alanine aminotransferase (AlAT), gamma glutamyl transferase (GGT) and leucine aminopeptidase (LAP), are located in liver and kidney of phocid seals,[4,21] and are generally indicative of liver damage.[5,7,20]

MATERIALS AND METHODS

Three apparently healthy 3-week-old harp seal pups were captured by hand on the ice in the Gulf of St. Lawrence, placed in burlap bags, and transported 25 min by helicopter to holding facilities in the Iles de la Madeleine, Quebec. The first blood samples were drawn immediately on arrival at the holding pens, approximately 30 min after capture. The seals were then subjected to 2.5 h of vigorous handling, i.e. pushing, prodding, rolling and crowding, in an attempt to simulate an extreme measure of handling stress. Blood samples were drawn

at the conclusion of the handling period. The seals were then placed in a secluded outdoor pen with ample room, and blood-sampled every 12 h for 2 days, and every 24 h for 2 additional days. Three days later, the seals were transported by commercial air carrier to the University of Guelph using standard procedures for animal transportation.[2] Blood samples were collected 24, 48 and 120 h after arrival.

Blood from the extradural intravertebral vein[12] or the interdigital vessels of the hind flippers[8] was drawn into heparinized Vacutainer tubes.[1] The plasma was removed after centrifugation within 1 h of collection. Plasma obtained in the field portion of the study was stored at ambient temperature (-4 to 0 C) for up to 24 h until frozen at -20 C. Samples from Guelph were frozen immediately. Plasma from all seals was stored at -20 C for 3 months prior to analysis.

Enzymes were assayed using a Coleman 55 spectrophotometer,[2] with a temperature controlled cuvette, using the following commercially available reagent kits: BMC 15721 (CK), 15793 (GGT), 15952 (LAP), 15955 (AspAT) and 15956 (AlAT).[3] Sigma test kit 750 [4] was used for aldolase determinations. Monitrol,[5] a lyophilized plasma preparation, was used as a control with each series of determination.

RESULTS AND DISCUSSION

Thirty minutes after capture and helicopter transportation to the field site, 2 of the 3 seals showed plasma CK activity approximately twice the upper limit of normal (Fig. 1), as judged from values in acclimatized harp seal pups.[3] After 2.5 h of additional handling stress, the most dramatic enzyme change was again seen in CK. Plasma activity rose to 4-7 times the upper limit of normal, placing it well within the pathological range. The values returned to normal within 12 h in two seals, and by 24 h in the third.

The 25 h transportation period to Guelph resulted in moderate plasma CK elevation in one seal at least. That sample was taken 24 h after arrival, which presumably was long enough for plasma enzyme no longer released from muscle[9] to be cleared. Therefore, the single elevated level suggests either prolonged release, or its residual presence from even higher levels during the 25 h transportation period.

These findings are consistent with changes in plasma CK following exercise in man[15] and simulated transportation stress in dolphins.[10] Peak levels observed in this study are comparable to those seen in disease states such as myocardial infarction[6] and primary myopathies[17] in man. However, the duration of the elevation in seals was much shorter, suggesting that for CK at least, serial samples must be obtained at frequent intervals, and the first sample within 12 h of the suspected insult. Furthermore, continued samplings are required to distinguish stress-induced from disease-induced elevations.[16]

Other enzymes found in high concentrations in seal muscle tissues include AspAT and ALD.[4,21] Only plasma AspAT was slightly elevated after capture and 3 h of handling; mean levels during the first 24 h after capture (31 ± 10 mU/ml, $n = 9$) were significantly higher ($p<.05$) than in subsequent samplings (24

[1] Becton, Dickinson and Co., Canada Ltd., Mississauga, Ontario, Canada.

[2] Perkin Elmer Corp., Oakbrook, Illinois, USA.

[3] Boehringer Mannheim GmbH., Mannheim, Germany.

[4] Sigma Chemical Co., St. Louis, Missouri, USA.

[5] Dade Division, American Hospital Supply Corp., Miami, Florida, USA.

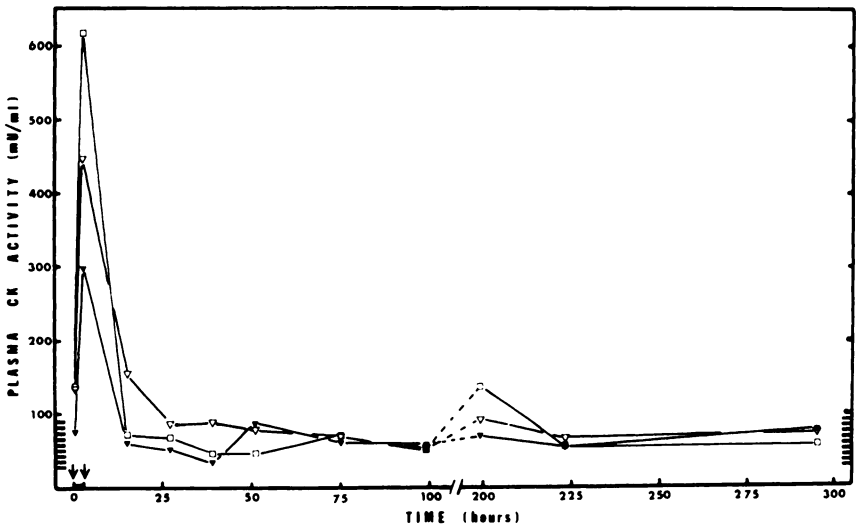

FIGURE 1. Plasma CK activity in three harp seals following capture (zero time) and 3 hours of handling stress (arrows). The seals were transported by commercial air carrier during hours 150-175. Normal range[3] indicated by shaded bars.

± 5 mU/ml, n = 24). No change was noted in plasma ALD activity. This further emphasizes the selective value of CK as an indicator of handling stress. Similar findings were obtained in oil ingestion and immersion studies on ringed seals, in which plasma CK and AspAT activities decreased from abnormally high post-capture levels to normal values at the end of the oil experiments.[14] Along these lines, the elevated AspAT values reported for fur seals, *Callorhinus ursinus*,[18] probably reflect muscle damage associated with herding just prior to death.

The remaining enzymes, AlAT, GGT and LAP, are used principally in the diagnosis of liver disorders.[19] In this study, they were not elevated above normal values,[1,21] suggesting that the stress which we induced either did not affect the liver, as we suspected it might due to trauma or abdominal compression, or that the damage was insufficient to cause the release of detectable enzyme levels.

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