# HAEMATOLOGY AND PLASMA CHEMISTRY OF CAPTIVE PINNIPEDS AND CETACEANS

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## *Introduction*

It is well recognized that the maintenance of marine mammals in captivity, like that of other exotic species, is a difficult problem at best. Any stresses imposed by the captivity itself may be compounded by the composition and quality of food and water (ENGELHARDT & GERACI, 1978; GERACI, 1972a, b), disease, and training or experimental regimes. In view of the value of the individual pinniped or cetacean, as well as their decreased availability, it becomes ever more important to confirm and maintain the health of these animals in captivity. A preferred method for health monitoring is the use of a regular scheduled health maintenance programme, which includes routine hematological and plasma chemistry tests. Any deviations in the clinical parameters from baseline values may then be assessed accordingly, possibly calling for more intensive testing of a suspect problem. Unfortunately, such baseline values are still ill-defined in marine mammals, resulting simply from a scarcity in the literature of information on healthy captive animals. The intended function of the values presented in this paper is to augment this information base by presenting clinically useful haematological and plasma chemistry data from a number of pinniped and cetacean species, examined by the author at various aquaria throughout eastern North America.

## *Materials and Methods*

Three species of phocid seals were examined: harp seals *(Phoca groenlandica)*, harbor seals *(P. vitulina)*, and ringed seals *(P. hispida)*. The seals were held in fresh or salt water, all on a diet of herring *(Clupea harengus)*. This diet was supplemented with at least vitamins E and B-complex, and usually additional vitamins such as A, D and C, iron, and salt. Salt supplementation with NaCl was always carried out if the seals were maintained in fresh water facilities. All the data presented here comes from animals neither in, nor near, molt, avoiding the complications associated with that seasonal phenomenon (ENGELHARDT, 1977a). Information was also obtained from three cetacean species: bottlenosed dolphins *(Tursiops truncatus)*, Pacific white-sided dolphins *(Lagenorhynchus obliquidens)*, and beluga *(Delphinapterus leucas)*. These were all held in either natural or artificial salt water. The bottlenosed dolphins were maintained on a diet of herring and smelt *(Osmerus mordax)*, the Pacific white-sided dolphins on mackerel *(Scomber scombrus)*, and beluga on a mixed diet of herring and mackerel. All were supplemented with vitamins as above.

Blood sampling was carried out on physically restrained non-anesthetized animals, using nets and pads to make the restraint as comfortable as possible for each animal. The animals were fasted for 14 - 18 hours prior to sampling. In the case of seals, blood was taken by using a Vacutainer assembly (Becton, Dickinson and Co., Canada, Ltd., Clarkson, Ontario) or needle and syringe from vessels in the plantar aspect of the hind flippers (GERACI, 1971). Bottlenosed dolphins were sampled from caudal vessels, at a lateral aspect of the tail stalk just below the transverse processes of the caudal vertebrae, or from an anterior dorsal fluke vessel. Both the Pacific white-sided dolphins and the belugas were sampled in the latter way. The blood was placed into K-EDTA for routine haematology, and into Li-heparin for plasma chemistry and blood osmotic fragility analyses. Plasma was stored at -20° C until analyzed, usually within 5 days to 2 weeks of storage. Haematologic analyses were carried out within one day of sampling (GERACI & ENGELHARDT, 1974; GERACI & MEDWAY, 1974).

Packed cell volumes (PCV) were determined by the microhemotocrit method. Red blood cell (RBC) counts and haemoglobin (Hb) determinations were made using a Coulter counter and haemoglobinometer, respectively (Coulter Electronics Inc., Hialeah, Florida). Erythrocytic indices were calculated: mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC). The osmotic fragility of RBC's was tested using a salinity gradient from 0.20% to 90% NaCl, in a ratio of 1 :500 blood to NaCl solution. White blood cell (WBC) counts were also carried out with a Coulter counter and WBC differential counts were also carried out with a Coulter counter and WBC differential counts were obtained from a blood smear stained with Wright's stain.

Plasma enzyme concentrations were determined colorimetrically or by fluorometry, as appropriate. Colorimetric techniques were used for measures of glutamic pyruvic transaminase (GPT), isocitric dehydrogenase (ICD), and aldolase, as outlined in Sigma Technical Bulletins Nos. 505, 175, and 750, respectively (Sigma Chemical Company, Saint Louis, Missouri). Creatine phosphokinase (CPK) was analyzed fluorometrically according to Sigma Technical Bulletin No. 80-F.

Flame photometry was used to determine the concentrations of electrolytes in plasma. Sodium and potassium ions were analyzed. The protein profile in plasma was assessed by a determination of total protein levels by the biuret method, and the distribution of albumin and globulins by densitometry following electrophoresis on cellulose acetate (Gelman Instrument Company, Ann Arbor, Michigan).

In order to verify that the plasma enzymes assessed were diagnostic of selected tissue damage, an analysis of major seal tissue types for enzyme specificity was carried out. For this purpose, samples of liver, heart muscle (left ventricle), skeletal muscle (psoas), kidney (cortex and medulla combined), brain (cerebrum), and blood cells were taken in the field from three wild harp seals. These tissues were frozen immediately and kept at -30° C until analysis. Analysis of the enzymes GPT, ICD, aldolase, and CPK was by methods as outlined for plasma, except that in the case of tissue the assays were on $Na_2HPO_4$-HCl buffered tissue homogenates, appropriately diluted with the buffer. Enzyme concentrations were calculated on the basis of wet tissue weight.

*Results*

*Haematology* Examination of the erythrocytic parameters (Table 1) showed a consistency among the three pinniped species in PCV values, at about 60% of blood volume. There was a trend for harp seals to have a lower RBC count at 5 million cells/$\mu$l, about 10% less than the other species of seals. On the other hand, harbor seals showed a lower Hb concentration, at an average of 22 g/dl, as compared to 24 to 26 g/dl in the other two phocids. These small species differences were reflected in the calculated erythrocytic indices, where harp seals showed the highest MCV, MCH, and MCHC values, except for ringed seal MCH and MCHC values which resembled those of harp seals. There appeared to be no significant differences related to age in harp and harbor seals.

The three dolphin species examined showed less consistency among them. The bottlenosed dolphin tended to have the lowest erythrocytic values (RBC, PCV and Hb). The Pacific white-sided dolphin showed the highest RBC count of these three species, which was reflected in its particularly low MCV and MCH values. The beluga had the highest MCV and MCH values of all species in this report, resulting particularly from the low RBC count.

Table 1

Erythrocytic parameters of captive pinnipeds and cetaceans, showing average values and ranges

| Species* | No. | Age | PVC (vol. %) | RBC (no. x $10^6/\mu l$) | Hb (g/dl) | MCV (fl) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|---|---|
| P.g. | 5 | 3 mo. | 61(52-68) | 5.0(4.3-5.5) | 26(23-28) | 123(118-129) | 51(50-54) | 42(39-44) |
| | 10 | 6 mo. | 59(49-65) | 4.8(4.1-5.4) | 24(18-27) | 123(112-133) | 50(41-54) | 40(36-42) |
| | 5 | 3-5 yr. | 62(55-65) | 5.1(4.6-6.3) | 26(25-28) | 121(107-130) | 51(44-56) | 42(40-43) |
| P.v. | 12 | 4-6 mo. | 59(52-63) | 5.5(4.7-6.1) | 21(17-24) | 106( 98-113) | 39(31-43) | 37(29-40) |
| | 3 | 9 mo. | 62(56-67) | 5.9(5.2-6.7) | 23(18-27) | 106( 93-112) | 38(34-43) | 37(29-41) |
| | 10 | 1-2 yr. | 55(51-62) | 5.1(4.6-5.7) | 20(19-23) | 111(103-115) | 41(36-43) | 37(35-40) |
| P.h. | 3 | 2 yr. | 63(61-66) | 5.5(5.0-5.9) | 26(24-27) | 115(110-122) | 47(46-48) | 41(39-43) |
| T.t. | 7 | 4+ yr. | 43(40-47) | 3.4(3.1-3.8) | 16(14-17) | 127(117-142) | 46(42-51) | 36(34-38) |
| L.o. | 2 | adult | 49(49-49) | 5.5(5.3-5.8) | 18(18-18) | 89( 85- 93) | 31(31-31) | 37(37-37) |
| D.l. | 4 | adult | 54(48-60) | 3.2(2.9-3.3) | 21(19-24) | 169(159-184) | 67(64-72) | 40(39-40) |

*Pg. = *Phoca groenlandica*, harp seal; P.v. = *Phoca vitulina*, harbor seal;
P.h. = *Phoca hispida*, ringed seal; T.t = *Tursiops truncatus*, bottle-nosed dolphin; L.o. = *Lagenorynchus obliquidens*, Pacific white-sided dolphin; D.l. = *Delphinapterus leucas*, beluga.

Table 2

Leukocytic parameters of captive pinnipeds and cetaceans, showing average values and ranges

| Species* | No. | Age | WBC (No. x $10^3/\mu l$) | Neutrophils (%) | Eosinophils (%) | Basophils (%) | Lymphocytes (%) | Monocytes (%) |
|---|---|---|---|---|---|---|---|---|
| P.g. | 5 | 3 mo. | 8.3(3.8-10.0) | 57(45-77) | 2( 1- 4) | 0(0-1) | 27(18-33) | 14(4-18) |
| | 5 | 6 mo. | 12.2(9.3-17.4) | 60(57-66) | 2( 1- 3) | 0(0-0) | 33(26-41) | 4(3- 5) |
| | 5 | 3-5 yr. | | 52(44-60) | 0( 0- 1) | 0(0-1) | 39(33-60) | 9(7-12) |
| P.v. | 12 | 4-6 mo. | 9.7(5.2-17.5) | 63(47-61) | 2( 1- 7) | 0(0-1) | 32(23-46) | 6(2-20) |
| | 3 | 9 mo. | 7.9(6.3-10.5) | | | | | |
| | 10 | 1-2 yr. | 10.2(7.8-16.3) | 55(45-73) | 3( 1- 5) | 0(0-1) | 38(23-47) | 4(3- 8) |
| P.h. | 2 | 2 yr. | | 65(61-72) | 1( 0- 1) | 2(1-3) | 30(27-37) | 2(1- 3) |
| T.t. | 7 | 4+ yr. | 10.6(7.8-13.1) | 59(53-62) | 2( 1- 3) | 1(0-2) | 34(31-40) | 4(1- 5) |
| L.o. | 2 | adult | 8.0(6.5- 9.5) | 57(54-60) | 20(17-23) | 0(0-0) | 21(20-22) | 2(2- 3) |
| D.l. | 4 | adult | 7.5(5.6- 9.1) | 66(63-73) | 5( 1- 9) | 0(0-0) | 27(19-34) | 2(2- 2) |

* Species identification as in Table 1.

Table 3.

Levels of four diagnostic enzymes in selected tissues of the harp seal, *Phoca groenlandica*, in activity units x $10^3$/g wet weight tissue.

| Tissue | GPT | ICD | Aldolase | CPK |
|---|---|---|---|---|
| Liver | 14.9 | 233.0 | 2.6 | 2.0 |
| Heart | 1.9 | 84.0 | 13.9 | 40.8 |
| Skeletal Muscle | 2.8 | 77.0 | 16.0 | 32.6 |
| Kidney | 1.8 | 79.3 | 2.9 | 3.3 |
| Brain | 1.0 | 30.0 | 5.3 | 13.9 |
| Blood Cells | 1.5 | 7.0 | 0.2 | 0.1 |

Table 4

Plasma protein in two species of pinnipeds, showing average values and ranges for total protein in g/dl and electrophoretic fractions in percent.

| Species* | No. | Age | Total Protein | Albumin | α-Globulin | ß-Globulin | *y*-Globulin | Albumin/ Globulin |
|---|---|---|---|---|---|---|---|---|
| P.g. | 5 | 10 mo. | 6.6(6.3-7.0) | 41(36-46) | 26(12-35) | 24(14-38) | 9( 6-14) | 0.71(0.56-0.91) |
| | 4 | 3-5 yr. | 7.6(7.3-8.3) | 38(34-39) | 28(24-30) | 20(16-22) | 16(13-18) | 0.61(0.55-0.70) |
| P.v. | 6 | 6 mo. | 7.8(6.3-8.9) | 37(33-41) | 37(22-41) | 17(13-23) | 9( 5-13) | 0.62(0.45-1.03) |
| | 13 | 15 mo. | 6.4(5.5-8.6) | 42(36-47) | 38(28-58) | 13( 9-16) | 9( 6-16) | 0.74(0.51-0.90) |

*Species identification as in Table 1.

Table 5.

Plasma enzyme and electrolyte concentrations in captive pinnipeds and cetaceans, showing average values and ranges

| | | | Enzyme (units/ml) | | | | Electrolyte (meq/1) | |
|---|---|---|---|---|---|---|---|---|
| Species* | No. | Age | GPT | ICD | Aldolase | CPK | $Na^+$ | $K^+$ |
| P.g. | 5 | 3 mo. | 42( 21- 57) | 482(318-623) | 9( 7-11) | 10( 5-17) | 149(140-158) | 4.1(3.7-4.5) |
| | 5 | 6 mo. | 155(146-164) | 552(303-720) | 29(23-25) | 15(12-19) | 158(157-161) | 4.9(4.6-5.3) |
| | 5 | 3-5 yr. | 95( 42-184) | 333(156-543) | 36(17-72) | 9( 6-12) | 159(149-167) | 3.8(3.4-4.2) |
| P.v. | 12 | 4-6 mo. | 58( 25- 87) | 335(234-517) | 15( 9-20) | 6( 5- 7) | 154(145-164) | 4.7(3.5-6.0) |
| | 3 | 9 mo. | 40( 28- 52) | | 14(11-18) | 13(10-16) | | |
| | 10 | 1-2 yr. | 58( 32-101) | 382(300-475) | 15( 6-20) | 6( 5- 7) | 166(160-168) | 5.0(4.5-5.4) |
| P.h. | 3 | 2 yr. | 44( 33- 62) | | | | 157(154-159) | 4.4(4.1-4.8) |
| T.t. | 7 | 4+ yr. | 28( 24- 29) | 75( 56- 99) | 13(11-17) | 5( 4- 6) | 159(154-163) | 3.7(3.1-3.4) |
| L.o. | 2 | adult | 44( 40- 48) | | 3( 2- 4) | 12( 9-15) | 162(161-162) | 3.5(3.3-3.7) |
| D.l. | 4 | adult | 13( 8- 18) | | 5( 4- 7) | 9( 7-12) | 162(159-165) | |

*Species identification as in Table 1.

Total leukocyte numbers in both seals and cetaceans were of the average order of 7 to 12 thousand cells/ $\mu$l (Table 2). There was a trend for an increased WBC count with age shown in the harp and harbor seals. The WBC differentials indicated that more than one-half to two-thirds of the WBC's were composed of neutrophils, about one-third were lymphocytes,and the remainder was distributed among eosinophils, basophils, and monocytes. One obvious exception to this common differential distribution pattern was the case of the Pacific white-sided dolphin, which showed a markedly elevated eosinophil fraction and somewhat lower lymphocyte component.

The osmotic fragility test carried out on erythrocytes showed lysis to start in 0.45 to 0.55 percent NaCl solution in the case of seals, and at 0.55 to 0.65 in the three cetacean species.

*Plasma chemistry* Tissue identification and relative compartmentalization of the four clinical enzymes showed that GPT and ICD were found in their highest concentration in liver tissue (Table 3). In the cases of aldolase and CPK, their highest levels were found in the muscle tissues, both heart and skeletal muscle. Although not as great as in muscle, significant concentrations of CPK were also found in brain tissue.

The liver enzymes GPT and ICD had a generally higher plasma concentration in seals, particularly in the case of the harp seal, than was the case in the dolphin species (Table 5). The same ralationship appears to hold for the muscle enzyme aldosterone, although CPK, the other muscle enzyme examined, showed no such clear differences in concentration. No age-dependent trends could be recognized.

Plasma levels of $Na^+$ were distributed at average values of 149 to 166 meq/l in the seals and cetaceans. There was a possible trend for the dolphin $Na^+$ values to be slightly greater than those for seals. The converse was seen for $K^+$ concentrations, which averaged lower in the cetaceans than in the seals.

The concentrations and distributions of plasma proteins were assessed only in harp and harbor seals (Table 4). Total protein was found at an average concentration of 6.4 to 7.8 g/dl, of which albumin about 40% $\alpha$-globulin about 27% in the harp seal and higher at 37% in the harbor seal. Beta-globulin fractions showed the reverse relationship: harp seals showed higher quantities at 22%. Gamma-globulin levels were similar in the two species. The average albumin/globulin ratio was calculated to be 0.67.

*Discussion*

Haematological parameters of pinnipeds and cetaceans have been reviewed recently by LENFANT (1969) and MACNEILL (1975). The following are additional selected sources of information which were not included in the reviews or are more recent additions to the marine mammal literature and are useful for comparative purposes. Pinniped blood values are presented by BRYDEN & LIM (1969), ENGELHARDT & GERACI (1978), GERACI & ENGEL - HARDT (1974), GERACI & SMITH (1975), LANE et al. (1972), KRAFT (1966), RIDGWAY (1972), RIDWAY et al. (1975), and RONALD et al. (1969). Hematological reports on cetaceans for comparative information may be obtained from GERACI & MEDWAY (1973, 1974), GERACI et al. (1968a), MEDWAY & GERACI (1964, 1972), MEDWAY et al. (1970), RIDGWAY (1972), and RIDGWAY et al. (1968).

Although the volume of data on marine mammal haematology is large, particularly for the more common species, there is often a great variation among authors and it is at times difficult to accept reported values as captive normal or base values. This may be attributed to the vary-

ing conditions under which the information was obtained. The literature reports haematological information from animals of different or unknown age, from wild animals, from animals of unknown clinical condition, or of otherwise unstated physiological status. It is certainly recognized that haematological values correlate with the status of the animal, such as with age (BRYDEN & LIM, 1969; ENGELHARDT, 1977b; GERACI & SMITH, 1975), disease (ENGELHARDT & GERACI, 1978; RIDGWAY, 1972), molt (ENGELHARDT, 1977a) and other stress responses (GERACI & MEDWAY, 1973; MEDWAY & GERACI, 1964; MEDWAY et al., 1970). A further complication to interpreting the literature information is that the conditions of blood storage and anticoagulant used may have had a significant effect on measured and calculated blood indices (GERACI & ENGELHARDT, 1974; GERACI & MEDWAY, 1974). When it was possible to make an assessment of the condition of the animals and sampling methods, it was found that the results from this study corresponded closely with those of healthy animals, whether captive or wild.

In contrast to the large haematological information base, that of clinical plasma chemistry is much less so, in particular for pinnepeds. The enzymes GPT and ICD were identified as liver specific, or at least predominantly so, in the harp seals of this study. Where as low levels of these enzymes may be found in plasma resulting from liver cell turnover or cell leakage, an increase in plasma concentrations is usually associated with hepatic abnormalities, and is a sensitive indicator of cell damage (KANEKO & CORNELIUS, 1970). A similar situation exists for the enzymes CPK and aldolase, but in this case is particularly specific for heart and/or skeletal muscle abnormalities, as specified by the tissue distribution pattern in harp seals. There is then good correlation between the anticipated specificity of the four enzymes as based on human standards, and that of the harp seals. For lack of other evidence, this may be extrapolated to other species of marine mammals, but should probably be done with some caution. St. AUBIN & GERACI (1977), for example, found that the enzyme aspartate aminotransferase (AspAT) showed a predominance in cardiac tissues in harp and grey seals, but was of greatest concentration in the liver of the ringed seal. Its diagnostic specificity, and perhaps that of other clinical enzymes in marine mammals, is thus not absolute. An important recent paper which identifies a large number of diagnostic enzymes in cetaceans, giving their tissue sources, is by GERACI & St. AUBIN( 1979) and identifying CPK to be of predominantly muscle origin.

The GPT levels described here for the phocids examined are appreciably higher than the human normal. They are also greater than those reported for the northern fur seal, *Callorhinus ursinus* (HUNTER & MADIN, 1976), although similar to values for the California sea lion, *Zalophus californianus* (RIDGWAY, 1972). Comparatively, GPT values in cetaceans were lower than in seals, and for the bottle-nosed dolphin corresponded to those reported by RIDGWAY et al. (1968) and RIDGWAY (1972). The GPT levels for the Pacific white-sided dolphin and beluga were lower than reported (GERACI et al. 1968b; RIDGWAY, 1972). For the case of CPK as well, the concentrations determined in the plasma of the three species in this study were lower than those reported for cetaceans by GERACI & MEDWAY (1974) and GERACI & St. AUBIN (1979). It may be that these differences, as well as those seen for other enzymes, can be the result of the different methods of analysis used. In general, little comparative marine mammal information is available for this enzyme, or for the other muscle specific enzyme, aldolase. Further, the enzyme ICD, useful in the diagnosis of liver problems, does not appear frequently in the marine mammal literature. As compared to human baseline standard concentrations determined by Sigma methods, the values for plasma GPT, ICD, and aldolase are higher in seals, at times markedly. The levels found in the cetacean plasma are comparable to human values. Plasma CPK levels may be considered like human normal in all instances.

Plasma concentrations of $Na^+$ and $K^+$ were found to be of the same range of values in both seals and cetaceans as those reported by ENGELHARDT & GERACI (1978), GERACI et al. (1968b), GERACI & MEDWAY (1974), RIDGWAY (1972), and RIDGWAY at al. (1968), and thus may be called base values. The value in $Na^+$ determinations in particular lies in the findings that many stress-related responses are characterized by an acute decrease in $Na^+$ values (ENGELHARDT & GERACI, 1978; GERACI, 1972).

The value of plasma protein concentrations and albumin-globulin differentials for diagnostic and physiological assessments is well recognized (KANEKO & CORNELIUS, 1970). It is another area, however, which will require a much more extensive information base to allow diagnostic interpretations and effective comparisons to be made among marine mammal species. It appears that the globulin distribution may be species specific, and that albumin/globulin ratios in seals are lower than those reported for cetacean species (GERACI et al., 1968b; RIDGWAY, 1972).

The intent of this report was to increase the available information on blood and plasma constituents of marine mammals, information which will be useful to veterinarians and researchers working with these diverse species. This may permit a yet better interpretation of the health status of the animal, necessary both for effective maintenance and experimental studies.

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