SERUM CHEMISTRY OF THE MINKE WHALE FROM THE NORTHEASTERN ATLANTIC

Authors: Tryland, Morten, and Brun, Edgar

Source: Journal of Wildlife Diseases, 37(2) : 332-341

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.2.332

Journal of Wildlife Diseases, 37(2), 2001, pp. 332–341

SERUM CHEMISTRY OF THE MINKE WHALE FROM THE NORTHEASTERN ATLANTIC

Morten Tryland,[1,3] **and Edgar Brun**[2]

[1]Department of Arctic Veterinary Medicine, the Norwegian School of Veterinary Science, N-9292 Tromsø, Norway.
[2]Section of Epidemiology, National Veterinary Institute, P.O. Box 8156 Dep., N-0033 Oslo, Norway.
[3]Corresponding author (e-mail: morten.tryland@veths.no)

ABSTRACT: Serum samples were collected from 42 harpooned minke whales (*Balaenoptera acutorostrata*) during commercial whaling off the coast of northern Norway (1997 and 1998) and analyzed for serum chemistry parameters in order to find clinical reference values for the northeastern Atlantic stock of this species. Mean and median values, as well as standard deviation and 90% central range, are presented for 28 different serum chemistry parameters. Lipemia is a common finding in marine mammals such as the minke whale, and chemical analysis of lipemic serum samples may produce artifacts. We found statistically significant elevated values of total protein, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium and chloride in strongly-lipemic compared to non-lipemic samples, all which may be artifacts due to interference of lipids with the methods used for analysis. In addition, we found significantly elevated levels of creatin kinase, lactate dehydrogenase (LDH), urea, uric acid and triglycerides, as well as a decrease in creatinine in the strongly lipemic samples. Reanalyzing serum samples after twelve mo storage at −20 C (n = 13) revealed reduction in the serum concentration of the enzymes ALT (42%), alkaline phosphatase (ALP; 10%), LDH (19%), gamma glutamyl transferase (17%) and amylase (11%), as well as for triglycerides (9%) and non-esterified fatty acids (16%). It is crucial that serum chemistry analysis is performed without delay after sampling. Possible changes in the values of some parameters due to the presence of high amounts of lipids or long term storage of samples must be considered when interpreting results from serum chemistry analysis in these animals.

Key words: *Balaenoptera acutorostrata,* baleen whale, cetacean, clinical serum chemistry, frozen sera, minke whale, rorqual.

INTRODUCTION

Serum chemistry data, combined with anamnestic information and clinical and necropsy findings, contributes to the understanding of diseases in marine mammals. To be able to interpret serum chemistry data from marine mammals, reference values for the species are crucial. Such information is available for several dolphin species that are kept in captivity (Gallien, 1970; Bossart and Dierauf, 1990). In addition, hematology and/or serum chemistry values have been reported for killer whales (*Orcinus orca*) (Cornell, 1983) and beluga (white) whales (*Delphinapterus leucas*) (Cornell et al., 1988), both toothed whales, and for the baleen species the bowhead whale (*Balaena mysticetus*) (Heidel et al., 1996) and the fin whale (*Balaenoptera physalus*) (Lambertsen et al., 1986).

Serum chemistry data has not been reported for the minke whale (*Balaenoptera acutorostrata*), the smallest of the family Balaenopteridae. The minke whale has a cosmopolitan distribution and is found from the polar ice edge to the tropics. Individuals belonging to the northeastern Atlantic stock migrate regularly southwards to breeding areas in the autumn and to feeding areas in the north in spring and early summer. The modern Norwegian minke whale hunt started in the late nineteen-twenties, and the hunt has been conducted by fishermen as a subsidiary source of income (see Christensen and Øien, 1990). In 1986, the International Whaling Commission (IWC) classified the northeastern Atlantic population of minke whales as a protection stock, and the commercial minke whale hunt was banned. The Norwegian hunt was provisionally stopped after 1987. A Norwegian marine mammal research program was conducted from 1988 to 1994, where questions concerning minke whale biology, stock sizes,

killing methods etc. were addressed. On the background on data collected during this period and from sightings surveys in 1995, the northeastern Atlantic stock of minke whales was estimated to approximately 112,000 individuals (Schweder et al., 1997). The Norwegian commercial minke whale hunt was resumed in 1993. Approximately 30 to 40 boats, most of them 10 to 12 m long have participated, and quotas of 580 and 671 individuals were given for the years 1997 and 1998, respectively.

Norwegian spring spawning herring is found to be one of the most important prey species for the minke whale in the sampled area, in addition to capelin, cod and other species (Haug et al., 1995). Prey species rich in lipids, like herring and capelin, may induce lipemia in the minke whales. This condition results in a whitish and opaque serum sample which may give false results in serum chemistry analysis (Benjamin, 1978; Bush, 1991). Samples obtained during commercial whaling have to be frozen aboard and stored until analysis can take place. Storage, especially long term storage, may cause changes in some serum chemistry parameters which have to be addressed (Hunter and Madin, 1978; Thoresen et al., 1995).

The aims of this study were to obtain information on serum chemistry parameters for the minke whale, which may serve as reference values for future studies. Further, we wanted to compare serum chemistry parameters in non-lipemic samples with samples with different degree of lipemia to see which parameters that may be affected. Finally, we wanted to investigate whether twelve mo storage of samples at −20 C had any effect on serum chemistry parameters of clinical importance.

MATERIALS AND METHODS

Sera were collected from 14 individuals in 1997 and from 28 individuals in 1998 during commercial whaling off the coast of Finnmark county (Norway; 70°00′–72°20′N, 25°00′–34°00′E), from the end of May to the beginning of July. A postmortem blood sample was taken during on-board necropsy within 1 hr after death. The blood was collected with a syringe, either from the heart or from a major blood vessel in the abdominal cavity, in order to avoid contamination with sea water, urine, fat, or intestine contents. The blood was transferred to a 10 ml plain vacutainer (Venoject, Terumo Corporation, Leuven, Belgium) and left to clot for approximately 60 min before preparation of serum by centrifugation. Serum was removed and frozen at −20 C until analysis, which took place a maximum of 7 wk after collection. Strongly lipemic samples were diluted from 1:5 to 1:20 prior to analysis. The samples were analyzed for the complete biochemical profile commonly used at Central Laboratory (The Norwegian School of Veterinary Science, Oslo, Norway). The serum analyses were conducted in a Technicon AXON System (Miles Inc., Tarrytown, New York, USA). The methods used to determine each parameter are listed in Table 1.

All parameters were analyzed with reagents from Bayer (Bayer Corporation, Tarrytown, New York, USA), except for glutamate dehydrogenase (GLDH; Boehringer Mannheim GmbH, Mannheim, Germany), free fatty acids (Wako Chemicals USA Inc., Richmond, Virginia, USA), bile acids (Nycomed Pharma AS, Oslo, Norway), chloride (Cl; Diagnostic chemicals limited, Oxford, Connecticut, USA), and iron (bioMerieux sa, Lyon, France). The serum samples were analyzed together with a standard (SETpoint™, Bayer) and controls (TESTpoint™ Assayed Chemistry Control 1 and 2, Bayer; Seronorm™, Nycomed Pharma). The lipemic status of the samples were evaluated with the Technicon AXON System. The samples were classified into four levels of lipemia according to lipid concentration (mg/dl): 0+ at <10; 1+ at ≥10 and <20; 2+ at ≥20 and <40; and 3+ at ≥40. Thirteen of the samples were reanalyzed 12 mo after the first analysis in order to evaluate the potential effect of twelve mo storage at −20 C.

All statistical analyses were performed with the SAS-PC System® Version 6.12 for Windows (SAS Institute Inc., Cary, North Carolina, USA; 1996). PROC UNIVARIATE was used to evaluate normality. The values from several of the parameters had to be transformed in order to achieve a normal distribution. Differences between groups were analyzed using variance analysis (PROC GLM) and all significant independent variables were included in the model. Differences in the values of parallels stored for 12 mo were performed as a one-sample *t*-test (PROC MEANS) on the respective differences being different from zero. Value of α was set as $P \leq 0.05$.

TABLE 1. Methods used to determine serum chemistry parameters in the minke whale.

Parameter	Method	References
AST[a]	IFCC[b] (without pyridoxal-5-phosphate)	Bergmeyer et al., 1978
ALT[a]	IFCC (without pyridoxal-5-phosphate)	Keiding et al., 1974
ALP[a]	SCE[c] (DEA buffer)	Keiding et al., 1974
CK[a]	IFCC (NAC activated)	Szasz et al, 1976
LDH[a]	SCE (Pyruvate → Lactate)	Keiding et al., 1974
GGT[a]	SCE	Szasz, 1976
GLDH[a]	DGKC[d]	Bergmeyer et al., 1970
Amylase	CNP-G3[e]	Winn-Deen et al., 1988
Lipase	kinetic UV turbidometric	Fossati et al., 1992
Protein (total)	biuret	Skeggs and Hochstrasser, 1964
Albumin	bromcresol green	Doumas, 1971
Globulin	(total protein—albumin)	
Urea	urease	Tiffany et al., 1972
Creatinine	alkaline picrate, kinetic	Rossignol et al., 1984
Uric acid	uricase	Fossati et al., 1980
Bile acids	enzymatic, colorimetric	Mashige et al., 1976
Bilirubin (total)	blanked diazo	Novros et al., 1979
Cholesterol	enzymatic, colorimetric	Allain, 1974
Triglycerides	enzymatic, colorimetric	Fossati and Prencipe, 1982
Non-esterified fatty acids	enzymatic, colorimetric	Wako NEFA C test kit
Glucose	hexokinase	Slein et al., 1950
Phosphorus	phosphomolybdate	Amador and Urban, 1972
Calcium	cresolphtalein	Gitelman, 1967
Magnesium	enzymatic	Fossati et al., 1989
Sodium	ion selective electrode	
Potassium	ion selective electrode	
Chloride	ferric thiocyanate	Schoenfeld and Lewellen, 1964
Iron	colorimetric	bioMerieux sa Ferrimat-Kit

[a] AST-aspartate aminotransferase; ALT-alanine aminotransferase; ALP-alkaline phosphatase; CK-creatin kinase; LDH-lactate dehydrogenase; GGT-gamma glutamyl transferase; GLDH-glutamate dehydrogenase.
[b] IFCC: According to the International Federation of Clinical Chemists.
[c] SCE: According to the Scandinavian Committee on Enzymes.
[d] DGKC: According to the Deutsche Gesellschaft für Klinische Chemie.
[e] CNP-G3: 2-chloro-4-nitrophenyl-α-maltotrioside.

RESULTS

All the animals were examined by a veterinary inspector aboard. No clinical signs of disease were observed and all were in good condition. Of the 14 animals caught in 1997, seven were males and seven were females, of which five were pregnant. Of the 28 animals caught in 1998, two were males and 26 were females, of which 17 were pregnant. Two of the nine males and nine of the 33 females were considered to be sexually immature due to their having body lengths less than 6.8 and 7.2 meters, respectively (Christensen, 1981) and in the females a lack of fetus or corpus luteum.

One serum sample having a Mg content of 3.43 mmol/l (8.3 mg/dl) was presumed to be contaminated with sea water (Lambertsen et al., 1986) and therefore excluded from further analysis. The other samples varied in Mg content from 0.79 mmol/l (1.92 mg/dl) to 2.27 mmol/l (5.51 mg/dl). One individual, a sexually mature but nonpregnant female, had a high serum content of creatinin (506 μmol/l; 5.7 mg/dl), more than twice the average found for the minke whales studied (204 μmol/l; 2.3 mg/dl). The serum samples were normally light yellow. One serum sample was rejected from analysis because of strong hemolysis, whereas seven samples had slight to moderate hemolysis as assessed by visual inspection. These samples had statistically significantly higher values of the pa-

rameters aspartate aminotransferase (AST) and creatin kinase (CK) compared to samples without hemolysis.

None of the sera collected in 1997 were lipemic, whereas 12 of the 28 sera collected in 1998 were categorized as moderately-lipemic (1+ and 2+) and 13 as strongly-lipemic (3+), the latter having a whitish and opaque appearance. Although a year-to-year variation in serum lipid content is demonstrated, lipemia seems to be quite normal in free-ranging minke whales during the intensive feeding period in the spring and summer. We therefore present mean and median values, as well as standard deviation and the 90% central range, of the measured parameters for non-lipemic, moderately-lipemic and strongly-lipemic serum samples respectively (Table 2) to indicate what kind of serum chemistry values that might be expected for minke whale serum samples with these characteristics.

Sera from females were more often and more severely lipemic (23 of 32; 72%) than sera from males (2 of 8; 25%), whereas no such differences were found between pregnant ($n = 21$) and non-pregnant ($n = 11$) females. Females ($n = 32$) showed significantly higher levels of creatinine ($P = 0.04$) and alkaline phosphatase (ALP; $P = 0.03$), and also had higher levels of calcium (Ca; $P = 0.07$) than males ($n = 8$). Non-pregnant females ($n = 11$) had higher levels of lipase and CK with P-values of 0.1 and 0.07, respectively, but lower levels of glucose than pregnant females ($n = 21$).

Comparing the parameters of the serum samples collected early during each hunt and thus stored for up to 7 wk before analysis with the latest samples obtained revealed no significant differences. None of the serum samples from the 13 minke whales that were caught and analyzed in 1997 and re-analyzed after 12 mo storage were lipemic, whereas three of these samples were slightly hemolytic. Comparing serum chemistry data before and after storage for 12 mo, with correction for hemolysis, indicated a statistically significant decrease in the concentration of the parameters alanine aminotransferase (ALT), ALP, LDH, gamma glutamyl transferase (GGT), amylase, triglycerides, and non-esterified fatty acids. These results are shown in Table 3.

Serum chemistry parameters of clinical interest that may be influenced by hemolysis, lipemia, and 12 mo storage at −20 C respectively are presented in Table 4.

DISCUSSION

The samples collected for this study are from wild individuals in their natural environment, and serum chemistry are thus not influenced by choice of food or other factors that may play a role in captivity. On the other hand, samples from dead harpooned animals is not optimal for serum chemistry analysis, and better sampling possibilities for the minke whale may be available in the future. When we compared the minke whale data obtained from the non-lipemic sera of 15 individuals (Table 2) with data obtained for a close relative of the minke whale, the fin whale (Lambertsen et al., 1986), and for the bowhead whale (Heidel et al., 1996), the serum chemistry values in general are within the same range. This, together with the good condition and lack of any sign of disease among the minke whales, makes the analysis seem sound and the values representative for the northeastern Atlantic stock of minke whales.

One of the non-pregnant females had a high creatinine content in serum, which may be an indication of kidney disease (Bossart and Dierauf, 1990). However, no other parameter pointed in the direction of renal insufficiency as were found in fin whales, where infection with the giant kidney worm (*Crassicauda boopis*) were assumed to be the cause (Lambertsen et al., 1986). It is possible that the explosive device used on the minke whales may cause hypovolemic shock, which may lead to elevated levels of creatinine as seen in dogs (Benjamin, 1978). Comparing creatinine levels with other reports from whales

TABLE 2. Serum chemistry values of non-lipemic (0+), moderately-lipemic (1+ and 2+) and strongly-lipemic (3+) serum samples from 40 minke whales. The data are presented as follows: mean (median) ± one standard deviation (90% central range).

Parameter	Non-lipemic sera: 0+ (n = 15)	Moderately-lipemic sera: 1+ and 2+ (n = 12)	Strongly-lipemic sera: 3+ (n = 13)
AST (U/l)	211 (167) ± 138 (110–681)	220 (209) ± 87 (109–440)	833 (427) ± 1,217 (48–4,711)
ALT (U/l)	19 (9) ± 28 (5–116)	15 (16) ± 7 (2–29)	81 (58) ± 87 (0–299)
ALP (U/l)	516 (566) ± 275 (158–1,043)	442 (421) ± 163 (147–690)	465 (437) ± 231 (222–1,032)
CK (U/l)	691 (346) ± 1,127 (44–4,435)	2,399 (482) ± 5,057 (49–17,924)	3,255 (1,426) ± 6,647 (167–24,920)
LDH (U/l)	617 (462) ± 505 (218–1,895)	1,234 (557) ± 1,989 (342–7,440)	1,630 (1,146) ± 1,692 (393–7,089)
GGT (U/l)	14 (9) ± 15 (4–67)	11 (9) ± 4.8 (6–22)	14 (13) ± 7.8 (5–35)
GLDH (U/l)	3.7 (0) ± 8.6 (0–32)	1.9 (0) ± 3 (0–9)	12.6 (0) ± 22 (0–69)
Amylase (U/l)	166 (171) ± 29 (110–226)	131 (127) ± 31 (81–187)	144 (142) ± 25 (94–187)
Lipase (U/l)	43 (34) ± 31.2 (0–119)	10 (10) ± 7.9 (0–23)	28 (22) ± 16 (8–56)
Protein, total (g/l)	66 (65) ± 6 (57–78)	66 (66) ± 5.8 (55–77)	84 (84) ± 8.1 (70–96)
Albumin (g/l)	34 (33) ± 3.2 (27–40)	29 (29) ± 2.6 (26–35)	33 (34) ± 3.5 (28–40)
Globulin (g/l)	32 (31) ± 4.5 (25–44)	37 (38) ± 4.2 (29–42)	50 (49) ± 7.4 (42–66)
Urea (mmol/l)	23 (22) ± 4.7 (15–31)	26 (26) ± 2.4 (22–30)	26 (26) ± 2.2 (23–30)
Creatinine (μmol/l)	204 (147) ± 110 (121–506)	157 (140) ± 40 (116–250)	165 (152) ± 37 (110–244)
Uric acid (μmol/l)	145 (138) ± 79 (59–380)	192 (204) ± 57 (83–272)	305 (305) ± 57 (182–399)
Bile acids (μmol/l)	13 (10) ± 8.6 (5–30)	15 (8.5) ± 22 (0–79)	9.3 (8) ± 7.9 (0–24)
Bilirubin, total (μmol/l)	0.7 (1) ± 0.5 (0–1)	0.5 (0.5) ± 0.5 (0–1)	0.7 (1) ± 0.6 (0–2)
Cholesterol (mmol/l)	11 (10) ± 2.3 (8–17)	11 (11) ± 1.2 (8–12)	10 (10) ± 1.4 (7.7–12)
Triglycerides (mmol/l)	3.2 (2.7) ± 1.4 (2–7.4)	3.9 (3.5) ± 1.4 (2.4–6.1)	5.6 (5.5) ± 1.8 (2.3–8.7)
Non-esterified fatty acids (mmol/l)	0.4 (0.3) ± 0.4 (0.1–1.7)	0.1 (0.1) ± 0.1 (0–0.3)	0.4 (0.4) ± 0.2 (0.2–0.9)
Glucose (mmol/l)	10 (8.2) ± 5.4 (5.3–22)	5.6 (7.9) ± 2.6 (4.6–13)	10 (8.3) ± 4.6 (5.8–22)
Phosphorus (mmol/l)	4.1 (3.4) ± 1.7 (2.4–9.3)	3.8 (3.4) ± 1.1 (2.5–6)	4.3 (4.2) ± 0.7 (3–5.4)
Calcium (mmol/l)	2.6 (2.5) ± 0.4 (2.1–3.6)	2.5 (2.5) ± 0.2 (2.1–2.9)	2.6 (2.7) ± 0.2 (2.3–3)
Magnesium (mmol/l)	1.3 (1.2) ± 0.3 (0.8–2.3)	1.2 (1.2) ± 0.2 (0.9–1.5)	1.4 (1.2) ± 0.4 (0.9–2.3)
Sodium (mmol/l)	158 (156) ± 7.4 (146–169)	167 (168) ± 8.1 (155–183)	165 (164) ± 7.1 (154–176)
Potassium (mmol/l)	8.2 (7) ± 3.6 (4.8–17)	8.1 (8.2) ± 1.8 (5.3–11)	8.4 (8.3) ± 2.6 (5.2–14)
Chloride (mmol/l)	113 (109) ± 9.5 (101–130)	116 (117) ± 5.5 (108–124)	127 (128) ± 8.1 (109–139)
Iron (μmol/l)	37 (35) ± 9.9 (23–54)	29 (28) ± 10 (15–49)	36 (39) ± 10 (16–45)

TABLE 3. Change in serum chemistry values of 13 minke whales after 12 mo storage of serum samples at −20 C.

Parameter	Mean decrease	% decrease	Standard error	*P*-value (Student *t*-test)
ALT	8 U/l	42	0.05	0.00
ALP	61 U/l	10	0.03	0.00
LDH	199 U/l	19	0.08	0.04
GGT	3 U/l	17	0.04	0.00
Amylase	21 U/l	11	0.03	0.00
Triglycerides	0.4 mmol/l	9	0.03	0.01
Non-esterified fatty acids	0.1 mmol/l	16	0.06	0.01

killed with explosive devices, the mean creatinine level in the minke whales (2.3 mg/dl) were higher than recorded for the fin whales (1.3 mg/dl) (Lambertsen et al., 1986), but considerably lower than reported for the bowhead whales (4.6 mg/dl) (Heidel et al., 1996).

Hemolysis is reported to elevate the levels of AST, LDH, inorganic P, and to some extent glucose in serum (Lambertsen et al., 1986; Bossart and Dierauf, 1990; Bush, 1991) and decrease Na level (Lambertsen et al., 1986; Heidel et al., 1996). In this study, seven of the 40 serum samples had slight to moderate hemolysis with a statistically significant elevation of AST. Additionally, a significant elevation of CK were observed in the sera with hemolysis (Table 4).

The protein profile (total protein, albumin, and globulin) showed only little variation compared with the fin whale and the bowhead whale. This also is the situation for the minerals P, Ca, Na, K, Cl, and Mg. The mean amount of Mg in serum of the minke whales was identical (1.3 mmol/l) with that of the fin whales (Lambertsen et al., 1986). Glucose levels in sera from min-

TABLE 4. Serum chemistry parameters for the minke whale of clinical interest that may be increased (↑) or decreased (⇩) by moderate hemolysis, moderate and strong lipemia, and 12 mo storage at − 20 C, respectively.

	Effect of:			
Parameter	Hemolysis (n = 7)	Moderate lipemia (1+ and 2+, n = 12)	Strong lipemia (3+; n = 13)	Storage (n = 13)
AST	↑		↑[a]	
ALT			↑[a]	⇩
ALP				⇩
CK	↑	↑	↑	
LDH			↑	⇩
GGT				⇩
Amylase				⇩
Protein			↑[a]	
Albumin				
Globulin		↑[a]	↑[a]	
Urea		↑	↑	
Creatinine		⇩	⇩	
Uric acid		↑	↑	
Triglycerides			↑	⇩
Non-esterified fatty acids				⇩
Sodium		↑[a]	↑[a]	
Chloride		↑[a]	↑[a]	

[a] Possible artificial elevation due to interference of lipids in the samples with the methods used.

ke whales were about twice as high (mean 10.2 mmol/l; 185 mg/dl) as that reported for the killer whale (6.5 mmol/l; 118 mg/dl), the beluga whale (5.7 mmol/l; 104 mg/dl) and the bowhead whale (4.8 mmol/l; 87 mg/dl), respectively (Cornell, 1983; Cornell et al., 1988; Heidel et al., 1996). Feeding normally raises glucose levels, and high amounts of glucose are reported to be a characteristic of lipemic sera (Bush, 1991; Duncan et al., 1994). The glucose levels in our data, however, showed a marked individual variation, most likely reflecting the degree of fasting and/or exertion prior to death and sampling, and the expected correlation with lipid content was not present.

Significantly higher serum concentrations of creatinine, ALP, and Ca were found in females ($n = 32$) compared to males ($n = 8$). This is not in agreement with Heidel et al. (1996), who reported lower creatinine levels and higher glucose in female bowhead whales compared to males. In our study, pregnant whales had significantly lower serum concentrations of lipase and CK but higher levels of glucose compared to non-pregnant whales. Although the results are based upon a restricted number of individuals, the differences in levels of lipase and glucose may reflect a generally increased energy consumption and metabolism in pregnant individuals to meet the metabolic demands of the new tissues that are produced. In bowhead whales, higher levels of total protein, albumin and triglycerides were found in pregnant whales (Heidel et al., 1996), whereas no significant differences for these parameters could be found in our data. The differences may also be influenced by age, since nine of the 11 non-pregnant whales were young and sexually immature individuals compared to the older and pregnant whales. Young captive killer whales had higher glucose levels than older individuals (Cornell et al., 1983), whereas in belugas, older individuals were reported to have higher levels of cholesterol and triglycerides and lower levels of total protein, albumin and ALP than younger individuals (Cornell et al., 1988). Altogether, our data does not fit well with previous reports on the influence of pregnancy and age on serum chemistry parameters, and no firm conclusions can be drawn.

Harpoons with explosive devices often cause severe muscle damage and enzymes from muscle tissue may enter into the blood. We found great individual variation in the serum concentrations of the enzymes AST and CK (Table 2), enzymes that usually increase during muscle disease or trauma (Bossart and Dierauf, 1990; Duncan et al., 1994). We also found high serum levels and a similar variation for the enzyme LDH. Individuals with high serum levels of AST and CK also had high levels of LDH. In marine mammals, LDH is reported to leak from skeletal muscles during diving and to have a great individual variation (Bossart and Dierauf, 1990). In general, high levels of this enzyme may be associated with cell damage or necrosis. The huge individual variation of AST, CK and LDH in serum from the minke whales may thus reflect abnormalities and be a result of the killing methods as suggested by Heidel et al. (1996). Unfortunately, data on the time from the harpoon entering the body to death (survival time) of the minke whales included in this study were not available, and we do not know whether there exists a correlation between the enzyme concentrations and the survival time. Lambertsen et al. (1986) could not find positive correlations between capture efforts (chase time) and any of the serum chemistry parameters measured on fin whales, but AST, CK, and LDH were not measured and a comparison with this species is therefore not possible. However, the levels of AST and CK in sera from bowhead whale, caught with explosive devices on hand-thrown harpoons (Heidel et al., 1996), were much higher than in sera from two toothed whale species, the killer whale (Cornell, 1983) and the beluga

whale (Cornell et al., 1988), which were sampled in captivity.

Serum samples from minke whales are often lipemic. Hypothyroidism and diabetes mellitus are disorders that may cause secondary hyperlipidemia in dogs (Bush, 1991). Since we have found no reports concerning such disorders in whales, the lipemia in the present material is regarded as exogenous lipids from diets rich in lipids such as herring and capelin, and not of secondary nature due to disease. Dietary lipids gives rise to the formation of chylomicra which are transported in the blood. In large amounts these chylomicra may cause a visible whitish and opaque appearance of serum samples. Since the serum chemistry parameters are measured photometrically, the degree of aberration caused by lipemia may be proportional to its intensity (Anonymous, 1994). The manufacturer of the Technicon AXON System analyzer suggests that the lipids in samples defined as 2+ (moderately-lipemic) may potentially interfere with one or more parameters, whereas in samples defined as 3+ (strongly-lipemic), significant interference may arise. We found a yr-to-yr difference in the presence of lipemia that may reflect differences in prey availability for the minke whales. Strongly-lipemic samples had statistically significant elevations of AST, ALT, CK, LDH, total protein, globulin, urea, uric acid, triglycerides, Na and Cl, and a depression of creatinine compared to non-lipemic samples (Table 4). While some of these characteristics should be regarded as real differences in chemistry levels, the level of total protein may be artificially elevated in strongly lipemic samples (Benjamin, 1978; Bush, 1991; Anonymous, 1994), which in turn also may give falsely high levels of globulin (globulin = total protein − albumin). The elevated levels of Na and Cl in moderate- and strongly-lipemic samples compared to non-lipemic samples may be artificial due to interference of lipids with the methods used (Anonymous, 1994), which is also the case for AST and ALT (Bossart and Dierauf, 1990). The elevated levels of triglycerides in strongly-lipemic samples were expected (Bossart and Dierauf, 1990).

A decrease in enzyme activity related to storage time has been reported for ALT, ALP, GLDH and amylase in sera from dogs that were stored for 90 and 240 days (Thoresen et al., 1995), and for the enzymes ALT, ALP, AST and LDH in sera from northern fur seals after storage of sera for 107 to 166 days (Hunter and Madin, 1978), and decreased enzyme activity was considerably greater for the longest periods studied compared to the shortest. No significant changes in enzyme activity could be demonstrated when comparing samples that were stored for up to 7 wk with samples obtained by the end of each hunt and thus stored for a shorter period of time. However, when reanalyzing samples stored at −20 C for twelve mo, statistically significant decrease of the enzymes ALT, ALP, LDH, GGT and amylase, as well as for triglycerides and non-esterified fatty acids were demonstrated (Table 4). As far as we know, decreases in triglycerides and non-esterified fatty acids during storage have not been previously reported. In our study these parameters decreased with 9% and 16% respectively, but due to the wide range recorded in the minke whale depending on the feeding situation previous to sampling, these changes should not be regarded as clinically important. For the enzymes ALT, ALP, LDH, GGT and amylase, however, it is obvious that twelve mo storage of samples at −20 C may give falsely depressed levels of clinical importance.

This study shows that lipemia may be a normal condition in the minke whale during the intensive feeding period on prey species rich in lipids in the spring and summer, and that serum chemistry analysis of moderate and strongly lipemic samples may give falsely elevated levels of a number of chemistry parameters. Due to the changes caused by long term storage, serum chemistry analysis should be con-

ducted without delay after sampling, and results from analysis conducted on long term stored frozen samples should be interpreted with care.

ACKNOWLEDGMENTS

We thank T. Haug, K. T. Nilssen, N. E. Skavberg, J. Teigland and T. Våg for help during sampling of the whales, and the crew of the whaling vessels for patience and cooperation. We also thank H. Morberg and D. Griffiths for assistance in interpreting the results and for providing constructive comments on the manuscript, respectively.

LITERATURE CITED

ALLAIN, C. C., L. S. POON, C. S. G. CHAN, W. RICHMOND, AND P. C. FU. 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry 20: 470–475.

AMADOR, E., AND J. URBAN. 1972. Simplified serum phosphorus analyses by continuous-flow ultraviolet spectrophotometry. Clinical Chemistry 18: 601–604.

ANONYMOUS. 1994. Technicon AXON System. Methods manual, publication No. TH9-4720-00. Miles Inc., Tarrytown, New York, 249 pp.

BENJAMIN, M. M. 1978. Outline of veterinary clinical pathology. 3rd Edition. The Iowa State University Press, Ames, Iowa, 351 pp.

BERGMEYER H. U., H. BÜTTNER, G. HILLMANN, F. H. KREUTZ, H. LANG, D. LAUE, W. RICK, E. SCHMIDT, F. W. SCHMIDT, D. STAMM, AND G. SZASZ. 1970. (Die Kommisionen für Enzymdiagnostik und Standardisierung). Empfehlungen der Deutschen Gesellschaft für Klinische Chemie: Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. Zeitschrift für Klinische Chemie und Klinische Biochemie (Berlin) 8: 658–660.

———, M. HØRDER, AND D. W. MOSS. 1978. (International Federation of Clinical Chemistry, Commitee on Standards, Expert Panel on Enzymes). Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes, Part 3, Revised IFCC method for aspartate aminotransferase. Clinical Chemistry 24: 720–721.

BOSSART, G. D., AND L. A. DIERAUF. 1990. Marine mammal clinical laboratory medicine. *In* CRC handbook of marine mammal medicine: Health, disease, and rehabilitation, L. A. Dierauf (ed.). CRC Press, Boca Raton, Florida, pp. 1–52.

BUSH, M. 1991. Interpretation of laboratory results for small animal clinicians, Blackwell Scientific Publications, Oxford, UK, 515 pp.

CHRISTENSEN, I. 1981. Age determination of minke whales, *Balaenoptera acutorostrata,* from laminated structures in the tympanic bullae. Report of the International Whaling Commission 31: 245–253.

———, AND N. ØIEN. 1990. Operational patterns of the Norwegian minke whale fishery. Report of the International Whaling Commission 40: 343–347.

CORNELL, L. H. 1983. Hematology and clinical chemistry values in the killer whale, *Orcinus orca* L. Journal of Wildlife Diseases 19: 259–264.

———, D. S. DUFFIELD, B. E. JOSEPH, AND B. STARK. 1988. Hematology and serum chemistry values in the Beluga (*Delphinapterus leucas*). Journal of Wildlife Diseases 24: 220–224.

DOUMAS, B. T., W. A. WATSON, AND H. G. BIGGS. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta 31: 87–96.

DUNCAN, J. R., K. W. PRASSE, AND E. A. MAHAFFEY. 1994. Veterinary Laboratory Medicine. Iowa State University Press, Ames, Iowa, 300 pp.

FOSSATI, P., L. PRENCIPE, AND G. BERTI. 1980. Use of 3,5-Dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinical Chemistry 26: 227–231.

———, AND ———. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry 28: 2077–2080.

———, M. SIRTOLI, G. TARENGHI, M. GIACHETTI, AND G. BERTI. 1989. Enzymatic assay of magnesium through glukokinase activation. Clinical Chemistry 35: 2212–2216.

———, M. PONTI, P. PARIS, G. BERTI, AND G. TARENGHI. 1992. Kinetic colorimetric assay of lipase in serum. Clinical Chemistry 38: 211–215.

GALLIEN, C. L., M. T. CHALUMEAU-LE-FOULGOC, AND J. M. FINE. 1970. Comparative study of serum proteins in four dolphin species (Cetacesodontocetes). Comparative Biochemistry and Physiology 37: 375–385.

GITELMAN, H. J. 1967. An improved automated procedure for the determination of calcium in biological specimens. Analytical Biochemistry 18: 521–531.

HAUG, T., H. GJØSÆTER, U. LINDSTRØM, K. T. NILSSEN, AND I. RØTTINGEN. 1995. Spatial and temporal variations in northeast Atlantic minke whale *Balaenoptera acutorostrata* feeding habits. *In* Developments of marine biology, 4: Whales, seals, fish and man, A. S. Blix, L. Walløe, Ø. Ulltang (eds.). Elsevier Science B. V., Amsterdam, The Netherlands, pp. 225–239.

HEIDEL, J. R., L. M. PHILO, T. F. ALBERT, C. B. ANDREASEN, AND B. V. STANG. 1996. Serum chemistry of bowhead whales (*Balaena mysticetus*). Journal of Wildlife Diseases 32: 75–79.

HUNTER, L., AND S. H. MADIN. 1978. Clinical blood values of the northern fur seal, *Callorhinus ur-*

sinus. II. Comparison of fresh versus stored frozen serum. Journal of Wildlife Diseases 14: 116–119.

KEIDING, R., M. HÖRDER, W. GERHARDT, E. PITKÄNEN, R. TENHUNEN, J. H. STRÖMME, L. THEODORSEN, J. WALDENSTRÖM, N. TRYDING, AND L. WESTLUND. 1974. (The Commitee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology). Recommended methods for the determination of four enzymes in blood. Scandinavian Journal of Clinical Laboratory Investigation 33: 291–306.

LAMBERTSEN, R. H., B. BIRNIR, AND J. E. BAUER. 1986. Serum chemistry and evidence of renal failure in the North Atlantic fin whale population. Journal of Wildlife Diseases 22: 389–396.

MASHIGE, F., K. IMAI, AND T. OSUGA. 1976. A simple and sensitive assay of total serum bile acids. Clinical Chimica Acta 70: 79–86.

NOVROS, J. S., T. R. KOCH, AND E. C. KNOBLOCK. 1979. Improved method for accurate quantitation of total and conjugated bilirubin in serum. Clinical Chemistry 25: 1891–1899.

ROSSIGNOL, B., D. ROSSIGNOL, AND C. PETITCLERC. 1984. Improvement of creatinine measurement on RA-1000. Clinical Biochemistry 17: 203–204.

SCHOENFELD, R. G., AND C. J. LEWELLEN. 1964. A colorimetric method for determination of serum chloride. Clinical Chemistry 10: 533–539.

SCHWEDER T., H. J. SKAUG, X. K. DIMAKOS, M. LANGAAS, AND N. ØIEN. 1997. Abundance of northeastern Atlantic minke whales, estimates for 1989 and 1995. Report of the International Whaling Commission 47: 453–484.

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

SLEIN M. W., G. T. CORI, AND C. F. CORI. 1950. A comparative study of hexokinase from yeast and animal tissues. Journal of Biological Chemistry 186: 763–780.

SZASZ, G. 1976. Reaction-rate method for γ-glutamyltransferase activity in serum. Clinical Chemistry 22: 2051–2055.

———, W. GRUBER, AND E. BERNT. 1976. Creatin kinase in serum: 1. Determination of optimum reaction conditions. Clinical Chemistry 22: 650–656.

THORESEN, S. I., A. TVERDAL, G. HAVRE, AND H. MORBERG. 1995. Effects of storage time and freezing temperature on clinical chemical parameters from canine serum and heparinized plasma. Veterinary Clinical Pathology 24: 129–133.

TIFFANY, T. O., J. M. JANSEN, C. A. BURTIS, J. B. OVERTON, AND C. D. SCOTT. 1972. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC Fast Analyzer. Clinical Chemistry 18: 829–840.

WINN-DEEN E. S., H. DAVID, G. SIGLER, AND R. CHAVEZ. 1988. Development of a direct assay for α-amylase. Clinical Chemistry 34: 2005–2008.

Received for publication 25 August 1999.