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Source: Journal of Wildlife Diseases, 19(3) : 259-264

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-19.3.259

Journal of Wildlife Diseases, 19(3), 1983, pp. 259–264

HEMATOLOGY AND CLINICAL CHEMISTRY VALUES IN THE KILLER WHALE, *ORCINUS ORCA* L.

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ABSTRACT: Clinical hematology and blood chemistry values are reported for the killer whale. These represent a panel of 13 hematological and 21 serum chemistry measurements determined on killer whales maintained for display at Sea World facilities. The values have been collected over a 10-yr period from 14 active, clinically normal individuals, six males and eight females. The cumulative normal values for each of these animals fall into well-defined clusters from which central tendencies and the range of values can be established. No significant male–female differences were observed for any measurement. There were consistent differences among the killer whales in hemoglobin, hematocrit and red blood cell count. A decrease in total white blood cell counts was associated with age and/or changes in parasite loads. Younger animals exhibited higher glucose levels and lower total protein levels. Serum urea nitrogen (BUN) and creatinine were elevated in older and larger males. Lactic dehydrogenase activity was lower in all animals of Pacific origin, as compared to animals from the Atlantic, regardless of age or sex. These "normal" differences emphasize the importance of establishing an animal's individual hematologic and blood chemistry profile by routine sampling.

INTRODUCTION

The killer whale is the largest marine mammal kept for public display. They have been maintained successfully at 11 locations in North America, as well as at several locations in Europe, Japan and Australia. The long-term maintenance of these animals has provided a unique opportunity to examine a variety of physiological and clinical features in a species which is difficult to study in the wild. It has also necessitated rigorous programs of health monitoring and care.

Few reports of normal and abnormal clinical laboratory test results have been published for the killer whale. Studies on respiratory function are reported by Spencer et al. (1967), Lenfant et al. (1968) and Dhindsa et al. (1974). Hematologic and blood chemistry values for two young males have been reported by Ridgway et al. (1979), hematologic values for two males are reported by MacNeill (1975), and additional hematologic comparisons are given in Lenfant (1969). There are brief reports of diseases and parasites in captive and wild killer whales (Tomes, 1873; Colyer, 1938; Tomilin, 1957; Ridgway, 1972; Taylor and Farrell, 1973; Ridgway, 1979). The need for normal clinical information, especially blood values used in the diagnosis and treatment of disease, has prompted this summary of hematologic and serum chemistry values from hundreds of clinical records kept over a 10-yr period on killer whales displayed at Sea World facilities in Washington, California, Ohio and Florida. These animals are kept on active training programs and are monitored routinely, including blood sampling, in order to anticipate possible changes in health status.

Received for publication 14 August 1979.

MATERIALS AND METHODS

Fourteen sub-adult to adult killer whales, six males and eight females, were included in this study. These animals ranged in weight from 350 kg to over 2,500 kg and in length from 275 cm to 620 cm. All have been maintained for at least 3 yr; three of the males have been maintained for 10–15 yr. The whales were collected either in the Pacific Northwest (primarily Puget Sound) or in the North Atlantic near Iceland, as previously described (Goldsberry et al., 1976).

Blood samples were taken at varying intervals of 24 hr to several months, allowing the evaluation of both short-term and long-term normal ranges in the blood values of individual animals. Sampling was most often done on a biweekly basis for hormone studies and other projects. Hematology and serum chemistry panels were performed on these samples as routine checks. Blood was taken by venipuncture from the tail fluke using a plastic disposable syringe with a 4 cm (18 or 20 ga) needle, and placed either in heparinized or EDTA Vacutainer tubes and in clot tubes. The whales were conditioned to present the tail fluke for blood sampling and the majority of samples were obtained in this manner. Alternately, blood samples were taken when the pool water was lowered for cleaning or transfer of animals to different areas. At these times, the whales were laid on foam rubber pads on the bottom of the pool or were held in hammock-like lifting slings and kept wet with a water spray. The whales were remarkably tolerant of both procedures. Serum was separated by centrifugation within 1 hr of collection. Hematology and serum

TABLE 1. Methods used for analysis of killer whale blood.

Test	Method
I. Hematology	
RBC, Hct, MCV, WBC	Coulter ZB1 (Coulter Electronics; calibrated daily)
Hemoglobin	Coulter hemoglobinometer (standardized daily)
MCH, MCHC	Calculated
Leukocyte differential	Routine microscopic procedure (100 cells/10,000 WBC)
II. Blood serum chemistry	
Glucose	1. Gilford system, reagents—hexokinase/G-6-PD method 2. Clinicard (Harleco)—hexokinase/G-6-PD method
Serum urea nitrogen (BUN)	1. Gilford system, reagents—urease/oxidation of NADH 2. Clinicard (Harleco)—diacetyl monoxime method
Creatinine	1. Gilford system, reagents—alkaline picrate reaction 2. Clinicard (Harleco)—alkaline picrate reaction
Bilirubin, total	1. Gilford system, American Monitor reagents—diazotized sulfanilic acid method 2. Clinicard (Harleco)—Erlich sodium nitrite/sulfonilic acid method
Cholesterol	1. Gilford system, reagents—combined cholesterol esterase, cholesterol oxidase, peroxidase and trinder reagent method 2. Clinicard (Harleco)—ferric ion method
Total protein	1. Gilford system, reagents—colorimetric biuret method 2. Clinicard (Harleco)—colorimetric biuret method
Albumin	1. Gilford system, reagents—bromcresol green 2. Clinicard (Harleco)—bromcresol green
Globulin	1. Calculated from albumin and total protein
Alkaline phosphatase (ALP)	1. Gilford system, reagents—hydrolysis of phosphate esters 2. Clinicard (Harleco)—measurement of increase in *p*-nitrophenylate
α-amylase	1. Gilford system, reagents—coupled enzyme reaction utilizing maltotetraose 2. Clinicard (Harleco)—measurement of increase of NADH
Aspartate aminotransferase, serum glutamic oxalacetic transaminase (AST, SGOT)	1. Gilford system, reagents—oxidation of NADH 2. Clinicard (Harleco)—measurement of rate of decrease of NADH
Alanine aminotransferase, serum glutamic pyruvic transaminase (ALT, SGPT)	1. Gilford system, reagents—oxidation of NADH 2. Clinicard (Harleco)—measurement of rate of decrease of NADH
Creatine kinase (CK)	1. Gilford system, reagents—oxidation of G-phosphogluconate 2. Clinicard (Harleco)—coupled enzymatic method/measurement of increase in NADH
α-Hydroxybutyric dehydrogenase (HBD)	1. Gilford system, reagents—oxidation of NADH
Lactic dehydrogenase (LDH)	1. Gilford system, reagents—reduction of NAD 2. Clinicard (Harleco)—measurement of increase in NADH
Calcium	1. Gilford system, reagents—cresolphthalein complexone reaction 2. Clinicard (Harleco)—chlorophosphonazo-III dye method
Inorganic phosphorus	1. Gilford system, reagents—phosphomolybdate method
Iron	1. Gilford system, American Monitor reagents—bathophenanthroline reaction
Potassium	1. Flame photometry
Sodium	1. Flame photometry
Chloride	1. Gilford system, reagents—thiocyanate/ferric ion method

1. is the preferred method

chemistry values were determined within 24 hr. Procedures are listed in Table 1.

Due to the routine nature of the sampling, it was possible to examine each animal repeatedly over a long period of time (3–15 yr). This allowed determination of long-term stability in an individual's values as well as detection of trends of change. For this summary of blood values, samples taken when the whales were judged by behavior, appearance or food consumption to be abnormal, have been excluded. Samples were also excluded for periods where there was marked and persistent deviation in any hematologic or chemistry value indicative of a change in health status. These abnormal changes were easily apparent upon comparison of the records. All other samples were considered to be representative of "normal" values. Frequency distributions of these "normal" values for all hematologic and chemistry measures were made on each killer whale. The median value was taken as the measure of central ten-

TABLE 2. Hematologic values for 14 captive killer whales.

Test		No. studied males females	No. of samples	Central tendency[a]	Normal range[a]
Erythrocytes					
Hemoglobin (g dl)	High[b]	1 1	142	17.1	16–19
	Low	5 7	550	15.0	13–16
Hematocrit (%)	High	1 1	144	49	44–55
	Low	5 7	581	43	37–49
RBC count (10^6/mm^3)	High	1 1	143	4.3	3.8–5.0
	Low	5 7	575	3.8	3.2–4.3
Mean corpuscular volume (MCV) (μm^3)		6 8	682	112	94–123
Mean corpuscular hemoglobin (MCH) ($\mu\mu g$)		6 8	667	39	35–45
Mean corpuscular Hb conc. (MCHC) (%)		6 8	684	35	32–38
White blood cells					
Total WBC count (per mm^3)		6 7	851	7,800	4,500–11,000
Differential					
Neutrophils	Rel. %	6 8	907	74	54–86
	Abs. no.[c]			5,772	
Bands	Rel. %	6 8	897	0	0
Lymphocytes	Rel. %	6 8	893	21	8–32
	Abs. no.			1,638	
Monocytes	Rel. %	6 8	906	3	0–6
	Abs. no.			234	
Eosinophils	Rel. %	6 8	822	2	0–8
	Abs. no.			156	
Basophils	Rel. %	6 8	667	0	0

[a] Determination of central tendency and normal range are explained in the text

[b] See discussion for explanation of high and low categories of hemoglobin, hematocrit and red blood cell count. MCV, MCH and MCHC were the same for both groups.

[c] Absolute number has been calculated from the median total WBC count and the relative percentage of each cell type in the differential. rel. % is used by Sea World Labs.

dency because it was felt to be less distorted by an occasional extreme value on either side of the normal range (Simpson et al., 1960). High, median and low values for all individuals were plotted. These plots made it possible to establish the upper and lower limits of the normal ranges for each measurement and to compare the distribution of individual animals within these ranges. Normal range is here defined as those limits in which the values for all animals fell. An occasional single value falling far outside the range of all the other animals was not considered to be representative of normal in these animals and was therefore not used to define normal range.

RESULTS AND DISCUSSION

Normal values are given in Table 2 (hematology) and Table 3 (serum chemistry). An overall median value and the upper and lower limits of the normal range for each measurement are given, along with the total number of determinations and the number of animals (males/females) used in each instance. There was evidence in some tests of sub-grouping within the overall range by age/size classes or by animals of different origins. These will be discussed below.

Hematology: Persistent differences in hemoglobin concentrations, hematocrits and red blood cell counts can be seen among the killer whales. These differences were not related to age or sex and were maintained throughout the entire period of observation. They have been reported previously in the bottlenose dolphin, *Tursiops truncatus* (Duffield et al., 1983), and in the killer whale (Cornell et al., 1981). In the bottlenose dolphin, it has been suggested that the differences in hemoglobin values, hematocrits and red blood cell counts represent coastal vs. off-shore ecotypes, and off-shore form having the higher values for all three measurements. Captive-bred crosses between the two forms resulted in animals with intermediate hematologic values, indicating that there is a genetic basis for these differences in this species. Intraspecific hematologic differences were observed in killer whales as well, although it has not yet been possible to test the genetic basis for such differences. The high (off-shore?) and low (coastal?) hemoglobin, hematocrit and red blood cell values for the killer whales in this study are

TABLE 3. Serum chemistry values for 14 captive killer whales.

Test	Test proc.[a]	No. studied (M/F)	No. of samples	Central tendency[b]	Normal range[b]
Glucose (mg/dl)	1,2	6/7	551	118[c]	90–150
Serum urea nitrogen (mg/dl) (BUN)	1	4/6	289	34[c]	20–50
(BUN)	2	5/8	213	28	20–50
Creatinine (mg/dl)	1,2	6/8	517	1.3[c]	0.5–2.0
Bilirubin, total (mg/dl)	1,2	6/8	488	0.2	0.1–0.4
Cholesterol (mg/dl)	1	4/6	261	193	110–280
	2	6/7	257	267	135–365
Total protein (g/dl)	1	5/6	316	7.1	5.5–8.9
	2	6/8	244	8.1	6.4–9.5
Globulin (g/dl)	1	5/6	309	3.4	2.0–6.0
	2	6/8	162	4.6	2.2–6.7
Albumin (g/dl)	1,2	6/8	598	3.4	2.5–4.4
Alkaline phosphatase (U/liter)	1,2	6/8	427	192	50–350
Amylase (U/liter)	1	5/6	298	17	5–35
	2	5/6	134	57	20–80
Aspartate aminotransferase (AST, SGOT) (U/liter)	1	5/6	319	27	14–36
	2	5/7	226	48	20–70
Alanine aminotransferase (ALT, SGPT) (U/liter)	1,2	6/8	440	9	3–18
Creatine kinase (CK) (U/liter)	1	5/5	241	61	11–110
	2	6/6	154	153	42–240
α-Hydroxybutyric dehydrogenase (HBD) (U/liter)	1	5/6	270	340	220–440
Lactic dehydrogenase (LD) (U/liter)	1	5/6	291	198[c]	115–240
	2	5/6	218	300	175–425
Calcium (g/dl)	1	5/6	277	8.4	6.8–9.5
	2	6/7	218	9.1	7.7–10.1
Phosphorus (mg/dl)	1	5/6	262	6.1	3.8–7.2
Iron (μg/dl)	1	4/6	259	107	50–220
Potassium (mEq/liter)	1	4/3	48	4.2	3.7–4.8
Sodium (mEq/liter)	1	3/3	37	154	139–157
Chloride (mEq/liter)	1	6/3	209	107	91–124

[a] Gilford Systems procedures are coded 1; Clinicard procedures are coded 2; see Table 1.
[b] Determination of central tendency and normal range are explained in the text.
[c] Sub-grouping of individual animals within the normal range by age, size or place of origin was seen; see text.

indicated separately in Table 2. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) did not appear to be different between killer whales of high or low hematologic profile and were grouped together. They were similar to values reported for MCV, MCH and MCHC by MacNeill (1975). There was no apparent distinction based on hematologic type in any of the other clinical values reported here.

Erythrocytes appeared as biconcave discs of approximately 7 μm in diameter. Routine blood smears were carefully examined for abnormal red cell morphology and for inclusion bodies. The occasional occurrence of abnormal cell types such as polychromasia, microcytes, spherocytes and piokilocytosis was noted. Persistent increases in any of these were considered abnormal. Howell-Jolly bodies and nucleated RBC's were also occasionally seen and considered normal. Any consistent increase in these or the presence of other types of inclusion bodies were taken to indicate a change in health status.

Total white blood cell counts (Table 2) ranged from 4,500 to 11,000/mm^3 with medians about 7,900/mm^3. There were no consistent differences between males and females. However, there was an apparent decrease in WBC count with age. Young animals had median counts around 9,500/mm^3, whereas older animals had much lower counts around 6,000/mm^3. It was not clear whether this change was entirely age-related. A drop in the number of eosinophils was observed following collection of the animals. As it has been suggested that increased eosinophilia may be associated with parasitism

(Tomilin, 1957; Ridgway, 1972), the observed decrease in WBC count along with the drop in eosinophils may also be reflective of a gradual lowering of the high initial parasite loads that are often seen when the animals are first brought from the wild (Cornell, unpublished data). The most predominant of these parasites are tapeworms, possibly belonging to the order Cyclophyllidea, and were observed to be shed over time.

As reported for the bottlenose dolphin (Ridgway et al., 1970), killer whales showed a strong leukocytic response to infections. Killer whales with mediastinal, lung and mandibular abscesses, bacterial pneumonia and urinary infection have shown increases of WBC counts to 20,000–30,000/mm^3 prior to treatment.

In a routine differential, 100 cells for every 10,000 WBC were counted. Platelet counts were made and normally ranged from 10–30/1,000× field. The differential leukocyte counts (Table 2) were similar to those reported previously (MacNeill, 1975; Ridgway et al., 1979), except that no bands were detected from the "normal" samples in these animals. Basophils were also rarely seen in killer whale differential counts. Killer whales, in our experience, and as reported by MacNeill (1975) and Ridgway (1979), have a significantly lower percentage of eosinophils (median, 2%) than dolphins such as *Tursiops* and *Lagenorhynchus* (mean values summarized in MacNeill as 17 ± 9% and 22 ± 12%, respectively). However, differentials performed at collection and later during abnormal periods showed that the eosinophil percentages in killer whales could rise as high as 17–23%. The exact clinical association of changes in total WBC count and changes in relative frequencies of WBC types with parasitism or changes in health status has yet to be fully investigated in killer whales.

Serum chemistry: Serum chemistry tests were initially performed using Clinicard procedures (Harleco, 480 Democrat Rd., Gibbstown, New Jersey 08027, USA). These were later replaced by Gilford Systems spectrophotometric procedures as technological improvements were developed (Gilford Instrument Laboratories Inc., Oberlin, Ohio 54074, USA). Without exception, values determined using the Gilford systems were more consistent and exhibited smaller ranges. The chemistry values reported in Table 2 reflect the Gilford tests. The Clinicard values are presented where these procedures gave different levels than the Gilford tests for comparison with data still taken by Clinicard procedures and exemplify the variation which can be introduced into data comparison by differences in clinical methods.

There were no apparent differences in any of the serum chemistry values between males and females. For five of the values, however, comparison of individual animal low, median and high values revealed sub-grouping of animals within the normal range by age, size or place of origin. These are as follows:

Glucose: The younger animals were found consistently in the upper part of the normal range of 90–150 mg/dl.

Creatinine: Creatinine levels were elevated in the older, larger males. While all other animals exhibited values in the lower part of the normal range, the older and much larger males had normal values in the upper part of the range, frequently extending as high as 2.5 mg/dl. It was assumed that this difference in level was due to the tremendous change in size seen with sexual maturity in male killer whales, the increase in quantity of creatinine reflecting increased muscle mass (Finco, 1980).

Serum urea nitrogen: BUN also appeared to be elevated in the much larger males (Gilford System median values 43–50 mg/dl) compared to medians of 29–38 mg/dl in the other animals (Clinicard, 40–46 mg/dl vs. 23–35 mg/dl). Whether this was also reflective of the increased size of these males or was related to the massive daily intake of protein is not known.

Total protein: The younger animals were consistently found in the lower part of the normal range of 5.5–8.9 g/dl.

Lactic dehydrogenase: Increased total LD values are frequently seen with lung inflammation in the absence of elevations in other serum chemistry, measurements, particularly those reflecting heart, muscle and liver function, in cetaceans (Cornell, unpubl. data) and are, in my opinion, of considerable diagnostic use in these animals. In the plots of low, median and high normal LD values, it was noticed that the LD values in all animals of Pacific origin were lower than those in ani-

mals of Atlantic origin, over all age groups examined. Intraspecific differences in total LD activity have also been observed in sampling wild bottlenose dolphins from different areas. This distinction in killer whales may be indicative of population differentiation between the animals of the Atlantic vs. Pacific oceans.

No other chemistry values showed this clear sub-grouping by age, size or genetic differences. However, it must be emphasized that, as this report of normal range was not designed to examine individual changes over time and as the animals are usually over 4 yr of age when acquired, subtle differences in values with age would not be seen within the overall normal range.

The normal ranges in serum chemistry values reported here were in general agreement with those given in another study on two other killer whales (Ridgway, 1979). Glucose, cholesterol, aspartate aminotransferase (AST, SCOT), alanine aminotransferase (ALT, SGPT) and phosphorus levels were lower in this study; alkaline phosphatase, calcium and iron were higher. The differences may be due to individual animal variation and/or to differences in the tests used, but in either case, the ranges of the two killer whales were not inconsistent with those of the animals reported here.

This panel of tests has given invaluable information for the diagnosis of disease and for monitoring health in our killer whales. Routine sampling to establish individual profiles within these normal ranges is strongly recommended. This allows detection of subtle shifts in values before having to deal with an acute medical problem.

ACKNOWLEDGMENTS

We would like to thank Dr. Sam H. Ridgway for his encouragement and help in preparing this manuscript; Drs. Debbie Duffield and Deane Clarkson for their help in data compilation and analysis; Bernice Stark and Gregg Case and the laboratory personnel working for them at Sea World for the fine work that they do; and the Cetacean Behaviorists and Animal Care Staff at Sea World without whom none of this would have been possible.

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