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DISTRIBUTION OF TISSUE ENZYMES IN THREE SPECIES OF PINNIPEDS

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Abstract: In domestic animal medicine, changes in serum enzyme levels are routinely used as diagnostic tools to detect liver disease. Hepatic disease occurs in pinnipeds, but limited data are available on the tissue distribution of serum enzymes in marine mammals. The objectives of this study were to determine the tissue distribution of seven serum enzymes in three pinniped species. Enzymes evaluated were alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) in tissues from California sea lions (*Zalophus californianus*) ($n = 5$), harbor seals (*Phoca vitulina*) ($n = 5$), and northern elephant seals (*Mirounga angustirostris*) ($n = 5$) that stranded and then died at a rehabilitation center. Samples were evaluated in duplicate from liver, skeletal muscle, cardiac muscle, kidney, adrenal, spleen, pancreas, lung, lymph node, and intestine. Patterns of tissue enzyme distribution were similar in all species, with SDH activity highest in liver and kidney, CK activity highest in skeletal and cardiac muscle, ALP activity highest in adrenal, and GGT activity highest in the kidney. Aspartate aminotransferase and LDH activities were less specific, with high activity in multiple tissues. Tissue ALT activity was high in the liver of all species, but was also high in cardiac muscle (California sea lions), skeletal muscle (harbor seals), and kidney (elephant seals). These results suggest that concurrent analysis of SDH, ALT, and CK would provide high specificity and sensitivity for the detection of hepatic lesions, and allow differentiation of liver from skeletal muscle lesions in pinniped species.

Key words: Alanine aminotransferase, creatine kinase, liver enzymes, liver pathology, pinnipeds, sorbitol dehydrogenase.

INTRODUCTION

Hepatic disease has been identified in several species of pinnipeds stranded along the California coast.[5] Gulland et al. found urogenital carcinoma with metastases to the liver in 10% (37 of 370) of stranded subadult and adult California sea lions (*Zalophus californianus*).[7] Herpes virus infection with hepatic necrosis was found in 19% (31 of 162) of harbor seal pups (*Phoca vitulina*) that stranded and died along the California coast.[6] Hepatic disease also has been associated with liver fluke (*Zalophatrema hepaticum*) infections in captive sea lions.[12] Liver disease can be associated with nonspecific clinical signs such as anorexia, fever, abdominal pain, weakness, and vomiting. None of the signs, including the more specific signs of icterus and ascites, is pathognomonic for hepatic disease. Therefore, further diagnostic tests are needed to assist in the identification, management, and prognosis of liver damage or dysfunction.

In domestic animals, serum biochemical analysis is used to detect and monitor liver disease. Routine serum tests include serum bilirubin concentration, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) activities.[1]

The main factors affecting serum enzyme activity are organ specificity; intracellular location; rate of removal from the plasma; and the type, severity, and duration of the injury or stimulus.[9] In domestic animals, serum enzyme tests are grouped into those that indicate hepatocellular leakage due to hepatocyte damage (ALT, SDH, AST, and lactate dehydrogenase [LDH]), and those that reflect increased enzyme production caused by impaired bile flow or drug induction (ALP and GGT). Serum activity of the cytosolic enzymes, alanine aminotransferase, and SDH may increase even with mild alterations in hepatocyte permeability. Aspartate aminotransferase is found in the cytosol as well as mitochondrial membranes; therefore, alterations in AST activity may be caused by sublethal changes in mem-

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brane permeability, such as inflammation, as well as more lethal changes such as necrosis. Alkaline phosphatase and GGT are found in many tissues and are membrane bound. Increases in serum ALP and GGT activity are dependent on increased production by the tissue in which they are located, and may be seen with cholestasis, hepatic inflammation, bone growth in young animals (ALP), colostrum ingestion, and glucocorticoid administration.[3]

Tissue enzyme activity may be species specific. For example, cow and horse hepatocytes have low ALT activity, whereas cat and dog hepatocytes have high ALT activity.[2] Thus, serum ALT activity is more sensitive for detecting hepatic disease in cats and dogs compared with cows and horses. Sorbitol dehydrogenase is liver specific in all species evaluated and is more useful in diagnosing liver disease in cattle, horses, and sheep than ALT.[9]

In ringed seals (*Phoca hispida*), St. Aubin and Geraci reported that AST activity was highest in liver, but in harp (*Phoca groenlandica*) and grey seals (*Halichoerus grypus*) AST activity was highest in cardiac muscle.[11] Alanine aminotransferase and SDH activities were highest in the liver of all three pinniped species. Overall, very limited data are available regarding the tissue distribution of serum enzymes in pinnipeds with previous studies concentrating on arctic species. In addition, the effects of hepatic disease on serum enzyme activities in pinniped species are unknown. The main reason for this paucity of data is the impossibility of inducing lesions experimentally in pinnipeds and subsequently monitoring changes in serum enzymes, because of current legal, ethical, and logistical constraints. This study approached the problem using data from opportunistically sampled stranded animals, so natural variations in physiology and health status occurred in the study animals.

The purpose of this investigation was to determine the tissue distribution of seven serum enzymes in the California sea lion, harbor seal, and northern elephant seal (*Mirounga angustirostris*) in order to refine diagnostic tools for veterinarians caring for marine mammals. This study was conducted with the use of stranded animals admitted to a rehabilitation center; therefore, patterns of tissue enzyme distribution may be slightly different in healthy, free-ranging animals.

MATERIALS AND METHODS

Animals and tissue samples

Tissue samples were obtained from five California sea lions, five harbor seals, and five northern elephant seals that stranded and died at The Marine Mammal Center (TMMC), Sausalito, California, USA. All animals sampled were juveniles with limited clinical disease and minimal tissue damage on histopathologic examination. Most animals received antibiotics or corticosteroids prior to death. California sea lions received penicillin, amoxicillin, enrofloxacin, and prednisone (four received from one to five doses of penicillin or amoxicillin; and one received one dose each of enrofloxacin, penicillin, and prednisone). Harbor seals received penicillin, amoxicillin, enrofloxacin, and diazepam (one received no drugs; one received one dose of enrofloxacin; one received two doses of amoxicillin; one received one dose each of enrofloxacin, penicillin, and amoxicillin; and one received 12 doses of diazepam). Northern elephant seals received erythromycin, doxycycline, enrofloxacin, prednisone, aspirin, and ketoprofen (two received no drugs; one received two doses each of erythromycin, doxycycline, and prednisone; one received four doses of aspirin; and one received nine doses each of enrofloxacin and ketoprofen). Tissues were collected during post mortem examinations within 6 hr of death and were frozen at −80°C. Tissue samples were collected from liver (parenchyma), skeletal muscle, cardiac muscle, kidney, adrenal, spleen, pancreas, lung, lymph node, and intestine.

Homogenization of tissues

Tissues were homogenized according to the techniques derived from Herzfeld and Greengard[8] and St. Aubin and Geraci.[11] Samples were allowed to thaw before homogenization, and structural components were removed. Samples of tissue weighing 0.5–1.5 g were placed immediately into 20 ml of cold phosphate-buffered saline (PBSS, pH 7.45–7.55). The tissues were chopped coarsely, and then homogenized in bursts for 30–60 s at 5,000–30,000 rpm, with the use of a tissue homogenizer (Omni Tissue Homogenizer, Omni International, Marietta, Georgia 30066, USA). The homogenate volume was brought to 40–45 ml with cold PBSS. A solubilizing agent (Triton X-100, Mallinckrodt Baker, Inc., Phillipsburg, New Jersey 08865, USA) was added to the homogenates to a final concentration of approximately 0.5% (v/v), and the homogenates were incubated for 30 min at 0–4°C. The homogenization was completed with the use of a sonicator (Blackstone Ultrasonics, Inc., Sheffield, Pennsylvania 16347, USA) at maximum power for 30 sec to ensure complete lysis of all membrane-bound structures. Homogenates were then centrifuged at 2,600 rpm for 15 min to remove fibrous components. The supernatants were decanted and stored at 4°C for approximately 1–3 hr until analysis.

Table 1. Mean (±SD) activities of enzymes in tissues (IU/mg protein) collected from five juvenile California sea lions.[a]

Tissue type	ALT	AST	SDH	LDH	CK	ALP	GGT
Liver	2.40 ± 0.88	12.7 ± 3.99	2.21 ± 0.96	15.7 ± 5.94	3.45 ± 1.00	0.57 ± 0.16	0.55 ± 0.18
Skeletal muscle	1.31 ± 0.43	10.6 ± 2.54	0.02 ± 0.00	21.3 ± 4.80	470 ± 115	0.00 ± 0.00	0.00 ± 0.00
Cardiac muscle	4.33 ± 0.70	22.2 ± 3.95	0.54 ± 0.12	27.3 ± 3.46	296 ± 56.7	0.02 ± 0.01	0.03 ± 0.01
Kidney	1.10 ± 1.11	4.69 ± 2.62	0.69 ± 0.56	16.2 ± 3.65	4.51 ± 1.30	1.69 ± 0.97	5.51 ± 3.34
Adrenal	0.34 ± 0.15	8.64 ± 2.02	0.03 ± 0.01	5.12 ± 0.71	3.29 ± 1.08	13.2 ± 3.51	0.02 ± 0.01
Spleen	0.08 ± 0.03	1.33 ± 0.35	0.12 ± 0.05	9.99 ± 2.29	5.67 ± 2.56	0.65 ± 0.22	0.80 ± 0.16
Pancreas	1.05 ± 0.56	3.21 ± 0.25	0.07 ± 0.02	6.20 ± 0.88	1.09 ± 0.42	0.19 ± 0.07	0.65 ± 0.23
Lung	0.04 ± 0.02	0.61 ± 0.25	0.01 ± 0.01	4.07 ± 3.20	2.30 ± 1.22	0.17 ± 0.07	0.17 ± 0.13
Lymph node	0.17 ± 0.09	2.41 ± 0.79	0.09 ± 0.04	18.0 ± 6.10	1.71 ± 0.80	1.14 ± 0.56	0.30 ± 0.10
Colon	0.21 ± 0.09	2.38 ± 0.52	0.07 ± 0.04	20.4 ± 6.51	74.1 ± 42.6	1.55 ± 1.85	0.40 ± 0.32
Jejunum	0.40 ± 0.32	3.71 ± 0.74	0.11 ± 0.04	23.7 ± 3.80	95.7 ± 42.5	3.45 ± 3.05	0.36 ± 0.23

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; SDH, sorbitol dehydrogenase; LDH, lactate dehydrogenase; CK, creatine kinase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.

Enzyme assays

Tissue homogenates were analyzed for ALT, AST, SDH, LDH, CK, ALP, and GGT activities. All enzyme analyses were performed in duplicate on a Hitachi 912 chemistry analyzer (Roche Diagnostics, Indianapolis, Indiana 46250, USA), with standard methods and reagents in, the Veterinary Medical Teaching Hospital Clinical Pathology Laboratory at the School of Veterinary Medicine, University of California, Davis, California, USA. Tissue enzyme activity was reported as IU/mg protein. Protein was determined by measuring microprotein in the supernatants. Samples for which results exceeded the upper limit of linearity of the assay were diluted and reanalyzed.

Statistical analysis

Absolute means and standard deviations were calculated for each tissue enzyme activity. Means were compared among tissue types in each species with the use of the Mann-Whitney test.[13] Results were considered significant at P values < 0.05. Statistical calculations were performed with SPSS 11.0 software (SPSS Inc., Chicago, Illinois 60606, USA).

RESULTS

In California sea lions, cardiac muscle had significantly higher levels of ALT activity then other tissues ($P \leq 0.001$; Table 1). Cardiac muscle had the highest AST activity ($P \leq 0.001$), and liver had the highest SDH activity ($P \leq 0.001$). Cardiac muscle had the highest LDH activity, but skeletal muscle, colon, and jejunum also had appreciable LDH. Skeletal muscle and cardiac muscle had significantly higher levels of CK than all other tissues tested ($P \leq 0.001$). Alkaline phosphatase activity was highest in the adrenal gland ($P \leq 0.001$), whereas GGT activity was highest in the kidney ($P \leq 0.001$).

Harbor seals had similar patterns of enzyme distribution among tissues as California sea lions for AST, CK, ALP, and GGT activities (Table 2). Liver, skeletal muscle, and kidney had the highest activity of ALT in harbor seals. Sorbitol dehydrogenase activity was significantly higher in liver than in other tissues except for kidney ($P \leq 0.001$). Cardiac muscle had the highest LDH activity, but skeletal muscle, liver, colon, and jejunum also had significantly higher LDH activity.

Northern elephant seals had similar patterns of enzyme distribution as California sea lions and harbor seals for SDH, CK, ALP, and GGT activities (Table 3). Cardiac muscle, adrenal, and liver had higher AST activity than other tissues. Kidney had higher ALT activity than all other tissues in this species except for liver ($P \leq 0.001$). Lactate dehydrogenase activity was significantly higher in cardiac muscle than in other tissues ($P \leq 0.001$).

DISCUSSION

California sea lions, harbor seals, and northern elephant seals had similar patterns of tissue enzyme distribution. Although animals with limited tissue damage were selected for determination of enzyme tissue distribution, all were rehabilitated animals that had died during treatment. Therefore, although these patterns of tissue enzyme distribution were similar among the animals and species tested, slightly different enzyme activities or distributions may exist in tissues of healthy, free-ranging animals. Additionally, several animals were given an-

Table 2. Mean (±SD) activities of enzymes in tissues (IU/mg protein) collected from five juvenile harbor seals.[a]

Tissue type	ALT	AST	SDH	LDH	CK	ALP	GGT
Liver	2.16 ± 1.59	11.0 ± 4.86	1.63 ± 1.99	24.8 ± 12.40	2.33 ± 1.30	0.88 ± 0.41	0.72 ± 0.36
Skeletal muscle	1.43 ± 0.63	11.6 ± 6.17	0.02 ± 0.01	33.9 ± 23.4	479 ± 215	0.01 ± 0.00	0.00 ± 0.00
Cardiac muscle	0.79 ± 0.32	21.4 ± 7.15	0.12 ± 0.05	35.9 ± 11.7	323 ± 109	0.02 ± 0.01	0.01 ± 0.00
Kidney	0.89 ± 0.31	5.47 ± 1.76	0.96 ± 0.18	16.9 ± 1.76	5.85 ± 2.37	1.57 ± 0.92	6.42 ± 1.21
Adrenal	0.49 ± 0.17	7.16 ± 2.02	0.06 ± 0.02	6.72 ± 1.80	2.35 ± 0.98	31.1 ± 11.6	0.03 ± 0.02
Spleen	0.10 ± 0.02	1.61 ± 0.23	0.02 ± 0.01	9.05 ± 2.54	4.03 ± 0.91	0.84 ± 0.67	0.03 ± 0.01
Pancreas	0.17 ± 0.08	2.65 ± 1.25	0.23 ± 0.09	6.53 ± 1.63	1.46 ± 0.31	0.24 ± 0.11	0.72 ± 0.19
Lung	0.05 ± 0.15	0.72 ± 0.20	0.03 ± 0.02	4.25 ± 1.00	1.36 ± 0.49	0.49 ± 0.23	0.02 ± 0.01
Lymph node	0.20 ± 0.08	2.73 ± 0.92	0.08 ± 0.04	15.5 ± 1.46	3.92 ± 0.98	0.95 ± 0.74	0.06 ± 0.03
Colon	0.27 ± 0.14	3.41 ± 1.40	0.21 ± 0.12	25.0 ± 10.0	89.9 ± 52.2	1.6 ± 1.19	0.10 ± 0.05
Jejunum	0.53 ± 0.14	4.21 ± 1.25	0.21 ± 0.10	21.9 ± 2.28	81.4 ± 17.4	5.20 ± 2.34	0.32 ± 0.06

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; SDH, sorbitol dehydrogenase; LDH, lactate dehydrogenase; CK, creatine kinase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.

tibiotics and/or steroids for short periods prior to death. Of the medications administered, only erythromycin, enrofloxacin, ketoprofen, and prednisone have been found to cause increases in liver enzymes in other species, mainly dogs.[10] Enzyme activities in liver tissue from the elephant seal that received nine doses each of enrofloxacin and ketoprofen were ALT = 2.09 IU/mg protein, SDH = 1.29 IU/mg protein, ALP = 0.48 IU/mg protein, and GGT = 0.84 IU/mg protein. These values were within one standard deviation of the mean of all animals tested (ALT = 2.01 ± 1.24 IU/mg protein, SDH = 1.66 ± 1.39 IU/mg protein, ALP = 0.81 ± 0.40 IU/mg, and GGT = 0.61 ± 0.30), as well as the mean of the other four elephant seals (ALT = 1.34 ± 1.14 IU/mg protein, SDH = 1.01 ± 0.92 IU/mg protein, ALP = 1.02 ± 0.54 IU/mg, and GGT = 0.55 ± 0.39), suggesting that the short exposure to these medications did not affect enzyme activity.

High enzyme activity in certain tissues makes it more likely that damage or stimulation of that tissue will result in increased serum enzyme activity (i.e., high sensitivity), and a narrow range of tissue distribution makes it more likely that the serum activity of an enzyme is specific for the target organ. However, other variables, such as tissue mass, type of damage, and rate of enzyme excretion or catabolism may affect the amount of enzyme in the serum, such that conclusions regarding diagnostic specificity and sensitivity in different disease conditions require additional research. Nonetheless, the results of this study provide valuable guidelines that can be used to strategize laboratory testing, based on the potential likelihood that serum enzyme activity will increase in response to disease in certain tissues.

Alanine aminotransferase activity was high in the liver of all species examined, although it also was high in skeletal muscle, cardiac muscle, and kidney.

Table 3. Mean (±SD) activities of enzymes in tissues (IU/mg protein) collected from five juvenile northern elephant seals.[a]

Tissue type	ALT	AST	SDH	LDH	CK	ALP	GGT
Liver	1.49 ± 1.04	5.89 ± 3.89	1.10 ± 0.82	9.71 ± 2.83	1.71 ± 0.48	0.91 ± 0.53	0.61 ± 0.36
Skeletal muscle	0.46 ± 0.17	5.78 ± 1.99	0.02 ± 0.01	20.1 ± 5.17	317 ± 71.2	0.02 ± 0.01	0.00 ± 0.01
Cardiac muscle	0.56 ± 0.15	10.5 ± 2.68	0.11 ± 0.03	35.7 ± 5.90	320 ± 80.9	0.17 ± 0.16	0.00 ± 0.00
Kidney	1.54 ± 0.47	3.99 ± 1.54	0.84 ± 0.24	14.5 ± 1.48	9.89 ± 4.13	2.31 ± 1.06	6.44 ± 2.96
Adrenal	0.29 ± 0.07	7.58 ± 1.71	0.04 ± 0.01	5.00 ± 0.67	1.81 ± 0.54	6.92 ± 2.48	0.01 ± 0.00
Spleen	0.02 ± 0.01	0.49 ± 0.11	0.01 ± 0.01	2.37 ± 1.57	0.73 ± 0.23	0.13 ± 0.21	0.02 ± 0.01
Pancreas	0.31 ± 0.09	3.02 ± 1.05	0.07 ± 0.02	5.84 ± 1.89	10.8 ± 3.24	0.22 ± 0.09	0.02 ± 0.01
Lung	0.05 ± 0.03	1.01 ± 0.53	0.02 ± 0.01	3.01 ± 1.39	1.78 ± 0.50	0.56 ± 0.20	0.01 ± 0.00
Lymph node	0.14 ± 0.03	2.77 ± 0.42	0.09 ± 0.04	16.3 ± 2.60	2.59 ± 0.59	2.20 ± 1.48	0.03 ± 0.01
Colon	0.28 ± 0.08	2.64 ± 0.60	0.08 ± 0.02	17.1 ± 2.78	99.7 ± 14.6	0.98 ± 0.20	0.04 ± 0.01
Jejunum	0.38 ± 0.17	3.67 ± 1.34	0.16 ± 0.07	18.7 ± 2.95	81.4 ± 34.9	2.77 ± 1.16	0.05 ± 0.05

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; SDH, sorbitol dehydrogenase; LDH, lactate dehydrogenase; CK, creatine kinase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.

Because ALT activity was high in skeletal muscle, serum ALT should be measured and interpreted in combination with CK to differentiate hepatocellular leakage from muscle damage or necrosis. Based on its predominant location in skeletal and cardiac muscle, serum CK should be a specific and sensitive indicator of myocellular damage.

The most liver-specific enzyme was SDH, especially in California sea lions. Sorbitol dehydrogenase activity also was high in kidney tissue, but increases in serum enzyme activity have not been documented secondary to renal damage; rather, enzymes are lost into the urine, where they may be measured to assess renal injury.[4] In addition to high specificity, SDH also had substantively higher levels in the liver, suggesting it would be the most sensitive indicator of hepatocellular damage in the pinniped species examined. The low organ specificity of AST and LDH suggests that the serum activities of these enzymes would have minimal clinical utility in localizing hepatocellular (or other tissue) damage in these pinniped species.

Several tissues, including bone and gall bladder, were not examined, because of budgetary constraints. In other species, these tissues are high in ALP and GGT activity, respectively. As the gall bladder may be affected in conditions involving the liver, it would be useful in future to be able to differentiate hepatic and biliary sources of GGT. In our study the adrenal gland had high ALP activity, suggesting serum ALP levels may have value as an indicator of adrenal hyperplasia. Gamma-glutamyl transferase activity was highest in the kidney such that urine GGT levels may be useful for evaluating renal tubular disease.

In summary, based on the results of this study, serum SDH would be expected to be the most specific and sensitive indicator of hepatocellular damage in California sea lions, harbor seals, and northern elephant seals. Additional studies are needed to correlate serum enzyme activities with disease in specific tissues. Alanine aminotransferase activity also may be useful in detecting hepatocellular damage; however, because of its low specificity, it must be evaluated in conjunction with SDH and CK to differentiate hepatocellular from myocellular damage. Activities of AST and LDH lack both the sensitivity and specificity needed for accurate hepatic disease diagnosis.

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