

ONTOGENETIC AND SEASONAL VARIATION IN BLOOD PARAMETERS IN SOUTHERN ELEPHANT SEALS

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ABSTRACT

Blood samples were collected from 156 free-ranging southern elephant seals (*Mirounga leonina*) from Península Valdés to study the variation in blood parameters related to ontogeny and the annual cycle. Samples ranged from newborn pups to adults. Interactions between age and sex showed different trends in ontogenetic changes of blood parameters (MANOVA, Wilkis' Lambda_{12,243} = 0.75, $P < 0.01$). The hematocrit (HCT) and hemoglobin concentration (HB) reached adult levels early in life. Weanlings had 14 % higher HCT than pups, and similar levels to juveniles and adults (HCT range = 46%–62 %). HB of males were below those of females from weaning (18.4 vs. 20.3 g/dl) to adulthood (19.8 vs. 22.3 g/dl). Red blood cell counts (RBC) did not change significantly from pups to juveniles ($2.8\text{--}3.2 \times 10^6/\mu\text{l}$), but varied for adults at different times of the annual cycle. Breeding females had higher RBC than molting females ($3.1 \text{ vs. } 2.4 \times 10^6/\mu\text{l}$). Changes in blood parameters are related to the development of diving capabilities from pups to juveniles. Changes in adults were associated with different stages of the annual cycle, and these may be the result of the requirements imposed by pregnancy and fasting duration.

Key words: ontogeny, hematology, southern elephant seal, *Mirounga leonina*, blood values.

Previous studies of blood values in elephant seals (*Mirounga* sp.) summarize descriptive data of general ontogenetic changes related to diving (Lenfant 1969, Bryden and Lim 1969, Lane *et al.* 1972). Hematological data are available for free-ranging individuals of the southern elephant seal (*M. leonina*) at Macquarie Island (Bryden and Lim 1969, Lane *et al.* 1972). Early work on this species showed that blood volume, hematocrit (HCT) and hemoglobin concentration (HB) increased from pups to adults (Bryden and Lim 1969, Lane *et al.* 1972). This paper reports changes in hematological parameters of southern elephant seals of different age categories in the context of the development of oxygen storage capacity and seasonal changes.

Hematological studies provide an understanding of the relationship between oxygen storage and transport capacity of the blood to diving capabilities (Kooyman 1989, Hedrick and Duffield 1991, Hindell *et al.* 1992). Both southern and northern (*M. angustirostris*) elephant seals are deep divers that have developed hematological and cardiorespiratory adaptations for prolonged diving (Castellini *et al.* 1986, Wickham *et al.* 1989, Hedrick and Duffield 1991, Blackwell and LeBoeuf 1993, Andrews *et al.* 1997). In northern elephant seals changes in HB, HCT, blood volume, and myoglobin concentrations increase oxygen storage capacity during the first 10 wk of life (Thorson and LeBoeuf 1994). Dive duration and dive depth increases with age and experience (LeBoeuf *et al.* 1996). Total oxygen stores increase by 14.2% over the first five-month trip to sea, culminating in oxygen stores that reach 68.8 ml O₂/kg (LeBoeuf *et al.* 1996). Crucial cardiorespiratory adaptations related to diving during the first months of life were also described for both elephant seal species (Blackwell and LeBoeuf 1993, Castellini *et al.* 1994, Andrews *et al.* 1997, Falabella *et al.* 1999).

Blood is the most important oxygen storage site in phocid seals, containing approximately 65% of the total oxygen stores, followed by the muscle and the lungs (Kooyman 1989). When the lean mass and lungs are fully developed, oxygen stores may continue to increase due to changes in blood parameters, because the latter is a dynamic storage site. We also summarize variation in hematological parameters in adult southern elephant seals at different times of the year. Present hematological data in adult elephant seals (Bryden and Lim 1969, Lane *et al.* 1972) do not allow differentiation between individuals that have spent several weeks ashore from those that have recently arrived.

METHODS

This study was conducted at the southern elephant seal colony of Península Valdés, Argentina (Campagna and Lewis 1992, Lewis *et al.* 1996), between 1993 and 1999. The annual cycle of elephant seals at this colony is similar to that described for other populations (LeBoeuf and Laws 1994). During the breeding season (September–November) adult females stay on land for a mean of 28 d, give birth 6 d after arrival, and nurse their pups for 22 d (Campagna *et al.* 1993). Males spend 57–80 d ashore fasting and defending harems. Pups are weaned when their mothers go to sea to forage. Two months later, adult

females return to shore for about one month to molt. After the molt, a long pelagic period precedes the subsequent breeding season.

Blood samples were obtained from 156 animals: 37 pups, 43 weanlings (24–40 d), 23 weanlings older than 60 d, 10 juveniles of ages 1–3 yr and 43 adults (Table 1). To determine differences between stages of the annual cycle (breeding and molt), adult females were sampled 3–4 d postpartum ($n = 12$), postlactating ($n = 15$) and during the molt ($n = 7$). Molting females were selected for comparisons with other sex and age categories, and adult males were sampled only at the end of breeding. Females were sampled once, except for 11 breeding individuals sampled 3–4 d postpartum and 1–3 d before weaning their pups. Pups were sampled 1–3 d after birth; 12 were also sampled immediately before weaning. All pups and most weanlings and juveniles were known-age animals tagged at birth. Adult females were marked with a black dye or a bleaching agent at their arrival on the coast.

Adults, juveniles, and 51 weanlings were anesthetized with tiletamine-zolazepam (Telazol®) at doses of 0.8–1.3, 1.6–1.8, and 1.2–1.4 mg/kg, respectively. Pups and 15 young weanlings were manually restrained and blood was drawn by rear flipper venipuncture. All samples from anesthetized animals were collected from the extradural vein in heparinized syringes and placed in plain 10-ml blood collection tubes and 9.5-ml serum separator tubes (Vacutainer, Benton Dickinson, Rutherford, NJ). Samples were then stored on ice until processing. Blood samples were taken during eupnea periods (Castellini *et al.* 1986, 1996). In individuals chemically restrained we used the stimulant doxapram hydrochloride (Viviram®, Holliday Scott, Beccart, Argentina) when an animal did not breathe for more than three minutes (Blackwell and LeBoeuf 1993).

Standard length (L) and maximum girth (G) were related to body mass (M) in pups and weanlings using an equation described in previous studies: $M = 12.1 + 37.7 (LG^2/10^6)$ (Lewis 1989, Table 1). Mass for older animals was estimated using L (Table 1) in the equation summarized for juveniles and adult females by Bell *et al.* (1997), juveniles: $M = 32.2 (L^{2.8})$; postlactation females: $M = 29.8 (L^{2.7})$. Adult male body mass was estimated following Haley *et al.* (1991) for northern elephant seals: $M = 31.3 (L^{3.0})$.

The samples obtained during 1993–1996 were processed within 48 h of collection. The HCT (%) was obtained by microcentrifugation. HB concentration in whole blood (g/dl of blood) was determined using automated cyanmethemoglobin standard methods by spectrophotometric measurement (Hemoglobinometer, Coulter Corporation, Hialeah, FL). Red blood cell counts (RBC: 10^6 cells/ μ l) were determined by Coulter-counter techniques (Coulter D2N, Coulter Corporation, Hialeah, FL).

In 1997–1999, samples were processed in the field within 3–8 h of collection. HCT (%) was obtained following centrifugation of capillary tubes at 1,500 rpm for 15 min in a portable 12-V centrifuge (Mobilespin, Vulcan Technologies, Grandview, MO). The resulting HCTs were read on a microhematocrit scale. RBC counts were conducted in the field using a commercial dilution system, following the manufacturer's instructions (Unopette Test

Table 1. Length, girth (mean \pm 1 SD), and number of samples collected from different age classes of southern elephant seals. N.A.: not applicable.

| Age class | Standard length (cm) | Axillary girth (in cm) | n | Time of year | Fasting time (d) |
|----------------|----------------------|------------------------|----|--------------|-------------------|
| Pups 1-3 d | 135 \pm 5.3 | 88.4 \pm 4.2 | 37 | At birth | Nursing |
| Weaned 24-40 d | 144 \pm 10.1 | 141.9 \pm 7.3 | 43 | Postweaning | 7-17 |
| Weaned >60 d | 147 \pm 9.8 | 121.5 \pm 10.6 | 23 | Postweaning | >35 |
| Juveniles | 186 \pm 29.5 | N.A. | 10 | Molt | 1-10 ^a |
| Adult females | 272 \pm 15.4 | N.A. | 27 | Breeding | 1-30 |
| Adult males | 401 \pm 13.1 | N.A. | 9 | Postbreeding | >50 ^a |

^a Fasting duration estimated.

5877 and RBC W/Pipettes 200/cs 1:200, Benton-Dickinson Vacutainer Systems, Rutherford, NJ). To compare different analysis techniques, replicated blood samples from 14 adults were stored on ice and shipped to the human diagnostic laboratory for HCT and RBC analysis. In duplicate samples the HCT values were similar to those processed in the field laboratory (HCT = 54.5% *vs.* 53.1%; $t = 2.1$, $P > 0.05$). There was no difference in cell counts by Coulter-counter and the Unopette Test (RBC = $2.7 \times 10^6 \mu\text{l} \pm$ *vs.* $2.8 \times 10^6 \mu\text{l}$; $t = 1.7$, $P > 0.05$).

HCT, HB, and RBC were examined for normal distribution (Levene's test) and then tested by a 2-way MANOVA for differences between groups (five age categories and sex). A 1-way ANCOVA with the sex as a factor was performed to detect the changes in HB during development. The Scheffé multiple comparisons test was used for *post hoc* testing for significant differences of sample means. A 1-way ANOVA with each blood parameter as a dependent variable was used to determine differences in adults between stages of the annual cycle. The Tukey test was used for *post hoc* testing of the differences in means. Females sampled more than once during the breeding season were compared using a repeated measures ANOVA. Statistical analyses were performed with the Statistica for Windows software, Release 4.5, 1993 (Statsoft, Inc.).

RESULTS

Ontogenetic Changes in Hematology

Blood values related to oxygen transport capacity changed with age (Fig. 1). The categories of age and sex had significant effects on blood values when we tested the following: pups at birth, young weanlings, old weanlings, juveniles, and adults (MANOVA for HCT, RBC, and HB as dependent variables, with age categories and sex as factors, $n = 104$, interaction Wilkis' Lambda_{12,243} = 0.75, $P < 0.01$). Interactions between age and sex were significant in HB concentration of pups and weaned males and females of the same age (Scheffé $P < 0.01$, Fig. 1).

Analysis of the effects of sex and age on each variable showed a significant increase in HCT from nursing pups to adults (2-way ANOVA for HCT with age categories and sex as factors $F_{4,119} = 14.4$; $P < 0.01$). By the time weanlings were ready to depart for their first foraging trip, their HCT level was 14% higher than animals nine weeks younger (Scheffé tests $P < 0.01$) and similar to older animals (Scheffé tests $P > 0.05$).

Younger animals had a higher RBC count, showing more RBC than adults (2-way ANOVA for RBC with age categories and sex as factors $F_{4,116} = 4.7$; $P < 0.01$). Recently weaned pups had a 17% higher RBC count than adults of both sexes ($P < 0.01$), but the count was similar to that of nursing pups, older weanlings, and juveniles.

HB concentration increased with different trends in each sex. From birth to the weaning time, HB concentration in female pups increased 12%, but

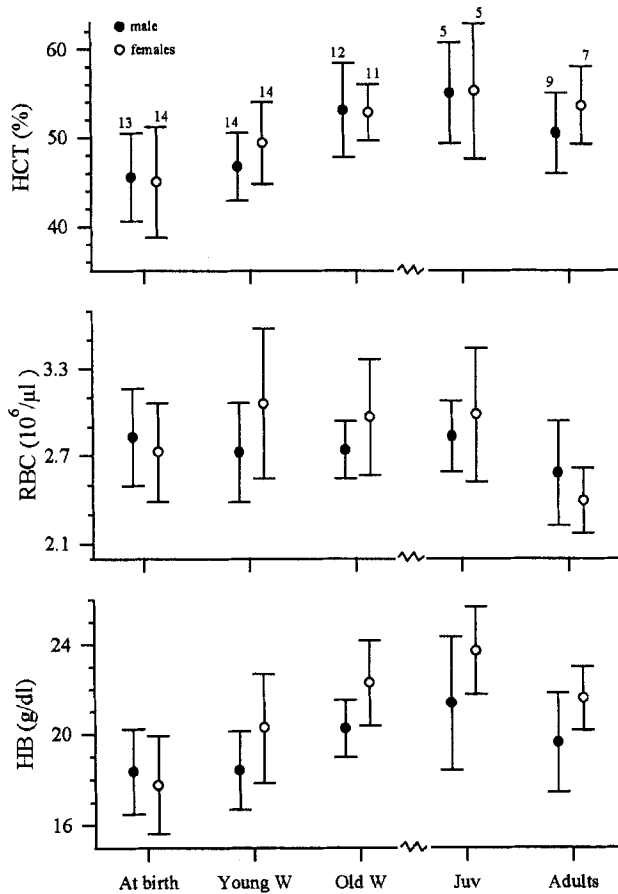


Figure 1. Hematocrit (HCT), erythrocyte counts (RBC), and hemoglobin concentration (HB) for southern elephant seals as function of age categories. Values are means ± 1 SD and numbers on top of bars are sample sizes.

male pups had similar concentrations to that of neonates (2-way ANOVA with age categories (pups and weanlings) and sex as factors: $F_{1,52} = 5.7$, $P < 0.05$). In categories older than weanlings, sex had a more significant effect on HB concentration than age (sex: $F_{1,43} = 13.4$, $P < 0.01$; age: $F_{2,43} = 3.2$, $P = 0.05$). The effect of development on HB was analyzed with the sex as covariates and age as a factor. The corresponding 1-way ANCOVA revealed a significant increase in HB concentrations ($F_{4,99} = 7.9$, $P < 0.01$). Weanlings ready to depart to sea and juveniles had significantly higher HB concentrations than pups and young weanlings (Scheffé $P < 0.01$).

The HCT levels of postpartum mothers were 12% higher than their pups' levels at birth ($t = -4.6$, $df = 40$, $P < 0.05$). Differences were even more marked at the end of lactation (58.6 vs. 47.7 %; $n = 10$ $U = 2.5$, $P < 0.01$). RBC counts in mothers and pups were similar at birth and the end of lactation.

Hemoglobin concentrations were similar between mothers and their pups at birth (27.1 *vs.* 23.3 g/dl, $t = -1.5$, $df = 16$, $P > 0.05$), but higher in the mothers at the end of lactation compared to the pups values at the time of weaning (27.3 *vs.* 19.8 g/dl, $t = -2.5$, $df = 7$, $P < 0.05$).

Variation in Blood Parameters During the Annual Cycle

Adult females at the end of lactation had higher HCT values compared to males at the end of the reproductive season (ANOVA Table 2, Tukey $P < 0.05$). RBC counts were lower in molting females compared to breeding females and males. Females that came ashore to give birth had RBC counts 22% higher than adult females that came ashore during the molting season (ANOVA Table 2, Tukey $P < 0.05$). Females during the breeding season (Table 2) had higher HB concentrations than adult males after fasting two times longer than females (postpartum: Tukey $P < 0.01$; end of lactation: Tukey $P < 0.05$).

Females sampled more than once during the lactation period showed a 10% increase in the HCT over the course of lactation (53.0% *vs.* 58.6%, $F_{1,9} = 6.5$, $P < 0.05$). RBC counts and HB concentration did not change during the same interval (RBC: $3.0 \times 10^6/\mu\text{l}$ *vs.* $2.9 \times 10^6/\mu\text{l}$, $F_{1,11} = 0.2$; HB: 23.3 *vs.* 23.9 gr/dl, $F_{1,4} = 0.7$, $P > 0.05$).

DISCUSSION

Our results show that blood parameters related to the oxygen storage capacity of southern elephant seals increase from birth to juveniles as has been found previously (Bryden and Lim 1969, Lane *et al.* 1972). Adults have the highest values for HCT in marine and terrestrial mammals (Kooyman 1989, Hedrick and Duffield 1991). Variation in blood parameters for the first months of life occur simultaneously with the development of cardiorespiratory adaptations related to diving (Falabella *et al.* 1999). During the first 11 wk of life, breath-holding capabilities increase 2.7 times to a 4.6% increase in blood oxygen stores due to changes in the HCT and HB (Kooyman 1989). Similar results were reported for the northern elephant seal (Balckwell and LeBoeuf 1993, Thorson and LeBoeuf 1994, Andrews *et al.* 1997).

In our work, the increase in HCT during postweaning fasting may be ontogenetic in nature, because handling stress and long apnea (Castellini *et al.* 1986, 1996) could affect only pups and younger animals that were manually restrained. HCT increase in weanlings prior to their departure is probably independent of dehydration. Data from healthy phocids, and specifically for northern elephant seals, suggests that body water content does not change while fasting, as fat catabolism supplies nearly all energy and water requirements (Ortiz *et al.* 1978, Reilly and Fedak 1991, Castellini and Rea 1992). Plasma protein concentrations from 1997/1998 blood samples during the 20 d of postweaning fast remained unchanged (9.0 to 8.6 g/dl, $n = 14$, Uhart, unpublished data).

Table 2. Blood values of adult females at different stages of annual cycle and adult males at end of breeding season. Sample size in parenthesis.

| Blood parameters | Postpartum | Females End of lactation | Molt | Males End of breeding | F | P |
|-------------------------|--------------------|--------------------------|-------------------|-----------------------|-----|-------|
| HCT (%) | 55.9 ± 4.4 (12) | 56.6 ± 6.1 (14) | 53.6 ± 4.3 (7) | 54.4 ± 4.5 (9) | 3.1 | >0.05 |
| RBC 10 ⁹ /μl | 3.1 ± 0.6 (10) | 2.9 ± 0.6 (14) | 2.4 ± 0.2 (7) | 2.6 ± 0.4 (9) | 3.3 | <0.05 |
| HB (g/dl) | 23.3 ± 1.1 (6) | 22.3 ± 1.6 (8) | 21.6 ± 1.4 (7) | 19.8 ± 2.3 (8) | 6.3 | <0.01 |

Sex differences in blood parameters were not detected in previous studies on elephant seals (Bryden and Lim 1969, Lane *et al.* 1972, Thorson and LeBoeuf 1994). We found that male HB concentrations were below those of females of similar age from weaning time to adulthood. Similar findings are reported for Galápagos fur seals (*Arctocephalus galapagoensis*) and harbor porpoises (*Phocoena phocoena*) (Horning and Trillmich 1997, Koopman *et al.* 1999). Weanling elephant seals do not show sex differences in weight (McCann *et al.* 1989, Campagna *et al.* 1992, Fedak *et al.* 1994), body composition (Rea and Costa 1992), or diving performance during their first foraging trip (Thorson and LeBoeuf 1994). The reason for these sex-specific differences in HB concentrations is unknown.

Hematology profiles for southern elephant seals increase as a function of oxygen demand. To our knowledge, there are no previous reports of how blood values for elephant seals vary during breeding, molting, or fasting. Fully developed adults have steady blood volume. However, adults will experience changes in their blood parameters which produce effects on individual aerobic capacity. For example, all blood parameters were higher in breeding females. An insignificant but slight increase of about 2 g/dl of HB found between postpartum and molting females would translate into a 7% increase in blood oxygen stores (Kooyman 1989). Increased HCT and RBC counts for breeding females would compensate for the increased oxygen consumption during pregnancy (Elsner *et al.* 1970). Pregnant females dive longer than non-pregnant females and take more time to reach the same depths (LeBoeuf 1994). HCT levels were not affected by fasting duration. The 10% increase in HCT observed in females over lactation was not due to dehydration (Sepúlveda *et al.* 1999), as it accompanied a decrease of plasma protein concentration (Uhart, unpublished data). Postbreeding adult males with more than 50 d ashore showed lower values of HCT than females at the end of lactation and similar values to females recently arrived to breed or molt. Sex differences in HB during breeding could also be related to the time that adults spend on land. When adult elephant seals are on land, they begin to breathe irregularly and experience sleep apneas. During breeding, sleep apnea is more truncated in adult males than in younger animals and lactating females. Social behavior does not allow the males to rest for long periods of time (Blackwell and LeBoeuf 1993). Consequently, with a smaller demand for oxygen, blood HB concentration might decrease.

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