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SERUM CHEMISTRY AND EVIDENCE OF RENAL FAILURE IN THE NORTH ATLANTIC FIN WHALE POPULATION

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ABSTRACT: Serum electrolytes, urea nitrogen, creatinine, albumin and globulin were studied in fin whales (*Balaenoptera physalus*) caught by commercial whalers in the North Atlantic (Denmark Strait area). Blood samples were obtained by catchment or cardiac puncture within 5–15 min of death and analyzed using automated spectrophotometric methods and flame photometry. Osmolality was determined for two serum samples by a vapor pressure method. Linear regressions determined for each measured serum variable vs. chase time suggested that pursuit of the whales prior to capture had no substantive effect on measured serum chemistry. As in other cetaceans, serum sodium, chloride, urea nitrogen and osmolality were distinctly higher in the fin whale than in terrestrial mammals. The total concentration of serum proteins, however, was 1.4–1.8 g/dl lower, on average, than reported in small toothed whales, and was similar to that of domesticated animals. One animal in this population showed alterations in serum chemistry which were consistent with renal failure.

INTRODUCTION

The rorquals, or "grooved" baleen whales represent an absolute extreme in the diversity of evolution. These mammals, which comprise the family Balaenopteridae, include the largest of any extant or extinct animal species. Past records for blue whales (*Balaenoptera musculus*), the largest rorqual, give out-of-water weights of over 120,000 kg, with some estimates as high as 170,500 kg (Laurie, 1933). The next largest species, the fin whale (*B. physalus*), can reach 70,000 kg and probably more.

As such, rorquals have been the subjects of numerous extrapolations by comparative physiologists concerned with fundamental life processes. Yet the size of these whales has made even basic physiological data difficult to obtain. Compounding the difficulties imposed by sheer size is the common problem of contamination of blood and other tissue samples with seawater prior to analysis (Fetcher, 1939).

In this study we describe the electrolyte, urea nitrogen, creatinine, albumin and globulin concentrations in blood serum of fin whales, a common rorqual found throughout the world's oceans. Our objectives were to determine normal concentrations of serum constituents and to examine the population for evidence of systemic disease. The samples were obtained through the auspices of the Icelandic Whales Research Laboratory, Hvalfjordur, Iceland, and came from whales caught in the North Atlantic by commercial whalers. To consider one problem associated with this type of study, possible relationships between the time spent pursuing a whale prior to its being caught and the measured chemical properties of the serum were evaluated. The results are compared with serum chemistry profiles of other cetaceans and domesticated land mammals.

MATERIALS AND METHODS

The fin whales sampled in this study were taken off the west coast of Iceland during July–August 1984, and in the same area during June–August 1985. Blood samples from 39 fin whales were collected by specially trained crew members on three steam-powered whale catcher ships. Blood was obtained by removing one-half of the tail fluke within 10–15 min of the death

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TABLE 1. Methods used to determine serum chemical values for the fin whale.

Test	Reaction product	Wavelength (nm)	References
Sodium (Na)	flame	589	Amador et al. (1972)
Potassium (K)	flame	766	Amador et al. (1972)
Chloride (Cl)	Hg-thiocyanate complex	525	Skeggs and Hochstrasser (1964)
Calcium (Ca)	cresolphthalein complexone	600	Frings et al. (1970)
Magnesium (Mg)	Mg-magon complex	515	Mann and Yoe (1956)
Phosphorus (P)	NH_4-molybdate	340	Mollo et al. (1977)
Urea nitrogen (SUN)	urease catalyzed glutamate dehydrogenation coupled to NADH-NAD conversion	340	Tiffany et al. (1972)
Creatinine	creatinine picrate	520	Fabiny and Ertingshausen (1971)
Total protein	biuret complex	550	Kingsley (1939)
Albumin	bromcresol green	600	Rodkey (1965)

of the animal, hanging the sectioned fluke from the rigging, drying the specimen with paper towels, sectioning the tip of the fluke and catching the subsequent drops of blood in freshly opened Vacutainer® collection tubes (Beckton-Dickinson, Rutherford, New Jersey 07070, USA). The whole blood was then stored on shipboard at 4–6 C for 16–30 hr prior to centrifugation at a land-based laboratory. The serum, which in some instances showed signs of hemolysis, was frozen at −30 C for 1–1.5 mo and then −70 C for 3–4 mo before analysis.

A second set of five serum samples was obtained to assess possible effects of hemolysis on serum chemistry values. Blood samples were taken at sea by the above procedure, but centrifuged within 30 min after collection on board the catcher ship to obtain the serum. The serum was kept at 4–6 C for 16–30 hr and then frozen in liquid nitrogen until analyzed.

Additional blood samples were obtained from two whales by cardiac puncture 5–10 min after their deaths. These were taken by means of a 1.6-m-long, 2.0-cm-bore, side inlet "needle" attached by a 2-cm-bore rubber hose to an evacuated 500-ml-glass jar. Contamination of the collection apparatus with seawater was prevented by inserting the needle, with the vacuum turned off, through an appropriate site on the portion of the whale's thorax which floated above the sea surface. This site was slightly dorsal and caudal to the whale's left or right axillary region.

Blood collected by cardiac puncture was also centrifuged on board the catcher ship. The serum was transported to land at 4–6 C. There it was frozen to −30 C 24–26 hr after the time of collection. Otherwise, the handling and deep freezing of these specimens was identical to the original 39.

Serum chemistry profiles were determined on the thawed serum specimens after centrifugation at 800 *g*. The specific methods employed for each chemical analysis are listed in Table 1. All measurements except sodium, potassium and osmolality were made using an Encore® centrifugal analyzer at 30 C equipped with an automatic pipettor-diluter (J. T. Baker Instruments, Inc., Allentown, Pennsylvania 18105, USA). Concentrations of sodium and potassium were measured separately using an IL 443 flame photometer (Instrumentation Laboratories, Wilmington, Massachusetts 01887, USA). Calibrations of all procedures were performed using authentic standards. Commercially available control materials (Validate® and Validate-A®, General Diagnostics, Inc., Morris Plains, New Jersey 07950, USA) were analyzed simultaneously to ensure the reliability of all chemistry measurements. Total serum globulins and albumin/globulin ratios were calculated from measured albumin and total protein values. Albumin determinations were calibrated by electrophoresis. Serum protein electrophoresis for protein calibrations was done on polyacetate strips (Sepratex®, Gelman Instrument Co., Ann Arbor, Michigan 48106, USA) at pH 8.6. Serum samples of 0.50 μl volume were electrophoresed at 200 V for 20 min, stained with ponceau S in 7.5% trichloroacetic acid, and rinsed with 5% acetic acid. After clearing the strips with 40% aqueous *N*-methyl pyrrolidone the strips were dried at 90 C. The separated proteins were then quantified using a ACD-15 scanning densitom-

eter (Gelman Instrument Co., Ann Arbor, Michigan 48106, USA). Osmolality was determined for the two samples obtained from the heart by the vapor pressure method of Jewel (1977).

Data from a particular whale were rejected from subsequent statistical analyses if the whale's serum showed disproportionately high concentrations of magnesium. Given the high magnesium content of seawater (Sillen, 1961), this was considered indicative of contamination with seaspray, a problem that was unavoidable during heavy weather. In practice, the decision to reject data from a sample was facilitated by the occurrence of two populations in the magnesium data separated by an apparent gap over the range of 4.9–6.0 mg magnesium per dl. Data from whales with greater than 6.0 mg magnesium per dl were rejected, while seawater contamination was considered minimal or absent in samples having magnesium concentrations of 4.9 mg/dl or less.

Data on chase time were obtained from the logbooks of the whaling company and represent the interval of time between sighting and catching a given whale. The data on whale length represent the linear distance from the tip of the rostrum to the notch of the fluke.

Possible relationships between each measured serum variable and chase time were assessed graphically using scatter plots. As these showed no resolvable short term fluctuations the data were subsequently reduced by linear regression (least squares method).

RESULTS

Ten data sets were rejected in this study on the basis of probable seawater contamination of the serum samples. Data from the remaining 29 whales that were sampled from the tail flukes, exclusive of those studied using electrophoretic methods, are given in Table 2. All samples rejected on the basis of high (>6.0 mg/dl) magnesium concentration also showed sodium and chloride concentrations well above or in the upper portion of the range of these variables in the other 29 whales. Since this would be expected with seawater contamination, it appears that the criterion used for rejection of samples was valid.

In evaluating the results in Table 2 one should consider the effects of red cell lysis. This was a problem with the first group of samples obtained from the flukes (those held 16–30 hr before centrifugation), as several were moderately or even severely hemolytic. Such hemolysis could affect serum electrolyte concentrations (see Discussion).

Scatter plots of the data for each serum constituent vs. chase time were examined and showed no resolvable pattern of variation. Complementing these findings, the results of linear regressions determined from the same data showed no appreciable relationship between any serum constituent and chase time. In all cases the slopes of the linear regressions approximated zero (range −0.031–0.003), and correlation coefficients were low (range −0.22–0.31). Thus this limited data base failed to show any effect of capture effort on the average concentrations of any of the serum constituents. The relevance of this observation is that it suggests that the results in Table 2 constitute a reasonable approximation of normal.

Table 3 gives the serum chemistry and osmolality data for 16.9-m-long male (Hv8-51-84) and 17.5-m-long female (Hv9-52-84) fin whales determined using samples of blood obtained from the heart. Both of these animals were sexually immature. Rapid collection and centrifugation of the blood samples from these whales virtually eliminated the hemolysis problem. The serum of Hv8-51-84 showed no visible evidence of hemolysis, and was creamy white in color, whereas the serum from Hv9-52-84 was faintly pink (slight hemolysis).

Notable differences from the preceding data are as follows. The serum urea nitrogen (SUN) of both animals was higher, especially that of the female (Hv9-52-84), which had the highest SUN of any of the whales we studied. Also, creatinine in this animal was the highest, and chloride the lowest, of any measured.

TABLE 2. Serum chemistry values of fin whales comparing rapid and delayed centrifugation of blood samples.

Group	Na^+ (mEq/liter)	K^+ (mEq/liter)	Cl (mEq/liter)	Ca^{++} (mg/dl)	Mg^{++} (mg/dl)	P (mg/dl)	SUN (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Delayed sample centrifugation[a]												
Mean	149	4.9	113	11.2	3.1	8.5	79.0	1.2	6.1	4.2	2.0	2.2
Range	135–160	3.6–7.3	103–123	10.0–12.9	2.0–4.7	5.3–12.1	57–96	0.3–1.9	5.2–7.3	3.5–4.8	0.9–2.7	1.6–5.1
SD	7.1	1.1	4.6	0.7	0.7	1.6	9.0	0.4	0.6	0.3	0.4	0.7
Rapid sample centrifugation[b]												
Mean	167	6.5	114	9.8	3.3	6.9	79.4	2.1	6.4	3.4	3.0	1.2
Range	153–188	4.0–9.3	103–124	8.7–11.0	2.2–4.4	4.7–7.9	61–98	1.4–3.0	5.5–6.9	2.8–3.8	2.1–4.1	0.7–1.6
SD	17.7	2.2	8.8	1.1	1.0	1.3	15.8	0.7	0.6	0.4	0.7	0.4
Combined values[c]												
Mean	152	5.2	113	11.0	3.1	8.2	79.1	1.3	6.2	4.0	2.2	2.0
Range	135–188	3.6–9.3	103–124	8.7–12.9	2.0–4.7	4.7–12.1	57–98	0.3–3.0	5.2–7.3	2.8–4.8	0.9–4.1	0.7–5.1
SD	11.7	1.4	5.8	0.9	0.7	1.6	10.1	0.6	0.6	0.4	0.6	0.8

[a] Samples centrifuged 22–30 hr postmortem, $n = 24$.
[b] Samples centrifuged within 30 min postmorten, $n = 5$.
[c] $n = 29$.

DISCUSSION

This study apparently provides the first published data on the serum chemistry of a population of rorquals. The one exception is the early work of Laurie (1933), who gives the chloride content of the serum of 10 fin and 10 blue whales as "constant to within 5 percent" of 119 mEq/liter. This is slightly higher than the mean chloride concentration of 113 mEq/liter determined here, and may reflect our decision to reject samples contaminated with seawater.

Other pertinent chemical data on rorquals relate to the composition of whole blood, not serum, and thus are not directly comparable to our results. As expected from the added contribution of red cell cytoplasm, analyses of whole rorqual blood (Sudzuki, 1924; Okahara, 1925) give lower sodium concentrations and much higher potassium concentrations than our average values for serum. Hemolytic release of low sodium/high potassium red cell cytoplasm into serum can be expected to have the same selective effects, although these effects should be smaller in magnitude. For this reason, and because certain of the serum samples we had to work with were hemolytic, true mean serum sodium concentrations may be slightly higher, and potassium concentrations lower, than we obtained.

The magnitude of such deviations, however, may be negligible. In the data in Table 2, there are no significant differences in serum sodium or potassium concentrations in rapidly processed samples (group 2) compared with those stored as whole blood for 16–30 hr before centrifugation ($P > 0.10$).

Along these same lines, one should note that the work of Okahara (1925) gives phosphate concentrations in fin whale blood of 34 mg/dl (four whales), much higher than we found in serum, and that a time dependent hydrolysis of the phosphate esters in red cells produces phosphorus that is measured by both Okahara's and our own methods (Okahara, 1925; Mollo et al., 1977). Taken in view of the hemolysis problem and the delay in centrifuging the first group of blood samples ($n = 24$), this indicates that the mean phosphate concentration in fin whale serum is probably lower than we obtained. The range of serum phosphate is perhaps closer to that of large ruminants (4.0–7.0 mg/dl; Blood and Henderson, 1979).

Other deviations in the results from ideal values for nonhemolytic serum can be inferred from controlled studies on the effects of hemolysis on serum chemistry. In spectrophotometric measurements (viz., Table 1), hemolysis induces changes in light absorbance associated with small artifactual increases in creatinine concentration (Dorner et al., 1981). Also, hemolytic release of red cell cytoplasm may directly increase total protein. Studies on other mammalian blood, however, suggest that these changes should be small, and perhaps insignificant, even with the moderate hemolysis problem we encountered when using blood samples taken from the flukes. For example, severe (cherry red) hemolysis of equine blood, corresponding to hemoglobin concentrations of 194 mg/dl, causes no significant increase in protein, whereas creatinine concentration is increased 0.3 mg/dl (Dorner et al., 1981).

Inasmuch as these deviations are small, attempts to apply correction factors are not warranted. Beyond this, not all of our samples were visibly hemolytic, and the creatinine and total protein values we obtained (Table 2) already fall within or slightly below the range of these variables in other mammals, including small and other large cetaceans (Medway and Geraci, 1965; Malvin and Rayner, 1968; Blood and Henderson, 1979; Medway, 1983). For this reason we feel that our data for these serum constituents were not elevated significantly by artifact. However, the complete absence of visible he-

molysis in the heart serum obtained from whale Hv8-51-84 may in part account for the below average creatinine concentration and the low total protein concentration found in this animal (Table 3).

Going one step farther, the observation that the sample from whale Hv9-52-84 was only slightly hemolytic indicated that the markedly elevated creatinine concentration in this whale, as well as its high urea nitrogen and abnormal electrolyte pattern, were likely real. Contrary to what was found, the very low amount of hemolysis in the sample from this whale should give a creatinine concentration nominally lower—not markedly higher—than the average value for the preceding population, other factors being equal. The results for whale Hv9-52-84 thus suggest, in accord with basic principles of clinical laboratory diagnosis (Goldberger, 1980), that this animal was suffering from renal insufficiency or renal failure at the time of capture. Obvious support for this conclusion is the elevated creatinine and urea nitrogen concentrations. Also supporting this diagnosis are the animal's high serum phosphate and potassium values—which occurred despite rapid handling of the sample—and the depressed serum calcium and chloride concentrations. Such deviations, the former of which from the osmolality and total protein values cannot be explained on the basis of simple dehydration, are associated with severe nephron disease in mammals and indicate, among other things, a grave prognosis (Blood and Henderson, 1979; Goldberger, 1980).

Considering these points, the data for whale Hv9-52-84 should not be viewed as representative of a healthy fin whale. Rather, they can be interpreted as representing an expected finding in any "normal" population of wild animals, in which some individuals are affected by disease. On the basis of other work the apparent disease condition of this young animal may

TABLE 3. Serum chemistry values for fin whales sampled by postmortem cardiac puncture.

Whale number	Chase time (min)	Na^+ (mEq/liter)	K^+ (mEq/liter)	Cl^- (mEq/liter)	Ca^{++} (mg/dl)	Mg^{++} (mg/dl)	P (mg/dl)	SUN (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Serum osmolality (mosmol/liter)
Hv8-51-84	5	151	5.3	112	10.0	3.4	8.1	71	1.9	5.0	3.5	1.5	2.3	359
Hv9-52-84	10	156	6.4	99	9.6	3.8	9.7	114	3.5	6.0	3.8	2.2	1.7	330

have been caused by severe parasitism of the kidneys. We consider this likely because 90–95% of the fin whales in the population studied are infected with the giant kidney worm, *Crassicauda boopis* (Nematoda, Spirurida), a parasite which commonly induces fibrotic obstructions of the whale's renal veins (Lambertsen, 1985, 1986). Furthermore, necropsy studies of over 85 fin whales in this population suggested that truly severe infections with *C. boopis* could cause mortality by inducing congestive renal failure (Lambertsen, 1986). Animals with diffuse renal disease were found with bilateral obstructions of their renal veins caused by heavy *Crassicauda* infection.

Beyond these considerations, our results indicated that serum sodium, chloride and urea nitrogen concentrations were higher in the fin whale than in terrestrial animals, as is also the case for the small toothed cetaceans (Medway and Geraci, 1965; Malvin and Rayner, 1968; Medway, 1983) and the sperm whale (*Physeter catodon*) (Sudzuki, 1924). Our single measurement of serum osmolality for an apparently healthy animal is 18 mosM higher than the average found in six toothed whale species by Malvin and Rayner (1968), but is within the broad range of serum osmolality given by others for bottlenosed dolphins (*Tursiops truncatus*) (Eichelberger et al., 1940; Medway and Geraci, 1965), pilot whales (*Globicephala melaena*) (Medway and Muldovan, 1966), bowhead whales (*Balaena mysticetus*) (Medway, 1983) and the sperm whale (Sudzuki, 1924). As in all cetaceans for which data are available, this serum osmolality is higher than normal in terrestrial mammals, a finding which presumably reflects the combined effects of the comparatively high concentrations of sodium, chloride and urea.

Our average value for total serum protein, in contrast, is 1.4–1.7 g/dl less than reported for small cetaceans (Fetcher and Fetcher, 1942; Medway and Geraci, 1965), whereas it is in rather close agreement with the values given by Medway (1983) for the bowhead, a distantly related (but equally large) baleen whale. The concentration of proteins in the serum of the fin whale thus is similar to that seen in domesticated animals (Blood and Henderson, 1979). In other respects our results showed no notable differences in the serum chemistry of fin whales from that of other cetaceans or terrestrial mammalian species.

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LITERATURE CITED

AMADOR, E., R. L. CECHNER, AND J. J. BARKLOW. 1972. Continuous recording flame photometry of Na and K in serum. Clin. Chem. 18: 668–671.

BLOOD, D., AND H. HENDERSON. 1979. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs and Horses, 5th Ed. Lea and Febiger, Philadelphia, Pennsylvania, 1135 pp.

DORNER, J. L., W. E. HOFFMAN, AND T. F. LOCK. 1981. Effects of *in vitro* hemolysis on equine serum chemical values. Am. J. Vet. Res. 42: 1519–1522.

EICHELBERGER, L., E. S. FETCHER, JR., E. M. K. GEILING, AND B. J. VOS. 1940. The distribution of water and electrolytes in the blood of dolphins (*Tursiops truncatus*). J. Biol. Chem. 133: 145–152.

FABINY, D. L., AND G. ERTINGSHAUSEN. 1971. Automated reaction-rate method for determination of serum creatinine with the centrafichem. Clin. Chem. 17: 696–700.

FETCHER, E. S., JR. 1939. The water balance in marine mammals. Quart. Rev. Biol. 14: 451–459.

———, AND G. W. FETCHER. 1942. Experiments on the osmotic regulation of dolphins. J. Cell. Comp. Physiol. 19: 123–130.

FRINGS, C. S., P. S. COHEN, AND L. B. FOSTER. 1970. Automated method for determination of serum calcium by use of alizarin. Clin. Chem. 16: 816–819.

GOLDBERGER, E. 1980. A Primer of Water, Electrolyte and Acid-Base Syndromes. Lea and Febiger, Philadelphia, Pennsylvania, 472 pp.

JEWEL, R. 1977. Serum osmolality, a CAP survey analysis. Am. J. Clin. Pathol. 68: 165–169.

KINGSLEY, G. R. 1939. The determination of serum total protein, albumin, and globulin by the biuret reaction. J. Biol. Chem. 131: 197–200.

LAMBERTSEN, R. H. 1985. Taxonomy and distribution of a *Crassicauda* species (Nematoda: Spirurida) infecting the kidney of the common fin whale (*Balaenoptera physalus* L. 1758). J. Parasitol. 71: 485–488.

———. 1986. Disease of the common fin whale: Crassicaudiosis of the urinary system. J. Mammal. 67: 353–366.

LAURIE, A. H. 1933. Some aspects of respiration in blue and fin whales. Discovery Rep. 7: 363–406.

MALVIN, R. L., AND M. RAYNER. 1968. Renal function and blood chemistry in Cetacea. Am. J. Physiol. 214: 187–191.

MANN, C. K., AND J. H. YOE. 1956. Spectrophotometric determination of magnesium with sodium 1-azo-2-hydroxy-3-(2,4-dimethyl-carboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate). Anal. Chem. 28: 202–205.

MEDWAY, W. 1983. Examination of blood and urine from Eskimo killed bowhead whales (*Balaena mysticetus*). Aquat. Mamm. 10: 1–8.

———, AND J. R. GERACI. 1965. Blood chemistry of the bottlenose dolphin (*Tursiops truncatus*). Am. J. Physiol. 209: 169–172.

———, AND F. MOLDOVAN. 1966. Blood studies on the North Atlantic pilot (pothead) whale, *Globicephala melaena* (Traill, 1809). Physiol. Zool. 39: 110–116.

MOLLO, E., J. STEFANEHIK, N. PARIKH, AND B. J. NEWMAN. 1977. Improved method for the measurement of serum inorganic phosphate on the centrifichem fost analyzer. Clin. Chem. 23: 1163.

OKAHARA, Y. 1925. Untersuchen uber Cetacea XV. Uber die Zusammensetzung des Finwalblutes. Jpn. J. Med. Sci. II Biochem. 1: 43–50.

RODKEY, F. L. 1965. Direct spectrophotometric determination of albumin in human serum. Clin. Chem. 11: 478–487.

SILLEN, L. G. 1961. The physical chemistry of seawater. *In* Oceanography, M. Sears (ed.). Am. Assoc. Adv. Sci. Publ. Washington, D.C. 67: 549–581.

SKEGGS, L. T., AND H. HOCHSTRASSER. 1964. Multiple automatic sequential analysis. Clin. Chem. 10: 918–936.

SUDZUKI, M. 1924. Untersuchen uber Cetacea III. Uber das Blut. Tohoku J. Exp. Med. 5: 419–427.

TIFFANY, T. O., J. M. JANSEN, C. A. BURTIS, J. B. OVERTON, AND C. D. SCOTT. 1972. Enzymatic kinetic rate and end-point analysis of substrate by use of a GEMSAEC fort analyzer. Clin. Chem. 18: 829–840.