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HEMATOLOGY AND SERUM CHEMISTRY OF HARP (*PHOCA GROENLANDICA*) AND HOODED SEALS (*CYSTOPHORA CRISTATA*) DURING THE BREEDING SEASON, IN THE GULF OF ST. LAWRENCE, CANADA

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ABSTRACT: Standard hematologic and serum chemistry parameters were determined from 28 harp seals (*Phoca groenlandica*) and 20 hooded seals (*Cystophora cristata*) sampled from 6 March 2001 to 13 March 2001 during the breeding season. Whole blood was collected immediately postmortem from harp seal mother–pup pairs and from six hooded seal pups, and from live-captured adult hooded seals and three hooded seal pups; blood was analyzed within 24 hr at a local human hospital. A certified veterinary laboratory validated subsamples of whole blood and analyzed all serum chemistry parameters. Significant interlaboratory differences in mean values of packed cell volume (PCV) and mean cell volume (MCV) were found. Significant differences were found between samples from the five seal groups (adult male hooded seals, lactating female hooded seals, unweaned hooded seal pups; lactating female harp seals, and unweaned harp seal pups) for hematology and most serum chemistry parameters. In general, age-class influenced mean values of PCV, hemoglobin (HB), red blood cell (RBC) counts, MCV, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and nucleated red blood cell (NRBC) counts per 100 leucocytes, but most age-related variations were species specific. Harp seal pups had significantly lower mean values of HB, PCV, MCH, and MCHC than did other seal groups, and significantly lower mean RBC counts than did hooded seal pups. Mean NRBC counts per 100 leukocytes were more than three times higher in harp seal pups than in hooded seal pups, but this difference was not statistically significant. Mean MCV were significantly lower in harp and hooded seal pups compared to those of adult harp and hooded seals. Differences in hemograms between pup species were likely because of the precocious development of hooded seal pups, which are weaned within 4 days, compared to 12 days for harp seal pups. Among adult seal groups, male hooded seals had significantly higher mean values of PCV and HB than did female harp and hooded seals, and significantly higher mean RBC counts than did adult female hooded seals. Among adult females, mean values of MCH and MCHC were statistically higher in hooded seals than in harp seals. Adult female harp and hooded seals did not differ significantly in other RBC parameters and mean leukocyte counts. Mean values of glucose, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, and albumin showed species-specific variations between adults and pups. Except for ALP, few significant differences in mean enzyme activities of aspartate aminotransferase (AST), ALT, creatine kinase and γ-glutamyltransferase were found between seal groups. Mean concentrations of electrolytes (calcium, phosphorus, sodium, potassium, chloride, magnesium, and total carbon dioxide) varied with age class, but variations in potassium and magnesium were species specific. Harp seal pups had significantly higher mean phosphorus and potassium levels compared to other seal groups.

Key words: Blood, breeding, *Cystophora cristata*, harp seal, hematology, hooded seal, *Phoca groenlandica*, serum chemistry.

INTRODUCTION

Hematology and serum chemistry are essential tools for assessing health in humans and animals. Among humans and domestic animals, samples may be drawn from clinically healthy individuals and reference values established. In field studies however, collecting blood from marine mammals and access to laboratory facilities are both problematic. Therefore, most published hematology and serum chemistry values are from captive or stranded animals, which result in biases

in sampling and analyses (Bossart and Dierauf, 1990; Gulland, 1999). Recently, the number of strandings and extralimital records of ice-breeding harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) have been increasing in the North Atlantic (McAlpine et al., 1999; Mignucci-Giannoni and Haddow, 2002; Lucas et al., 2003); however, few baseline values exist for these two species in their natural environment.

The harp seal is a medium-sized (100–130 kg) phocid of the North Atlantic (Innes et al., 1981; Chabot et al., 1996) with an estimated population of 5.2 million in the Northwest Atlantic (Healey and Stenson, 2000). Harp seals summer in the Canadian Arctic and along the Greenland coast and migrate south in the late fall to overwinter off the northeast coast of Newfoundland ("Front" herd), or in the Gulf of St. Lawrence ("Gulf" herd) (Sergeant, 1991). The hooded seal is a large, sexually dimorphic phocid (females: 187–230 kg; males: 257–334 kg), with an estimated population of 627,000 in the Northwest Atlantic (Hammill et al., 1997; Hammill and Stenson, 2000). They also migrate south from the Arctic in late fall to reproduce off the southeastern coast of Labrador and northern Newfoundland ("the Front") and in the southern Gulf of St. Lawrence (Hammill et al., 1992; Hammill, 1993).

Although both species are pelagic, seasonal migrants, and whelp on the drifting pack ice in March, they have evolved different reproductive strategies to cope with their transient whelping sites. Harp seals give birth earlier in March and their pups are smaller than those of hooded seals (Lydersen and Kovacs, 1999). Harp seal pups are born with white fur (lanugo) and no blubber. Lactation lasts approximately 12 days and pups gain approximately 2 kg/day, while the lactating females lose approximately 3 kg/day of body mass (Kovacs and Lavigne, 1985; Lydersen and Kovacs, 1996). Hooded seal pups are born approximately 2 wk later than harp seal pups, shed their lanugo *in utero*, and possess blubber at birth (Oftedal et al., 1991; Lydersen and Kovacs, 1999). Hooded seal pups are weaned in 4 days, with an average mass gain of approximately 7 kg/day, while their mothers lose approximately 10 kg/day (Bowen et al., 1985; Kovacs and Lavigne, 1992). After weaning, harp and hooded seal pups fast for 4 to 6 wk (Worthy and Lavigne, 1983; Bowen et al., 1987).

Because of their pelagic habits and remote breeding sites on drifting ice, limited hematology and serum chemistry data are available for wild harp and hooded seals. Geraci (1971), St. Aubin and Geraci (1977), and Nordøy and Thoresen (2002) published hematology, liver enzyme, and serum chemistry data, respectively, from wild harp seals sampled during the breeding season. Kavtsevich (2001) provided relative differential leukocyte counts in wild harp seals of various age classes and body conditions from northern Europe. In wild hooded seals sampled during the breeding season, Cabanac (2000) determined blood and plasma volume, "hematocrit" (packed cell volume, or PCV), and hemoglobin (HB) values of seals of both sexes and various ages, and Mellish and Iverson (2001) published blood urea nitrogen (BUN) and two other metabolites in adult females. Other studies were conducted on captive harp (Ronald et al., 1969; Vallyathan et al., 1969; Geraci and Engelhardt, 1974; St. Aubin et al., 1979; Worthy and Lavigne, 1982; Engelhardt, 1979; Medway and Geraci, 1986; Bossart and Dierauf, 1990; Reidarson et al., 2000) or hooded seals (Clausen and Ersland, 1969; Keiver et al., 1987; Bossart and Dierauf, 1990), often involving few seals or parameters. The objectives of the present study are to provide reference or baseline hematology and serum chemistry values in wild harp and hooded seals during the breeding period, and to examine the biological significance of these values in two different ice-breeding phocids.

MATERIALS AND METHODS

Seals were sampled opportunistically on the ice near the Magdalen Islands (47°23′N, 61°52′W) in the Gulf of St. Lawrence, Canada, from 6 March 2001 to 13 March 2001 during directed research activities, with a permit issued by Fisheries and Oceans Canada. All harp seals and most hooded seal pups were killed according to approved Canadian sealing methods (see Daoust et al., 2002). Blood was collected immediately postmortem from severed blood vessels of the head or neck of seals killed by gunshot (adults) or by a blow to the head (pups). Seals were measured and weighed. Adult hooded seals and three pups were live-captured on the ice using nets and blood was drawn from the epidural vertebral vein using an 18-gauge needle mounted on a 10-ml syringe for adult seals or a 20-gauge needle and Vacutainer® (Becton Dickinson, Franklin Lakes, New Jersey, USA) for pups. Blood was collected into untreated and potassium ethylenediaminetetracetic acid (K_3 EDTA)-treated Vacutainers® for serum chemistry and hematology, respectively. Some adult hooded seals were tranquilized with 0.35–0.45 mg/kg intravenous or intramuscular tiletamine–zolazepam (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) before being handled. Seals were classified as adults based on their presence in the breeding area and attending pups. Unweaned pups were identified by observation of nursing behavior. The heart, liver, lungs, and digestive tract of most seals were examined for parasites and gross lesions.

Blood samples were taken in the midmorning to midafternoon, kept between 5 C and 15 C in a cooler, and processed later in the day in a field laboratory. For serum chemistry determinations, clotted blood was centrifuged at 1228 × G for 20 min; the serum was collected in cryogenic vials and immediately frozen at −20 C, then later at −80 C until analyzed in a certified veterinary laboratory at the Faculté de médecine vétérinaire de l'Université de Montréal (FMV), Québec, Canada, 5 mo later with a Synchron CX®5 (Beckman Instruments Inc, Fullerton, California, USA). Estimated time from blood sampling to centrifugation ranged from 2 to 10.5 hr. For hematology determination, thin blood smears were made in the field laboratory and air-dried, and two to three heparinized microhematocrit capillary tubes were filled with whole blood. Microhematocrit capillary tubes were centrifuged for 10 min at 12,850 × G and PCV read using a microcapillary reader (I.E.C©. Cat. No. 2201, Damon/I.E.C. Division, Needham Heights, Massachussetts, USA). Readings were averaged for each seal. For hemograms, a sample of whole blood from each seal was analyzed within 24 hr of collection at a local human hospital, the Centre Hospitalier de l'Archipel (CHA) in Cap-aux-Meules on the Magdalen Islands, Québec, Canada, by impedance using a Sysmex™ NE-1500 (TOA Medical Electronics Co. Ltd., Kobe, Japan). Subsamples of whole blood from seven seals were shipped on ice packs, with blood smears, to the FMV laboratory for validation by impedance and flow cytometry with a Cell-Dyn® 3500 (Abbott Laboratories, North Chicago, Illinois, USA) and analyzed 2 (four samples) to 5 days (three samples) after sampling. Automated analyzers determined red (RBC) and white (WBC) blood cell counts, HB, and mean cell volume (MCV), and calculated hematocrit (HCT), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Because of falsely elevated WBC counts obtained from the human hospital, WBC counts were estimated from smears using a microscope (250× or 400×). The average number of WBC within 10 fields was multiplied by the square of the magnification of the objective, and the result divided by 1,000 to obtain the number of WBC in 10^9/l units. The average of two to three WBC counts was calculated for each seal. Although WBC counts estimated from smears ($\#WBC_{smear}$) were highly correlated with those obtained from subsamples sent to the FMV laboratory ($r=0.93$, $P< 0.01$), these $\#WBC_{smear}$ were lower ($t=3.72$, $P<0.01$) than those from the FMV laboratory. Therefore, $\#WBC_{smear}$ were corrected using parameter estimates from the linear regression: corrected $WBC=1.019(\#WBC_{smear})+1.154$. Relative differential WBC counts (%) were determined manually using a microscope (500×) from blood smears stained with Wright–Giemsa, and the percentage of each cell type determined. Absolute differential WBC counts were obtained by multiplying relative WBC counts (%) with corrected WBC counts.

Statistical analyses were performed using SAS® software (SAS; Statistical Analysis System, Release 8.2, Cary, North Carolina, USA). Interlaboratory differences in HCT, HB, MCV, RBC counts, MCH, and MCHC were assessed with *t*-tests. Effect of species (harp versus hooded seals), age class (unweaned pup versus adult), and sex (male versus female), and interactions between these effects, were analyzed with two-way analyses of variance (ANOVAs). Blood and serum parameters were also analyzed by one-way ANOVAs on seal groups (hooded seals: adult males, adult

females, pups; harp seals: adult females, pups). We used SAS/LAB® to determine the best response scaling and to meet the underlying assumptions for ANOVAs. Tukey's studentized range tests determined which group means differed significantly. Nonparametric ANOVAs on ranked data (indicated by F_{rk}) were used when transformations failed to meet the conditions of normality of the residuals and homogeneity of variances. The significance level was set at $\alpha=0.05$ for all analyses. Five data values, which differed from the mean by more than three standard deviations, were considered outliers and excluded from tables and calculations. Values from two hooded seals were excluded from statistical tests and Table 1 for parameters sensitive to hemolysis (PCV, RBC counts, MCH, MCHC, glucose, total bilirubin, enzymes, phosphorus, potassium, and magnesium).

RESULTS

Hematology

Packed cell volumes were determined for 28 harp and 20 hooded seals. Standard hematology values such as HCT, HB, RBC counts, MCV, MCH, MCHC, WBC counts, and the differential counts and proportions of neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E) and basophils (B) were determined from 42 of the 48 seals (Tables 1, 2). Platelet determinations were excluded from analyses because of observed platelet aggregation on blood smears. Two data values from two adult harp seals were considered outliers: proportion of basophils=1.5%, and MCHC=563 g/l; these were excluded from Table 2 and statistical tests. One harp seal pup with low HCT (0.25 l/l) and RBC count (1.96×10^{12}/l), and high reticulocyte count (90.7×10^{9}/l), was considered anemic and was excluded from statistical analyses and Table 2 for all parameters. Significant differences between laboratories were only found for HCT ($t=4.11$; $P<0.01$) and MCV ($t=8.05$; $P<0.01$). Packed cell volumes determined in the field laboratory were higher than HCT from the CHA laboratory ($t=3.14$; $P=0.02$) but lower than those from the FMV laboratory ($t=3.33$; $P<0.05$). These differences are likely because of time delays postsampling between laboratories and different methodologies (manual versus automated). Therefore, PCV were used for statistical analyses and tables.

Packed cell volume ($F=29.22$, $P<0.01$) and HB ($F=42.93$, $P<0.01$) differed between seal groups. Highest mean PCV and HB were recorded from adult male hooded seals, and the lowest values from harp seal pups. Adult female harp and hooded seals and hooded seal pups had comparable mean PCV and HB levels (Tables 1, 2). Effects of species, age class, sex, and interactions between these effects, were significant for HB and PCV. Seal groups also differed in mean RBC counts ($F=5.54$, $P<0.01$). Hooded seal pups had higher mean RBC counts than did harp seal pups, and adult male hooded seals had higher mean RBC counts than did adult female hooded seals (Tables 1, 2). Mean RBC counts were influenced by sex ($F=9.47$, $P<0.01$) and interactions of age class with species ($F=17.08$, $P<0.01$) and with sex ($F=12.13$, $P<0.01$). Mean cell volume differed between adult seals and pups ($F=12.62$, $P<0.01$) (Tables 1, 2). Age class ($F=9.85$, $P<0.01$) had the greatest effect on MCV, but this effect varied between species ($F=6.46$, $P<0.05$). Seal groups also differed in their MCHC ($F=38.70$ $P<0.01$) and MCH ($F=45.32$, $P<0.01$). Compared to other seal groups, mean MCHC and MCH were significantly higher in adult hooded seals and significantly lower in harp seal pups (Tables 1, 2). Age class ($F_{MCHC}=62.21$, $P<0.01$; $F_{MCH}=61.99$, $P<0.01$) and species ($F_{MCHC}=33.25$, $P<0.01$; $F_{MCH}=22.84$, $P<0.01$) affected MCHC and MCH, but these effects interacted for MCHC ($F=5.06$, $P<0.05$). Nucleated red blood cells (NRBC) were observed in the blood of pups only (Tables 1, 2). Although mean counts of NRBC per 100 leukocytes were more than three times higher in harp seal pups than in hooded seal pups, this difference was not statistically significant.

White blood cell counts varied between

seal groups ($F=7.96$, $P<0.01$). Adult harp and hooded seals had similar mean WBC counts, and those of harp seal pups were significantly lower than those of adults (Tables 1, 2). Age class had a significant effect on WBC counts ($F=10.70$, $P<0.01$), with adult hooded and harp seals having higher WBC counts than those of pups, although those of hooded seal pups were not statistically different from those of adults. Adult hooded seals had relatively higher N and lower L counts than other seal groups (Tables 1, 2). Harp seal pups had significantly lower E counts than other seal groups. Basophils were mostly found in adult hooded seals (Tables 1, 2). Counts of N, L, E, and B were mostly influenced by effects of age class and species.

Serum chemistry

Nineteen serum chemistry parameters were determined for 19 hooded seals and 28 harp seals (Tables 1, 2). Three values from adult female harp seals were considered outliers: aspartate aminotransferase (AST)=290 U/l, creatine kinase (CK)=8705 U/l, and sodium=134.5mmol/l, and were excluded from Table 2 and statistical analyses. Mean serum glucose concentrations differed between seal groups ($F=11.39$, $P<0.01$), with the highest and lowest levels recorded in adult female hooded seals and harp seals, respectively (Tables 1, 2). Both pup species had similar mean serum glucose levels. Overall, serum glucose concentrations were influenced by sex ($F=8.53$, $P<0.01$), species ($F=7.20$, $P<0.05$), and the interaction between species and age class ($F=4.14$, $P=0.05$). Blood urea nitrogen varied between seal groups ($F=11.29$, $P<0.01$). In general, hooded seals had slightly higher mean BUN levels than those of harp seals, the largest difference being found between pup species (Tables 1, 2). Serum BUN levels were influenced by species ($F=12.54$, $P<0.01$), but the effect of species was not the same for all age classes ($F=10.83$, $P<0.01$) and sexes ($F=4.14$, $P<0.05$). Seal groups differed in their serum creatinine concentration ($F=53.92$, $P<0.01$). Adult hooded seals had the highest mean serum creatinine levels, and those of adult males were significantly higher than those of females (Tables 1, 2). Only age class ($F=43.87$, $P<0.01$) and species ($F=20.12$, $P<0.01$) had a significant effect on serum creatinine levels.

Mean total bilirubin varied between seal groups ($F_{rk}=28.02$, $P<0.01$). Hooded seal groups had similar mean total bilirubin, whereas harp seal pups had significantly lower total bilirubin levels than all other seals groups (Tables 1, 2). Serum bilirubin concentrations were affected by species ($F_{rk}=31.04$, $P<0.01$), age class ($F_{rk}=10.81$, $P<0.01$) and interactions between these effects ($F_{rk}=6.30$, $P<0.05$). No significant differences in AST activities were found between seal groups ($F_{rk}=0.39$, $P>0.05$), but the interaction between species and sex was significant ($F=6.18$, $P<0.05$). Alanine aminotransferase (ALT) activity differed between seal groups ($F_{rk}=4.55$, $P<0.01$), and only species-specific variations with age class were significant ($F_{rk}=6.10$, $P<0.05$). Large differences in mean alkaline phosphatase (ALP) activity occurred between seal groups ($F_{rk}=110.6$, $P<0.01$) (Tables 1, 2), which formed three homogeneous seal groupings: pups, adult hooded seals, and adult female harp seals. Alkaline phosphatase activity was mostly influenced by age class ($F_{rk}=156.9$, $P<0.01$) and species ($F_{rk}=28.13$, $P<0.01$), but these effects interacted together ($F_{rk}=5.90$, $P<0.05$). Mean creatine kinase activity differed between some seal groups ($F=3.49$ $P<0.05$), and only sex had some effect on CK activity ($F=4.13$, $P=0.05$). No differences were found among seal groups in mean γ-glutamyltransferase (GGT) activity ($F_{rk}=1.78$, $P>0.05$). Activity of GGT was independent of species, age class, sex, and interactions between these effects ($F=2.03$, $P>0.05$).

Serum proteins varied between seal

TABLE 1. Hematology and serum chemistry values for wild hooded seals, *Cystophora cristata*, by age class and sex, sampled between 11–13 March 2001 during the breeding season in the Gulf of St. Lawrence, Quebec, Canada.

	Adult males[b]			Adult females[c]			Pups[d]		
Parameters[a] (SI units)	*n*	Mean (median)±SD	Range	*n*	Mean (median)±SD	Range	*n*	Mean (median ±SD	Range
HEMATOLOGY									
PCV (%l)	5	68 (69)±1	67–69	7	55 (56)±4	51–61	8	57 (58)±5	48–63
HB (g/l)	5	286 (288)±6	279–292	6	240 (241)±14	225–264	9	228 (229)±22	194–252
RBC ($\times 10^{12}$/l)	4	4.23 (4.25)±0.19	4.01–4.42	6	3.53 (3.52)±0.23	3.23–3.90	8	4.20 (4.25)±0.32	3.67–4.54
MCV (fl)	5	141 (141)±5	134–147	6	142 (142)±6	135–150	9	125 (125)±4	119–131
MCH (pg)	4	68 (67)±4	64–73	6	68 (67)±2	67–70	8	54 (54)±3	48–58
MCHC (g/l)	4	478 (475)±11	467–494	6	478 (474)±11	467–494	8	429 (428)±22	405–469
WBC ($\times 10^9$/l)[e]	5	7.9 (8.4)±1.4	6.4–9.5	6	7.7 (8.1)±1.1	6.2–8.7	9	5.8 (5.6)±2.0	2.8–8.8
Neutrophils ($\times 10^9$/l)	5	6.2 (6.3)±1.2	5.0–7.8	6	5.8 (5.9)±0.8	4.8–6.9	9	3.4 (2.8)±1.2	1.7–5.4
(%)		79 (78)±3.0	74–82		76 (75)±2	73–79		58 (60)±8	48–71
Lymphocytes ($\times 10^9$/l)	5	0.8 (0.6)±0.3	0.5–1.2	6	0.7 (0.6)±0.3	0.3–1.0	9	1.6 (1.5)±0.8	0.6–2.6
(%)		10 (8)±3	6–14		9 (8)±3	4–12		27 (30)±8	13–38
Monocytes ($\times 10^9$/l)	5	0.2 (0.2)±0.1	0.1–0.3	6	0.6 (0.5)±0.2	0.4–1.0	9	0.6 (0.6)±0.3	0.1–1.1
(%)		3 (2)±2	1–6		8 (7)±3	6–13		10 (8)±4	4–18
Eosinophils ($\times 10^9$/l)	5	0.5 (0.5)±0.2	0.3–0.7	6	0.5 (0.5)±0.2	0.2–0.6	9	0.3 (0.3)±0.2	0.0–0.6
(%)		7 (7)±2	5–8		6 (7)±2	2–8		4 (6)±3	0–7
Basophils ($\times 10^9$/l)	5	0.1 (0.1)±0.1	0.1–0.2	6	0.2 (0.2)±0.2	0.0–0.4	9	0.0 (0.0)±0.0	0.0–0.0
(%)		2 (2)±1	1–4		3 (3)±2	0–5		0 (0)±0	0–1
NRBC (per 100 WBC)	5	0 (0)±0	0–0	6	0 (0)±0	0–0	9	6 (1)±15	1–45
SERUM CHEMISTRY									
Glucose (mmol/l)	5	7.6 (7.6)±0.2	7.5–7.9	7	12.0 (10.8)±2.4	9.7–15.6	5	10.3 (8.6)±3.2	8.1–15.7
BUN (mmol/l)	6	19.8 (20.3)±3.6	13.7–24.0	7	18.9 (19.2)±3.2	13.3–23.0	6	24.4 (26.4)±5.8	15.2–29.9
Creatinine (μmol/l)	6	204 (210)±32	147–240	7	161 (157)±27	131–213	6	82 (76)±33	47–123
Total Bilirubin (μmol/l)	5	28.8 (31.5)±4.9	21.6–33.2	7	37.1 (34.6)±11.9	27.0–61.0	5	32.7 (35.3)±20.8	1.7–59.6
AST (U/l)	5	98 (120)±53	15–146	7	97 (95)±37	43–145	5	132 (79)±164	4–418
ALT (U/l)	5	42 (25)±47	12–125	7	18 (20)±5	9–25	5	168 (43)±296	13–696
ALP (U/l)	5	85 (75)±27	64–131	7	122 (115)±27	96–176	5	809 (772)±318	436–1288
CK (U/l)	5	1139 (727)±954	251–2406	7	296 (168)±234	124–682	5	1020 (961)±880	286–2478
GGT (U/l)	5	13 (10)±5	8–20	7	9 (9)±4	3–15	5	15 (15)±6	6–25
Total protein (g/l)	6	76.8 (75.6)±4.8	72.7–85.8	7	66.6 (64.2)±4.8	61.2–73.1	6	67.8 (66.7)±6.1	59.8–77.3
Albumin (g/l)	6	43.1 (42.3)±3.3	39.9–47.3	7	38.9 (37.6)±3.2	35.3–44.8	6	46.6 (47.3)±3.3	41.3–50.2
Globulin (g/l)	6	33.7 (33.8)±3.1	29.8–39.1	7	27.7 (27.6)±2.5	24.0–32.2	6	21.1 (18.4)±6.2	15.4–30.7

TABLE 1. Continued.

Parameters[a] (SI units)	Adult males[b]			Adult females[c]			Pups[d]		
	n	Mean (median)±SD	Range	*n*	Mean (median)±SD	Range	*n*	Mean (median ±SD	Range
Calcium (mmol/l)	6	2.28 (2.25)±0.14	2.15–2.49	7	2.27 (2.25)±0.13	2.14–2.48	6	2.97 (2.88)±0.23	2.77–3.36
Phosphorus (mmol/l)	5	1.76 (1.72)±0.23	1.55–2.14	7	1.79 (1.66)±0.42	1.42–2.69	5	1.57 (1.24)±1.20	0.30–3.53
Sodium (mmol/l)	6	154.8 (155.5)±3.5	150.3–158.8	7	153.2 (153.0)±2.0	151.1–157.0	6	151.8 (151.6)±2.3	149.6–155.7
Potassium (mmol/l)	5	3.78 (3.56)±0.41	3.46–4.46	7	4.13 (4.19)±0.16	3.88–4.31	5	4.49 (4.44)±0.52	4.00–5.34
Chloride (mmol/l)	6	104.2 (104.2)±2.1	100.9–106.7	7	103.4 (105.6)±4.0	97.5–107.4	6	98.6 (98.9)±3.7	94.3–103.3
Magnesium (mmol/l)	5	0.98 (0.99)±0.08	0.85–1.07	7	0.80 (0.77)±0.09	0.70–0.94	5	1.60 (1.57)±0.29	1.27–2.05
CO_2 (mmol/l)	6	24.2 (23.5)±2.1	22.4–27.5	7	24.0 (24.9)±4.5	17.8–29.2	6	29.5 (29.9)±2.0	26.7–32.1

[a] PCV = packed cell volume; HB = hemoglobin; RBC = red blood cell counts; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; WBC = white blood cell counts; NRBC = nucleated red blood cell counts per 100 leukocytes; BUN = blood urea nitrogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; CK = creatine kinase; GGT = γ-glutamyltransferase; CO_2 = total carbon dioxide.

[b] Adult males: mean standard length±SD (223.0±6.9 cm); mean body weight± SD (277±39.8 kg).

[c] Adult females: mean standard length±SD (195.7±13.7 cm); mean body weight±SD (190.9±15.3 kg).

[d] Pups: mean body weight±SD (27.2±5.7 kg); pups are ≤4 days old.

[e] Estimated WBC counts ($\times 10^9$/l). See text for details.

TABLE 2. Hematology and serum chemistry values for wild adult female harp seals, *Phoca groenlandica*, and unweaned pups sampled between 6 March 2001 and 9 March 2001 during the breeding season in the Gulf of St. Lawrence, Quebec, Canada.

	Adult females[c]			Pups[d]		
Parameters[a] (SI units)	*n*	Mean (median)±SD[b]	Range[b]	*n*	Mean (median)±SD	Range
HEMATOLOGY						
PCV (%)	17	55 (54)±4	49–61	11	47 (47)±3	42–53
HB (g/l)	14	230 (228)±15	205–254	8	173 (172)±14	154–192
RBC ($\times 10^{12}$/l)	14	3.85 (3.88)±0.43	2.96–4.64	8	3.66 (3.73)±0.21	3.33–3.88
MCV (fl)	14	135 (134)±6	121–145	8	127 (126)±7	119–138
MCH (pg)	14	60 (59)±5	46–74	8	47 (46)±2	45–52
MCHC (g/l)	13	434 (439)±17	392–458	8	372 (368)±21	349–410
WBC ($\times 10^9$/l)[e]	14	7.4 (7.2)±1.5	4.1–10.0	8	4.2 (4.0)±1.3	2.3–5.8
Neutrophils ($\times 10^9$/l)	14	4.4 (4.4)±1.2	2.1–6.4	8	2.7 (2.5)±1.0	1.4–4.1
(%)		59 (58)±6	50–68		64 (65)±8	55–78
Lymphocytes ($\times 10^9$/l)	14	1.9 (1.7)±0.7	1.1–3.7	8	1.1 (1.1)±0.3	0.7–1.4
(%)		26 (25)±9	14–42		26 (23)±7	18–39
Monocytes ($\times 10^9$/l)	14	0.6 (0.7)±0.2	0.2–0.9	8	0.3 (0.3)±0.2	0.1–0.7
(%)		8 (9)±3	2–12		8 (8)±3	3–12
Eosinophils ($\times 10^9$/l)	14	0.5 (0.4)±0.3	0.1–1.0	8	0.1(0.0)±0.1	0.0–0.3
(%)		7 (5)±4	2–14		1 (1)±2	0–6
Basophils ($\times 10^9$/l)	13	0.0 (0.0)±0.0	0.0–0.0	8	0.0 (0.0)±0.0	0.0–0.0
(%)		0 (0)±0	0–0		0 (0)±0	0–0
NRBC (per100 WBC)	14	0 (0)±0	0	8	21 (23)±19	0–50
SERUM CHEMISTRY						
Glucose (mmol/l)	17	7.1 (7.1)±1.4	4.9–9.6	11	9.2 (8.7)±1.5	7.1–12.1
BUN (mmol/l)	17	14.8 (14.9)±2.5	10.2–18.6	11	14.6 (15.5)±3.3	7.7–19.8
Creatinine (μmol/l)	17	100 (95)±16	81–132	11	55 (59)±15	15–71
Total Bilirubin (μmol/l)	17	19.0 (17.6)±4.8	12.8–30.7	11	2.1 (1.7)±1.4	0.1–4.9
AST (U/l)	16	88 (76)±30	53–143	11	78 (86)±59	0–188
ALT (U/l)	17	29 (28)±6	19–43	11	20 (24)±10	1–35
ALP (U/l)	17	40 (31)±21	17–95	11	536 (490)±193	282–847
CK (U/l)	16	1090 (632)±1064	264–4110	11	937 (712)±621	274–2170
GGT (U/l)	17	12 (12)±4	2–21	11	10 (10)±5	0–17
Total protein (g/l)	17	70.8 (71.5)±5.8	58.6–82.5	11	59.8 (59.5)±4.0	53.4–66.7
Albumin (g/l)	17	39.4 (39.4)±1.7	36.2–41.6	11	39.7 (39.5)±2.8	36.3–44.0
Globulin (g/l)	17	31.4 (30.6)±4.9	22.3–41.5	11	20.1 (21.5)±3.1	15.0–23.2

TABLE 2. Continued.

Parameters[a] (SI units)	Adult females[c]			Pups[d]		
	n	Mean (median)±SD[b]	Range[b]	*n*	Mean (median)±SD	Range
Calcium (mmol/l)	17	2.39 (2.33)±0.17	2.21–2.83	11	3.13 (3.14)±0.32	2.55–3.75
Phosphorus (mmol/l)	17	1.70 (1.75)±0.69	0.32–2.73	11	3.11 (3.19)±0.51	1.79–3.80
Sodium (mmol/l)	16	156.2 (155.3)±3.3	152.0–164.7	11	151.4 (150.3)±3.4	147.6–160.0
Potassium (mmol/l)	17	4.59 (4.60)±0.54	3.93–5.75	11	5.66 (5.80)±0.45	4.62–6.14
Chloride (mmol/l)	17	103.5 (104.5)±3.9	93.5–110.0	11	98.6 (98.1)±3.2	94.5–105.8
Magnesium (mmol/l)	17	1.10 (1.09)±0.12	0.88–1.34	11	1.52 (1.44)±0.38	0.96–2.29
CO_2 (mmol/l)	17	27.7 (28.3)±2.8	22.6–33.5	11	27.9 (28.0)±1.0	25.8–29.4

[a] PCV = packed cell volume; HB = hemoglobin; RBC = red blood cell counts; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; WBC = white blood cell counts; NRBC = nucleated red blood cell counts per100 leukocytes; BUN = blood urea nitrogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; CK= creatine kinase; GGT = γ-glutamyltransferase; CO_2 = total carbon dioxide.

[b] Excluding outliers (i.e., values greater or less than 3 SD from the mean).

[c] Adult females: mean standard length±SD (162.2±4.2 cm); mean body weight±SD (101.5±12.8 kg).

[d] Pups: mean body weight (BW)±SD (26.0±7.1 kg); pups are ≤12 days old. Excluding an anemic pup (mean BW =27.0 kg, PCV=24%, HB=160 g/l, RBC=1.96×10^{12}/l, reticulocytes=90.7×10^{9}/l)

[e] Estimated WBC counts ($\times10^{9}$/l). See text for details.

groups ($F=12.53$, $P<0.01$) and were influenced by age class ($F=12.78$, $P<0.01$), but the relationship was species specific ($F=6.33$, $P<0.05$), and by sex ($F=4.92$, $P<0.05$). The highest and lowest mean serum protein levels were recorded in adult male hooded seals and harp seal pups, respectively (Tables 1, 2). Hooded seal pups had intermediate serum protein levels, which were significantly different from those of adult male hooded seals and harp seal pups but similar to those of adult female seals. Serum albumin concentration varied between seal groups ($F=11.14$, $P<0.01$), being higher in adult male hooded seals and hooded seal pups, and lower in the other seal groups (Tables 1, 2). Serum albumin levels were influenced by interactions between species and age class ($F=13.30$, $P<0.01$), age class with sex ($F=7.65$, $P<0.01$), and sex ($F=4.81$, $P<0.05$). Serum globulins, which were calculated from total protein and albumin levels, differed between seal groups ($F=18.43$, $P<0.01$), being higher in adult seals than in pups (Tables 1, 2). Globulin levels were only influenced by age class ($F=21.35$, $P<0.01$).

Large variations in electrolyte concentrations observed between seal groups included calcium ($F=35.58$, $P<0.01$), phosphorus ($F=9.54$, $P<0.01$), potassium ($F=20.07$, $P<0.01$), sodium ($F=4.77$, $P<0.01$), chloride ($F=5.62$, $P<0.01$), total carbon dioxide (CO_2) ($F=5.83$, $P<0.01$), and magnesium ($F=29.17$, $P<0.01$). Of these, calcium ($F=36.56$, $P<0.01$), chloride ($F=10.53$, $P<0.01$), CO_2 ($F=6.03$, $P<0.05$), and sodium ($F=4.51$, $P<0.05$) concentrations were only influenced by age class. Both pup species had similar mean calcium and chloride concentrations, which were significantly higher (calcium) or lower (chloride) than those of adult harp and hooded seals, which formed a homogeneous group for these serum parameters. Mean sodium concentrations were lower in pup groups than in adult seal groups, but the only significant difference was found between adult female harp seals and both pup species. Mean total CO_2 and magnesium were higher in both pup species than in adult harp and hooded seals, although mean total CO_2 concentrations in pups were not statistically different from that of adult female harp seals. Magnesium levels were influenced by species ($F=7.78$, $P<0.05$) and age class ($F=20.71$, $P<0.01$), but the relationship was species specific ($F=10.93$, $P<0.01$). Mean serum concentrations of phosphorus and potassium were significantly higher in harp seal pups than in other seal groups (Tables 1, 2). Phosphorus levels were affected by species ($F=6.79$, $P<0.05$), but this effect depended on age class ($F=6.45$, $P<0.05$). For potassium, effects of age class ($F=17.87$, $P<0.01$), species ($F=10.35$, $P<0.01$), and the interaction between these effects ($F=4.32$, $P<0.05$) were significant.

DISCUSSION

There is an increased interest in wild marine mammal health assessments, but data are often lacking. Although strandings involving ice-breeding seals have recently been increasing in the North Atlantic (McAlpine et al., 1999; Mignucci-Giannoni and Haddow, 2002; Lucas et al., 2003), little work has been done on baseline blood and serum chemistry values for free-ranging harp and hooded seals, likely because of logistic constraints. During the breeding season, large numbers of seals of various ages are accessible for study in a relatively small geographic area. However, whelping patches are remote, leading to delays between sampling and analyses, and blood must be protected from freezing. In the present study, delays were minimized by daily transportation to whelping patches, but delayed separation of blood cells from serum may have influenced some blood parameters. Another problem is limited access to laboratories. Although blood analyzers in human hospitals have often been used for clinical

pathology of marine mammals, they can have limitations (Bossart and Dierauf, 1990; Goldstein et al., 1998). We used manual backups for blood parameters subject to preanalytical and analytical variations. In our study, sending some subsamples of blood and fresh smears to a reference laboratory proved valuable.

Overall, our hematology and serum chemistry values overlap ranges observed for captive or wild harp and hooded seals (Clausen and Ersland, 1969; Ronald et al., 1969; Vallyathan et al., 1969; Geraci, 1971; Engelhardt, 1979; Worthy and Lavigne, 1982; Medway and Geraci, 1986; Keiver et al., 1987; Bossart and Dierauf, 1990; Reidarson et al., 2000; Cabanac, 2000; Kavtsevich, 2001), but detailed comparisons with most of these studies may be confounded because of different methodologies, sampling conditions, and populations studied. It is known that many hematology and serum chemistry parameters vary with sex, age, season, physiological state, and methodology of sampling and analyses.

Hematology

In harp seals, many mean values of RBC parameters in this study differed from those of Geraci (1971) for wild adult female harp seals and pups ≤3 wk of age, but both studies revealed higher mean PCV, HB, MCV, MCH, and MCHC in adult females than in pups, and similar mean RBC counts between these two age groups. These differences were not seen in hooded seals, where pups had PCV and HB values similar to those of adult female harp and hooded seals, and RBC counts similar to those of adult male hooded seals. Selection pressures that favor producing a more precocious neonate, rapid deposition of blubber, and a shorter lactation period may be related to unstable ice conditions at the whelping sites (Bowen et al., 1985; Oftedal et al., 1991). Low PCV, HB, and RBC counts in harp seal pups compared to those of hooded seal pups may be related to rapid plasma expansion and body growth exceeding the rate of RBC production (Bryden and Lim, 1969) in harp seal pups. Although daily weight gain is greater in hooded seal than in harp seal pups, the longer lactation of harp seals results in greater core body growth (overall gain in lean weight) of harp seal pups compared to hooded seal pups (Oftedal et al., 1989). The precocious development of hooded seal pups during the short lactation period, with marked hematological differences compared to harp seal pups, probably indicates differences in reproductive strategy and life history between these two pagophilic phocid species.

Sex differences in PCV and HB observed in breeding adult hooded seals are similar to observations by Cabanac (2000) in "adult–subadult" hooded seals. Higher PCV, HB, and RBC counts in males compared to females could indicate some dehydration in males caused by fasting (Medway and Geraci, 1986; Kovacs et al., 1996), greater stress-induced splenic contraction and subsequent release of RBC (Medway and Geraci, 1986; Cabanac, 2000), recent parturition, or other physiological differences related to sex. In many domestic animals, HB (and by extension, HCT) levels are higher in males than in females (Jain, 1993). It is possible that males experienced more stress than did females during sampling. Diving capacity of adult male hooded seals could be greater than those of adult females as this species is sexually dimorphic, but Folkow and Blix (1999) found no sex-related differences in diving depth and duration.

Significant differences were found in RBC parameters between adult female harp and hooded seals. Adult female hooded seals had significantly higher mean MCH and MCHC, and slightly higher HB and MCV than did adult female harp seals. These differences could reflect the greater diving capacities of adult female hooded seals compared to harp seals (see Table 1 in Schreer and Kovacs, 1997). However, larger body size,

spleen size, blood volume, and myoglobin concentrations (Mottishaw et al., 1999) can also explain the greater diving performance of hooded seals relative to harp seals.

Leukocyte counts were calculated from blood smears because of erroneously elevated automated counts from the human hospital laboratory, which could be attributed to platelet aggregation, NRBC in pups, and perhaps to unlysed RBCs, because all seals had elevated WBC counts. In harp seals, our mean WBC counts are somewhat lower than those reported in studies from captive seals (Ronald et al., 1969; Engelhardt, 1979; Worthy and Lavigne, 1982; Medway and Geraci, 1986; Bossart and Dierauf, 1990; Reidarson et al., 2000) but remain within published ranges. However, captive seals have been shown to have higher WBC counts than those of wild seals (Geraci, 1971; Wickham et al., 1990; Goldstein et al., 1998; Lander et al., 2003). Our WBC counts in harp seals are similar to values reported in wild harp seals sampled during the breeding season (Geraci, 1971). Hooded seal pups had intermediate mean WBC counts, perhaps related to their advanced development compared to harp seal pups. In gray seals (*Halichoerus grypus*), Hall (1998) observed increasing WBC counts in pups up to weaning age, and higher WBC counts in yearlings compared to pups.

Absolute and relative differential WBC counts in captive harp seals have been published by Reidarson et al. (2000), and Englehardt (1979), respectively, and Kavtsevich (2001) published relative differential WBC counts in wild harp seals from the White Sea population. In general, our differential WBC counts in adult female harp seals are similar to those of Reidarson et al. (2000) and Engelhardt (1979) in captive harp seals. Discrepancies in the proportions of M and E in adult harp seals and proportions of N and L in 1-wk-old harp seal pups between Kavtsevich (2001) and this study cannot be explained, and may be related to methodology or population effects. In adult hooded seals higher and lower counts and proportions of N and L, respectively, than seen in other seal groups, are likely because of stress of capture (Medway and Geraci, 1986; Kavtsevich, 2001). Although sedation is known to reduce the effect of stress, we believe sedation had little or no effect on blood and serum parameters in our study because adult hooded seals were already stressed with capture and physical restraint when the drug was administered, and blood sampling occurred immediately after sedation in most cases. Little has been published on hematology of hooded seals (Clausen and Ersland, 1969; Bossart and Dierauf, 1990; Cabanac, 2000), thus precluding comparisons, and to our knowledge, this study is the first to provide baseline RBC counts, MCV, MCH, MCHC, and WBC counts with differentials in wild hooded seals.

Serum chemistry

Unlike hematology, serum biochemistry analyses can easily be completed on frozen samples. Like other marine mammals, serum glucose concentrations in harp and hooded seals are higher than those of domestic animals (Ridgway, 1972). Stress, fasting, and recent feeding can affect glucose levels, but interactions between these factors with other variables are difficult to assess (Geraci et al., 1979). The mean serum glucose level in adult female harp seals is similar to that reported by Nordøy and Thoresen (2002) in seals of similar physiological state and using similar sampling methods. However, when we used the method of Nordøy and Thoresen (2002) to establish a reference range for glucose, our minimum value (4.9 mmol/l) was somewhat lower than that (6.1 mmol/l) of Nordøy and Thoresen (2002) in killed adult female harp seals, probably because of delayed centrifugation of many of our samples. Our glucose levels in harp seal pups, which were sampled postmortem, are lower than those

measured by Nordøy and Thoresen (2002) in live-sampled harp seal pups. Higher serum glucose levels in live-captured compared to killed harp seals are believed to be because of stress-induced hyperglycemia (Nordøy and Thoresen, 2002) and delayed centrifugation of many of our samples. In ringed seals (*Phoca hispida*), Geraci et al. (1979) also found slight differences in plasma glucose between shot and live-captured (netted) seals, and between pups and older seals, but thought this had no biological significance. Slightly higher mean glucose levels in harp and hooded seal pups compared to adults (with the exception of adult female hooded seals) are likely because of recent ingestion of fat-rich milk (Greenwood et al., 1971) and interference from lipemic serum in pups (Bossart et al., 2001). However, levels of lipemia were unlikely to affect serum glucose significantly with the methodology used in the present study (Beckman Coulter Inc., 2001). Adult male hooded seals had significantly lower glucose levels compared to adult females despite the fact that both sexes were fasting and were subject to stress of capture which, in the case of many male hooded seals (which were large and difficult to handle) resulted in difficult bleeding and secondary hemolysis. Stress and hemolysis are known to increase serum glucose values (Bossart et al., 2001), but levels of hemolysis in this study were unlikely to cause significant variations in serum glucose determinations (Beckman Coulter Inc., 2001). It is possible that the different behavior of males compared to females during the reproductive period (see Kovacs et al., 1996) can affect glucose levels.

Blood urea nitrogen and serum creatinine levels are kidney-associated serum analytes. Urea is produced by the liver from protein catabolism and is excreted through glomerular filtration. Creatinine is a product of muscle metabolism and is another indicator of glomerular filtration (Bossart et al., 2001). Our results for harp seals are similar to those of Nordøy and Thoresen (2002) for harp seals sampled postmortem, but unlike these authors, we found no significant differences in mean BUN levels between adult female harp seals and their pups. Higher BUN levels in killed compared to live animals have been observed by Geraci et al. (1979) and by Nordøy and Thoresen (2002) for ringed and harp seals, respectively. Geraci et al. (1979) attributed high BUN levels in ringed seals sampled postmortem versus live-captured to recent feeding, but this may not apply in breeding harp seals whose metabolic needs exceeds their energy intake (Nordøy and Thoresen, 2002). In hooded seals, BUN levels in lactating females reported by Mellish and Iverson (2001) are similar to those of our study. No significant sex differences in BUN levels were found among adult hooded seals. Mellish and Iverson (2001) noted that BUN levels in adult female hooded seals remained constant throughout the short lactation period despite the loss of 7% of total body proteins during lactation, and they suggested that this is perhaps partly because of an increase in glomerular filtration rate and urea excretion. No serum creatinine levels have been published for harp or hooded seals. Our data suggest that serum creatinine levels in harp and hooded seals are related to body size. Creatinine levels are not affected by diet (Bossart and Dierauf, 1990), and variations in normal animals are because of differences in muscle mass (Duncan et al., 1994)

In domestic animals, the hepatobiliary system can be evaluated with serum enzymes such as AST, ALT, ALP, and GGT and with metabolites such as bilirubin. Analysis of these enzymes can also be useful in evaluating hepatocellular changes in marine mammals, although some exceptions occur (St. Aubin and Geraci, 1977; Bossart et al., 2001). In general, few significant differences in hepatobiliary markers (ALT, AST, and GGT) were found between seal groups,

except for total bilirubin and ALP. It is unclear why total bilirubin levels were high in all seal groups except for harp seal pups. High bilirubin levels can indicate accelerated destruction of erythrocytes, bile flow impairment, and liver disease (Dierauf et al., 1984; Bossart et al., 2001). Hemolysis and lipemia can also affect total bilirubin determinations (Morgan et al., 1998; Bossart et al., 2001, Beckman Coulter Inc., 2001). However, both pup species had lipemic serum, and therefore lipemia cannot explain the observed differences. It is possible that total bilirubin levels were higher in hooded seal pups because of their very young age, and that cholestasis was possibly present because of hepatic infiltration of fat. High total bilirubin levels in neonatal harbor seals have been interpreted as the liver not becoming fully functional until a few days after birth and by the rapid turnover and breakdown of fetal HB (McConnell and Vaughan, 1983; Dierauf et al., 1984). However, fetal HB has not been found in harp seals (Nævdal, 1965) or hooded seals (Nævdal, 1966), and knowledge of RBC characteristics in phocid neonates is lacking (Jørgensen et al., 2001). Although we did not examine livers of harp and hooded seal pups histologically, their color (orange-yellow) suggested that cholestasis was possible and this could contribute to higher mean total bilirubin levels in hooded seal pups compared to harp seal pups. At the end of lactation, the livers of hooded seal pups contain up to 30% fat whereas those of harp seal pups contain only about 3% fat (Oftedal et al., 1989). Our total bilirubin values for adult harp seals are higher than published ranges, except for those of harp seal pups (Bossart and Dierauf, 1990). We cannot explain the high total bilirubin levels in our adult seal groups; field necropsies revealed no macroscopic hepatic lesions or grossly visible fat infiltration associated with lipid mobilization in fasting adults. There are no previously published total bilirubin values in hooded seals.

Medway and Geraci (1986) state that ALP and glutamic pyruvic transaminase (or ALT) activities are liver specific in marine mammals. However, ALP is also found in other tissues. Levels of ALP increase because of a variety of physiologic processes such as bone growth in young animals (Bossart and Dierauf, 1990; Bossart et al., 2001), ingestion of colostrum in neonates (Center et al., 1991), and pathological processes such as liver disease and impaired bile flow (Bossart and Dierauf, 1990; Bossart et al., 2001). High ALP activity in harp and hooded seal pups is likely because of increased bone ALP activity, and perhaps because of colostrum ingestion. It is also possible that a fraction of ALP activity observed in hooded seal pups is due to some intrahepatic cholestasis caused by the lipid-rich milk, because total bilirubin levels were relatively high in this group (our study). Among adult hooded seals, the lower mean ALP activity observed in males (although not significantly different from that of females) could be caused by some hemolysis (Beckman Coulter Inc., 2001; Bossart et al., 2001), which occurred more frequently in males than in females.

No statistical differences were found in AST and GGT activity between seal groups. Similarly, Geraci et al. (1979) found no significant differences in AST levels with age in ringed seals. The significance of serum AST activity is difficult to interpret. Unlike ALT, AST is distributed in many tissues, especially muscles, and its tissue distribution can be species specific (St. Aubin and Geraci, 1977). Serum GGT is an enzyme associated with cellular membranes of many organs but, according to Bossart et al. (2001), it is a marker for passive immunoglobulin transfer in neonatal mammals because colostrum and milk have high GGT activity. In our study, GGT activity in pups was not significantly different from that of adults, perhaps because of small sample sizes and age of pups (see Center et al., 1991). No studies on

colostrum and milk GGT and ALP activity in harp and hooded seals, or their relationship with immunoglobulin transfer in seals, have been conducted.

Creatine kinase is found as tissue-specific isoenzymes in skeletal muscle, myocardium, and the brain in terrestrial mammals, but in marine mammals, most increases in CK levels are associated with muscle injury (Bossart et al., 2001), and St. Aubin et al. (1979) recommend its use as a sensitive indicator of handling stress in seals. Mean serum CK activity in harp and hooded seals were likely influenced by sampling method and seal behavior. Blood from harp seals and most pups was obtained postmortem from blood vessels severed when seals were killed, which could have led to elevated CK activity because of the release of muscle and brain isoenzymes. Difficult handling of large and aggressive adult male hooded seals could account for their higher CK activity compared to females. Adult female hooded seals had the lowest mean serum CK activity, probably because these lactating females never leave their pups unattended and spend most of their time idle or sleeping between nursing bouts (Lydersen and Kovacs, 1999). Therefore, skeletal muscle damage in these female hooded seals is unlikely.

Our ranges and mean values of serum total protein in harp and hooded seals are similar to published reference values using serum (Bossart and Dierauf, 1990; Nordøy and Thoresen, 2002) or plasma (Reidarson et al., 2000). Differences in mean serum total protein in harp seals by age class are due to lower mean globulin concentrations in pups compared to those in adults (Nordøy and Thoresen, 2002), probably because of greater immune stimulation in adults and perhaps because of reduced mother–pup transfer of immunoglobulins. Oftedal et al. (1989) suggest that the slow growth of the intestines of harp and hooded seal pups may reflect an absence of immunoglobulin absorption from the gut. Serum total protein and globulins increase with age in gray seal pups (Hall, 1998). This pattern was also observed in harp seal pups when weight was used as an indicator of age ($r=0.73$, $P<0.05$), but not in hooded seal pups ($r=0.03$, $P>0.05$), likely because of the short time interval from birth to weaning (4 days) in this species (see Bowen et al., 1985).

In general, mean values of electrolytes varied more between age groups than between species, but species-specific differences occurred with age class. In harp seals, significant differences in every electrolyte parameter were observed between adult females and unweaned pups, but Nordøy and Thoresen (2002) found age-related differences in harp seals for sodium and calcium only. Although our ranges for various electrolytes overlap those of Nordøy and Thoresen (2002), mean levels differed for some electrolytes. These differences are likely because of methodology. Significant interspecific differences were found for serum concentrations of phosphorus, potassium, and magnesium but not for calcium, sodium, and chloride. Interestingly, unweaned hooded seals had the lowest phosphorus levels (although not significantly different from those of adults) whereas levels in harp seal pups were significantly higher than those of all other seal groups. Young, growing animals have higher serum phosphorus concentrations than do adults (Bossart et al., 2001), suggesting that, at the time we collected our samples, bone formation may have been proceeding more rapidly in harp seal pups than in hooded seal pups. Oftedal et al. (1989) found that the longer lactation in harp seals resulted in greater total weight gains in this species. Electrolytes are under hormonal control, but dietary intake, diseases, hydration status, and sample quality can cause variations in these parameters.

In summary, differences in many blood parameters between harp and hooded seals likely reflect the great diving capacity of hooded seals (Schreer and Kovacs, 1997) and their short lactation during

which much of the mass gain of hooded seal pups occurs in the form of fat. Because of longer lactation, harp seal pups have more time to grow and to develop high HCT, HB, and other hematological adaptations commonly associated with diving (see Bossart and Dierauf, 1990; Hedrick and Duffield, 1991). Interspecific variations in hematology and serum chemistry were common, and many variations with age class were species specific. This emphasizes the clinical importance of using reference values by age group, physiological state, and species. In addition, interlaboratory variations in WBC counts indicated that human laboratories are not always familiar with analyses of wildlife blood. Therefore, a certified veterinary laboratory should be used whenever possible, along with manual backups. One goal of this study was to establish reference values for harp and hooded seals. Except for PCV and HB in wild adult hooded seals (Cabanac 2000) and BUN in wild adult female hooded seals (Mellish and Iverson, 2001), this study is the first to present data on hematology and serum chemistry in wild hooded seals. More research is needed to investigate the biological significance of many blood parameters (Lander et al., 2003), but establishing reference values is an important step in health assessment of wild harp and hooded seal populations.

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