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HEMATOLOGIC AND SERUM BIOCHEMICAL PROFILE OF THE NORTHERN ELEPHANT SEAL (*MIROUNGA ANGUSTIROSTRIS*): VARIATION WITH AGE, SEX, AND SEASON

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ABSTRACT: The foraging success, and thus the survival and reproductive success, of deep-diving pinnipeds such as the northern elephant seal, *Mirounga angustirostris*, depends on the ability to withstand repetitive breath-hold dives. Health parameters can be incorporated as potential explanatory variables for differences observed in diving and migratory performance of individual seals. Furthermore, biomedical samples from apparently healthy individuals can provide valuable baseline data for evaluating effects of natural or anthropogenic threats to individuals and populations. We evaluated 42 blood parameters in 134 northern elephant seals during the breeding and molting seasons (1992–1999) to test for age, sex, and seasonal differences and to develop reference ranges. Adult males sampled during the breeding season differed from all other adult groups for a suite of parameters often associated with inflammation, infection, or other stressors: lower hematocrit, higher white blood cell count, higher band neutrophils, higher neutrophil count, lower albumin, and lower serum iron. Adult females during the breeding season differed from all other adult categories for two parameters (lower platelet counts, lower alanine aminotransferase activity). Molting males had higher blood urea nitrogen than all other classes; creatinine did not differ between breeding and molting adult males, but was higher in males than in females in both seasons. We found significant differences among age classes for 24 of 42 parameters measured, including higher levels of triglycerides, total protein, calcium, and iron in pups than we found in juveniles or adults. Unlike other mammals which undergo substantial decreases in energy expenditure during prolonged fasting (e.g., hibernation), northern elephant seals defend territories, give birth and suckle large offspring, mate, and molt during their bi-annual fasts. Nonetheless, many studies have described physiologic homeostasis during fasting in elephant seals. The genus *Mirounga* is superbly adapted to going without feeding for extended periods, and this is reflected in our hematologic and serum biochemical profiles.

Key words: Biochemistry, elephant seal, hematology, *Mirounga angustirostris*, reference intervals, serum chemistry.

INTRODUCTION

The foraging success, and therefore the survival and reproductive success, of deep-diving pinnipeds such as the northern elephant seal, *Mirounga angustirostris*, depends on their ability to withstand repetitive, prolonged breath-hold dives (DeLong and Stewart, 1991; Stewart and DeLong, 1994, 1995). Evaluating the physical condition and health of seals has many applications, such as documenting the oxygen-carrying capacity of the blood of seals fitted with telemetry instruments. This allows health factors to be incorporated as potential explanatory variables for any inter-individual differences in diving and migratory performance that may be observed. Collecting samples from apparently healthy individuals also provides valuable baseline data that can be used to evaluate the impact of natural or anthropogenic health threats (e.g., pollutants, Mazet et al., 2000).

Although northern elephant seals spend most of their lives at sea, they come ashore twice each year during the breeding (mid-December to mid-March) and molting (juveniles and females, April–May; subadult and adult males, July–August) seasons. Despite energetically demanding activities such as parturition, lactation, mating, and molting, they fast for several weeks while on land (Deutsch et al., 1990; Worthy et al., 1992). The ability to maintain physiologic homeostasis during

the terrestrial segments of the elephant seal's annual cycle is dependent on the health of individual animals (e.g., renal function, Ortiz et al., 2000).

Previous studies of elephant seal hematology and biochemistry have focused generally on younger age classes (primarily nursing or weaned pups); on stranded animals (Roletta, 1993; Gulland et al., 1996; Goldstein et al., 1998; Ortiz et al., 2000; Reidarson et al., 2000; Larsen et al., 2002); on just a few parameters (Simpson et al., 1970; Adams and Osburn, 1972; Ridgeway, 1972; Costa and Ortiz, 1982; Castellini et al., 1986, 1990; Medway and Geraci, 1986; Wickham et al., 1990; Hedrick and Duffield, 1991; Gulland et al., 1996; Larsen et al., 2002); or on elephant seals (*M. leonina*) in the southern hemisphere (Bryden and Lim, 1969; Seal et al., 1971; Lane et al., 1972; Lewis et al., 2001; Englehard et al., 2002; Field et al., 2005). We developed reference ranges (Sasse et al., 1995; Solberg, 2001) for apparently healthy northern elephant seals sampled (1992–1999) during the course of our research on the ecology of seals and sea lions in the Southern California Bight.

MATERIALS AND METHODS

We collected blood samples from 41 adult (≥7-yr-old) male northern elephant seals at San Nicolas (33°14′N, 119°27′W) and San Miguel (34°01′N, 120°27′W) islands (34 during the breeding season, seven during the molting season); 30 adult (≥4-yr-old) females (21 breeding, nine molting); 19 juvenile females (12 during or just prior to the breeding season, five during the molt); 21 juvenile males (12 during or just prior to the breeding season, nine during the molt); and 10 female and 13 male weaned pups (all sampled during the breeding season). Juveniles included some yearlings, but most were 2–3 yr old.

Elephant seals are easily approached by cautious researchers and can be captured and immobilized without being chased. Blood samples were collected as soon after immobilization as possible (generally within 5 min) during eupnea (hematocrit increases during long-duration apnea in elephant seals; Castellini et al., 1986). Weaned pups and juveniles were physically restrained. Adult seals were chemically immobilized with ketamine hydrochloride by remote injection (Rydig, 1982).

Blood was collected from the extradural vein (Geraci and Smith, 1975) using a syringe and either a 8.9 cm, 18 gauge spinal needle (pups, juveniles, some adult females) or a 15.2 cm, 16 gauge spinal needle (some adult females, adult males). Whole blood was placed into serum separator Vacutainer® tubes (SST; Becton, Dickinson & Co., Rutherford, New Jersey, USA) and Vacutainer® tubes containing anticoagulant (ethylenediaminetetraacetic acid disodium salt [Na-EDTA]). Blood samples were kept cool and transported to a field laboratory within 4 hr for preliminary processing and preparation for transport. Serum was frozen (−20 F) and whole blood was kept refrigerated until hematologic and biochemical analyses were completed within 48 hr.

Hematologic and serum biochemical assays were performed by the SeaWorld San Diego Animal Care Laboratory (San Diego, California, USA). Hematocrit, erythrocyte counts, leukocyte counts, platelet counts, and mean corpuscular volume (MCV) were measured with a Coulter particle counter (model ZBI, Coulter Electronics, Hialeah, Florida, USA), calibrated for elephant seal cells. A Coulter hemoglobinometer (Coulter Electronics) was used to determine hemoglobin concentration. Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated. Microscopic blood-smear evaluation was used to make differential leukocyte counts, to rule out the presence of platelet clumps, and to evaluate cell morphology.

Biochemical assays were performed on a Ciba–Corning 550 Express Plus (Ciba-Corning Diagnostics Corp., Oberlin, Ohio, USA). Twenty-two serum chemistry parameters were measured including biomarkers for liver, kidney and muscle (alanine aminotransferase, ALT; alkaline phosphatase, ALP; aspartate aminotransferase, AST; total bilirubin; gamma glutamyltransferase, GGT; blood urea nitrogen, BUN; creatinine, CR; creatine kinase, CK; lactate dehydrogenase, LDH); nutritional status (glucose, cholesterol, triglycerides, total protein); inflammation–infection (albumin, globulin, serum iron); electrolytes (sodium, potassium, chloride), other ions (calcium, phosphorus); and total carbon dioxide as an estimate of bicarbonate.

Basic statistics (mean, median, range, standard deviation, and measurements of normal distribution) were calculated for all hematologic and serum biochemical parameters. Not all parameters were normally distributed. Median values are reported in Tables 1 and 2 (Borjesson et al., 2000). Kruskal–Wallis analysis of variance, followed by nonparamet-

TABLE 1. Adult elephant seal hematologic and serum chemistry values comparisons by sex and season.[a]

Variable (Units)	Breeding males	Molting males	Breeding females	Molting females
	Median	Median	Median	Median
Hematology[b]	(*n*=29–34)	(*n*=4–7)	(*n*=16–21)	(*n*=9)
Hemoglobin (gm/dl)	20.3 A	22.2 A,B	24.0 C	22.6 B,C
Hematocrit (%)	51 A	58 B	60 B	59 B
Red blood cells ($\times 10^6$/μl)	2.2 A	2.5 B	2.7 B	2.6 A,B
MCV (fl)	225 A	226 A	228 A	221 A
MCH (pg)	88 A	90 A	89 A	88 A
MCHC (gm/dl)	39 A	40 A	40 A	40 A
Platelets ($\times 10^3$/μl)	210 A	190 A	150 B	220 A
White blood cells (/μl)	14,500 A	8,200 B	9,700 B	10,050 B
Bands (no.)	288 A	0.0 B	0.0 B	0.0 B
Neutrophils (no.)	11,360 A	5,644 B	6,852 B	6,144 B
Lymphocytes (no.)	1,768 A	1,106 A	1,546 A	1,383 A
Monocytes (no.)	959 A	486 B	707 A,B	975 A,B
Eosinophils (no.)	681 A	639 A	632 A	700 A
Basophils (no.)	5 A	0 A	0 A	0 A
Serum chemistry[c]	(*n*=30–34)	(*n*=7)	(*n*=15–21)	(*n*=9)
Glucose (mg/dl)	86 A	112 B	110 B	123 B
BUN (mg/dl)	16 A	44 B	25 C	20 A,C
Creatinine (mg/dl)	1.8 A	2.1 A	1.2 B	0.8 B
Total bilirubin (mg/dl)	0.4 A	0.6 A,B	1.2 B	0.6 A
Cholesterol (mg/dl)	225 A	332 B	282 A,B	264 A,B
Triglyceride (mg/dl)	63 A	110 B	63 A	87 A,B
Total protein (gm/dl)	6.8 A	7.2 A	6.9 A	6.9 A
Albumin (gm/dl)	2.5 A	3.1 B	3.2 B	3.3 B
Globulin (gm/dl)	4.2 A	4.1 A,B	3.6 B	3.6 B
ALP (U/l)	138 A	194 B	155 A, B	165 A,B
ALT (U/l)	19 A	14 A	9 B	12 A
AST (U/l)	35 A	36 A	30 A	26 A
GGT (U/l)	13 A	15 A,B	16 A,B	22 B
CK (U/l)	2,143 A	1,027 A	476 B	296 B
LDH (U/l)	294 A	237 B	334 A	191 B
Calcium (mg/dl)	9.2 A	10.1 B	9.7 A,B	10.0 B
Phosphorus (mg/dl)	5.5 A,C	7.8 B	4.5 C	6.4 B
Sodium (mEq/l)	144 A	144 A	146 A	145 A
Chloride (mEq/l)	99 A	101 A	100 A	100 A
Potassium (mEq/l)	4.5 A	4.6 A	4.2 B	4.3 A,B
Carbon dioxide (mEq/l)	32 A	33 A	33 A	31 A
Iron (mcg/dl)	80 A	190 B	169 B	183 B

[a] Values within a row that do not share a common letter differ ($P<0.05$, Kruskal–Wallis analysis of variance and pairwise comparisons).

[b] MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

[c] BUN = blood urea nitrogen; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyltransferase; CK = creatine kinase; LDH = lactate dehydrogenase.

ric multiple comparison tests (Tukey test with ranked sums), was used to evaluate for age–sex class and seasonal differences; groups that differed from one another are identified with different letters in Tables 1 and 2. Differences in actual values of parameters were considered significant if $P<0.05$. We calculated 95% confidence limits and used these to define reference intervals. Outliers were defined as values that differed by >3 SD. These were discarded and statistics recalculated (if an outlier was excluded from the range, this is noted in the table). In all but one case (elevated CK in one pup), the outliers appeared to be transcription or reporting errors (e.g., one digit repeated).

TABLE 2. Elephant seal hematologic and serum chemistry values comparisons, by age.[a]

Variable (Units)	Adults (without breeding males) Median	Juveniles Median	Pups Median
Hematology[b]	(*n*=29–37)	(*n*=39–40)	(*n*=21–23)
Hemoglobin (gm/dl)	23.3 A	23.7 A	22.5 A
Hematocrit (%)	60 A	60 A	58 A
Red blood cells ($\times 10^6$/μl)	2.6 A	2.8 A	3.1 B
MCV (fl)	224 A	219 A	188 A
MCH (pg)	89 A	87 A	73 A
MCHC (gm/dl)	40 A	40 A	39 A
Platelets ($\times 10^3$/μl)	170 A	220 B	230 B
White blood cells (/μl)	9,700 A	12,500 B	11,400 B
Bands (no.)	84 A	0 A	134 A
Neutrophils (no.)	6,490 A	7,752 B	6,372 A
Lymphocytes	1,704 A	2,208 A	3,737 B
Monocytes (no.)	774 A	1,035 B	952 A,B
Eosinophils (no.)	757 A	688 A	990 A
Basophils (no.)	10 A	0 A	0 A
Serum chemistry[c]	(*n*=31–37)	(*n*=38–41)	(*n*=19–23)
Glucose (mg/dl)	107 A,B	100 A	119 B
BUN (mg/dl)	26 A	19 B	19 B
Creatinine (mg/dl)	1.4 A	1.0 A	1.0 A
Total bilirubin (mg/dl)	0.8 A	0.4 B	0.3 B
Cholesterol (mg/dl)	277 A	278 A	286 A
Triglyceride (mg/dl)	80 A	80 A	175 B
Total protein (gm/dl)	6.9 A	6.8 A	7.2 A
Albumin (gm/dl)	3.1 A	3.2 A	3.3 A
Globulin (gm/dl)	3.7 A,B	3.6 A	4.0 B
ALP (U/l)	196 A	142 A	164 A
ALT (U/l)	12 A	17 B	9 A
AST (U/l)	32 A	41 B	41 B
GGT (U/l)	19 A	19 A,B	25 B
CK (U/l)	664 A	750 A	512 A
LDH (U/l)	292 A	287 A	294 A
Calcium (mg/dl)	9.6 A	10.5 B	11.4 C
Phosphorus (mg/dl)	5.4 A	6.8 B	6.7 B
Sodium (mEq/l)	145 A	148 B	147 A,B
Potassium (mEq/l)	4.3 A	4.7 B	4.2 A
Chloride (mEq/l)	100 A	102 B	100 A
Carbon dioxide (mEq/l)	32 A	32 A	31 A
Iron (mcg/dl)	179 A	193 A	288 B

[a] Values within a row that do not share a common letter differ ($P<0.05$, Kruskal–Wallis analysis of variance and pairwise comparisons).

[b] MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

[c] BUN = blood urea nitrogen; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyltransferase; CK = creatine kinase; LDH = lactate dehydrogenase.

RESULTS

Sex and season variation

Hematologic and serum biochemical values for adults are compared by sex and season in Table 1. Adult males during the breeding season differed from all other adult groups (molting males, breeding and molting females) for several parameters: lower hematocrit ($P \leq 0.001$), higher white blood cell count ($P \leq 0.001$), higher band neutrophil count ($P \leq 0.004$), higher total neutrophil count ($P \leq 0.001$), lower albumin ($P \leq 0.013$), and lower iron

($P \leq 0.000$). Adult males during the breeding season also had lower serum glucose ($P \leq 0.009$). Creatinine levels were significantly higher in adult males than in adult females during both seasons ($P \leq 0.001$). Molting males had higher BUN ($P \leq 0.001$) than all other classes; creatinine did not differ between breeding and molting adult males, but was higher in males than in females in both seasons ($P \leq 0.001$). Females sampled during the breeding season had lower platelet counts ($P \leq 0.032$) and lower ALT activity ($P \leq 0.001$) than did all other adult groups.

Weaned pups were sampled only during the breeding season; no significant differences were observed between males and females. Juvenile males and females were sampled during the molt season or breeding season; no significant differences were detected between males and females.

Age class comparisons

We eliminated adult breeding males from age-class comparisons (Table 2) and considered them separately for reference ranges (Tables 3, 4) because of the number (eight of 42, [20%]) and magnitude of significant differences observed for the parameters we measured. Differences among molting adult males and the breeding and molting adult females were minimal (e.g., lower platelet counts and lower ALT activity in breeding females) and, therefore, we pooled these groups for comparing age classes. No significant differences were detected between male and female weaned pups, or between male and female juveniles, for any parameter; we also pooled these data for age class comparisons.

Significant differences in blood values among age classes are detailed in Table 2. Pups had higher ($P \leq 0.001$) red blood cell counts than did older seals, although there was no difference in hematocrit among age groups ($P = 0.84$). Northern elephant seal pups had lower hemoglobin levels than did adults or juveniles ($P \leq 0.05$). Adults had higher leukocyte counts than did the pups or juveniles ($P \leq 0.001$). Pups had more lymphocytes than either the juveniles or adults. Juveniles had more neutrophils

TABLE 3. Elephant seal hematology reference ranges.

Variable (Units)	Adults without breeding males Reference range	Adult breeding males Reference range	Juveniles Reference range	Pups Reference range
Hematology[a]	(n=28–37)	(n=29–34)	(n=38–39)	(n=20–23)
Hemoglobin (gm/dl)	18.8–27.0	12.7–25	19.2–28.5	17.2–26.0
Hematocrit (%)	50–69	34–62	49–69	45–66
Red blood cells ($\times 10^6$/μl)	2.0–3.1	1.4–2.9	2.1–3.2	2.5–3.5
MVC (fl)	193–236	184–250	190–238	179–229
MCH (pg)	72–100	70–94	73–95	68–90
MCHC (gm/dl)	37–42	36–49	38–43	38–40
Platelets ($\times 10^3$/μl)	60–330	130–330	70–360	80–320
White blood cells (/μl)	5,100–17,800	9,100–32,100	8,000–19,000	7,000–20,700[b]
Bands (no.)	0–712	0–2,673	0–1810	0–1,035[a,b]
Neutrophils (no.)	2,856–11,926	6,188–26,001	3,280–15,390	4,141–11,592[b]
Lymphocytes (no.)	676–4,123	648–3,750	729–4,158	1,260–5,983
Monocytes (no.)	279–2,525	273–3,280	414–2,540	228–3,312
Eosinophils (no.)	103–2,457	152–2,112	108–1,760	207–2,123
Basophils (no.)	0–388	0–177	0	0–114

[a] MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

[b] Outlier excluded from the range.

TABLE 4. Elephant seal chemistry reference ranges.

Variable (Units)	Adults without breeding males	Adult breeding males	Juveniles	Pups
	Reference range	Reference range	Reference range	Reference range
Serum Chemistry[a]	(*n*=31–37)	(*n*=30–34)	(*n*=38–40)	(*n*=19–23)
Glucose (mg/dl)	60–144	60–166	52–146	70–219
BUN (mg/dl)	10–57	9–38	9–35	9–30
Creatinine (mg/dl)	0.7–2.9	0.6–2.9	0.5–2.2	0.6–1.9
Total bilirubin (mg/dl)	0.2–2.0	0.1–2.0	0.1–0.9	0.2–0.7
Cholest (mg/dl)	99–399	121–355	157–366	121–349
Triglyceride (mg/dl)	41–209	33–351	51–161	76–301
Total protein (gm/dl)	5.3–8.2	4.4–8.5	6.1–8.2	5.9–8.8
Albumin (gm/dl)	2.1–3.8	1.7–3.4	2.7–4.0	2.4–3.8
Globulin (gm/dl)	2.6–4.5	2.6–6.5	2.7–5.2	2.4–5.0
ALP (U/l)	60–478	36–297[b]	72–1,483	115–341
ALT (U/l)	6–32	7–44	7–46	6–35
AST (U/l)	16–62	10–79	19–118	26–117
GGT (U/l)	4–38[a,b]	3–39	5–44	6–58
CK (U/l)	180–1,473	176–10,332	248–3,867	215–1,693[b]
LDH (U/l)	157–463	175–865	185–873	219–654[b]
Calcium (mg/dl)	7.8–11.6	6.8–11.2	9.0–13.9	10.2–12.6
Phosphorus (mg/dl)	2.1–8.8	2.3–7.3	4.1–8.6	2.4–8.0
Sodium (mEq/l)	131–151	133–154	142–154	140–151
Potassium (mEq/l)	3.0–5.1	3.2–6.3	4.0–5.9	3.4–4.7
Chloride (mEq/l)	87–106	90–108	98–111	95–104
Carbon dioxide (mEq/l)	23–37	28–37	27–36	27–35
Iron (mcg/dl)	60–292	35–336	75–360	96–505

[a] BUN = blood urea nitrogen; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyltransferase; CK = creatine kinase; LDH = lactate dehydrogenase).

[b] Outlier excluded from the range.

than did adults or pups ($P \leq 0.01$). Platelet counts were lower in adults than in pups or juveniles ($P \leq 0.005$).

Pups had significantly higher levels of triglycerides ($P \leq 0.001$), total protein ($P \leq 0.04$), calcium ($P \leq 0.001$), and iron ($P \leq 0.01$) than did juveniles or adults. Pups and juveniles had higher phosphorus ($P \leq 0.001$) than the adults. Juveniles had significantly higher potassium, chloride, and ALT activity ($P \leq 0.001$) than did adults or pups. Aspartate aminotransferase activity was lower in adults than in pups or juveniles. Calcium was lower in adults than in juveniles and lower in juveniles than in pups ($P \leq 0.001$). Phosphorus was lower in adults than in juveniles or pups ($P \leq 0.001$). Total bilirubin was higher in adults than in juveniles or pups ($P \leq 0.001$).

Reference ranges

Reference ranges for all parameters are presented in Tables 3 and 4. Two ranges are presented for adults: males sampled during the breeding season and pooled values for the other adult groups (females during the breeding season, males and females during the molt season). As described above, breeding-season males are reported separately because we considered the hematologic and serum biochemical changes numerous enough, and large enough, to be considered clinically significant (Riegelman, 1979; Sheehan, 1980). For 20% of the parameters reported, the magnitude (absolute value and percentage) of differences between adult breeding males and the next nearest adult group was generally several times greater than the maximum difference among the

remaining groups. For example, the neutrophil count for breeding males was 4,450, which is >30% higher than for the next nearest adult group, whereas the greatest difference among the three other adult groups was 1,850 (<20%). The serum iron value (80) for breeding males was less than half the value of the next nearest adult group (169), whereas the greatest difference among all other groups was 21. Separating out breeding males provides a better representation of "normal" values for adults.

DISCUSSION

Sex and season variation

Hematologic and serum biochemical values in northern elephant seals varied with sex and season. This was particularly evident for adult males, perhaps owing to variation in physiologic stress and social interactions (i.e., intraspecific competition and aggression) among breeding animals. Males fast for weeks and lose considerable mass during both the breeding and molting seasons. The number and magnitude of changes occurring in blood parameters during the breeding season are therefore likely to be due to something other than fasting, for which seals are well-adapted (Costa and Ortiz, 1982; Castellini and Costa, 1990; Castellini et al., 1990; Ortiz et al., 2000, 2003). The adult males we sampled during the breeding season were normal upon physical examination (no fresh wounds or obvious signs of dehydration, infection, or debilitation), suggesting that not all aggressive encounters during the breeding season result in obvious lesions (King, 1983). Males may challenge each other with vocalizations or stylized postures, or chase each other without making physical contact; in other cases, males push against each other or deliver closed-mouth blows. When severe bite wounds are inflicted, they generally heal quickly. Males had higher creatinine levels than females during both seasons ($P<0.001$), perhaps due to their much-larger muscle mass (the body mass of adult males can be more than three times the mass of adult females; Stewart and Huber, 1993). Platelet counts in human females do not decrease following normal parturition (Dahlstrom and Nesheim, 1994); therefore, it is interesting that northern elephant seals sampled during the breeding season had slightly lower platelet counts than all other adult categories. Although the difference is statistically significant, we did not consider it clinically significant; an animal with a platelet count of 150,000 would not be considered thrombocytopenic, and this value falls within the reference range we calculated for elephant seal adults.

Other than the changes noted above for breeding males, we did not find clinically significant seasonal differences among adults; therefore, molting males and breeding and molting females were pooled for our reference ranges. Statistically significant changes in blood values during the molt have been reported in some phocid seals (Phocidae; e.g., decreased erythrocyte counts and lower hemoglobin during the molt in harp seals, *Phoca groenlandica*, [Ronald et al., 1969]; higher triglyceride concentrations during the molt in southern elephant seals [Guilherme et al., 2004]), but other researchers have reported no differences. No consistent pattern was observed in molting Baikal seals (*Pusa sibirica*; Ronald and Kay, 1982) and no significant differences in total protein or cholesterol were observed between breeding and molting southern elephant seals (Guilherme et al., 2004).

Age class comparisons

Hematologic and serum biochemical values in northern elephant seals varied with age. We observed higher erythrocyte counts but not higher hematocrits in weaned pups. Others have reported higher packed-cell values in older animals. Lane et al. (1972) reported increases in packed cell volume (and in plasma proteins, hemoglobin, and MCHC), with age, in southern elephant seals and Kuiken

(1985) reported higher packed cell volume and hemoglobin concentration in older rather than in younger harbor seals. However, Morgan et al. (1998) reported that red blood cell counts were higher in juvenile than in adult harbor seals.

Pups had significantly higher levels of triglycerides, total protein, calcium, and iron than did juveniles or adults. High protein, calcium, and triglycerides are often reported in young mammals, including marine mammals (Geraci et al., 1979; Rea et al., 1998; Nordoy and Thoresen, 2002), due to their milk diet, and high triglycerides have been reported by others in northern elephant seal pups. Engelhard et al. (2002) reported increases in inorganic phosphate in southern elephant seals pups sampled more than once during the period from 2 days to 21 days postpartum; triglycerides increased, and then decreased, during this period.

Alanine aminotransferase activity was slightly but significantly lower in adults and pups than in juveniles, and AST activity was lower in adults than in pups or juveniles. However, decreases in the activity of these enzymes generally are not clinically significant, and clinically relevant increases are usually much larger than the differences we observed. In fact, they may exceed the upper value of the reference interval by several times (Stockham and Scott, 2002).

Other influences on blood values

We did not attempt to evaluate the effect of chemical immobilization on adult elephant seal blood parameters; juveniles and pups were handled using physical restraint only. Changes in some blood parameters with sample handling or prolonged anesthesia have been reported in pinnipeds (Geraci and Engelhardt, 1974; Castellini et al., 1986), but our handling times were short, and we collected our blood samples as soon as the animals were immobilized. Furthermore, Engelhard et al. (2002) did not detect differences in clinical chemistry values for mother–pup pairs related to intensity of handling (including tiletamine-zolazepam immobilization of females); intensity varied from seals handled once (control group), three times (moderate treatment group), or four to five times (high treatment group). McMahon et al. (2005) also reported no measurable effects on short-term (nursing period) or long-term survival in southern elephant seals that were repeatedly handled and sampled. St. Aubin et al. (1979) evaluated the effects of handling stress on several plasma enzymes in harp seal pups, but again the handling was prolonged (30 min transport followed by 2.5 hr of vigorous handling). The only significant change observed in these harp seal pups was an increase in CK; no significant changes were observed in AST, ALT, or GGT.

Unlike other mammals that undergo dramatic decreases in energy expenditure during prolonged fasting (e.g., hibernation [Nelson et al., 1973]), northern elephant seals defend territories, give birth and suckle large offspring to weaning, mate, and undergo a 'catastrophic' molt wherein they shed and replace superficial epidermal layers in addition to hair (Yochem and Stewart, 2002) during their bi-annual fasts. Nonetheless, many studies have described physiologic homeostasis during fasting in elephant seals (Costa and Ortiz, 1982; Castellini and Costa, 1990; Castellini et al., 1990; Ortiz et al., 2000, 2003). Once or twice during a normal annual cycle, northern and southern elephant seals may lose up to one-third of their body mass while fasting on land (McCann et al., 1989; Deutsch et al., 1990; Worthy et al., 1992) and then immediately resume a nearly continuous pattern (for months) of deep breath-hold diving upon leaving the beach (DeLong and Stewart, 1991; Stewart and DeLong, 1994, 1995). This indicates that the genus *Mirounga* is superbly adapted to going without feeding for extended periods, and this is reflected in our hematologic and serum biochemical profiles for northern elephant seals. Adult

males exhibited large and statistically significant changes in several blood parameters during the breeding season. Given the intensity of intraspecific aggression among adult males during the breeding season, we attribute these changes to an inflammatory response to physical exertion or recent tissue damage (e.g., newly healed scrapes or tears in the neck or chest, where many blows are struck). Some of the changes, as seen in leukocyte and neutrophil counts, also are consistent with a corticosteroid-induced neutrophilia, a condition which is sometimes referred to as a 'stress leukogram' (Aiello, 1998).

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