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PRELIMINARY INVESTIGATION: HEMATOLOGY AND SERUM CHEMISTRY OF THE YOUNG HAWAIIAN MONK SEAL, *MONACHUS SCHAUINSLANDI*

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ABSTRACT

Blood counts and serum chemistry analyses were performed as part of a health screening protocol on eight clinically normal Hawaiian monk seals up to approximately 1 year of age. This screening protocol was developed as part of a program to rehabilitate weaned female pups and enhance their early development to increase their chances of survival in the wild. The eight hematology and nine serum chemistry panels elucidated in this study were not remarkable for a young diving mammal.

INTRODUCTION

The breeding range of the endangered Hawaiian monk seal, Monachus schauinslandi, is limited to the Northwestern Islands from Nihoa Island to Kure Atoll in the Hawaiian Archipelago. In the past, human disturbance has been responsible for significant population declines (Kenyon and Rice 1959; Rice 1960; Johnson et al. 1982). Other environmental factors such as shark predation (Taylor and Naftel 1978; Balazs and Whittow 1979; Alcorn and Kam 1986), natural toxins (Gilmartin and Delong 1979; Gilmartin et al. 1980), injury of adult females and immature seals of both sexes from "mobbing" by adult male seals (Johnson and Johnson 1981; Alcorn 1984), entanglement in debris (Henderson 1984) and food availability (Kenyon 1973) may also limit the population. Total seal beach counts over the breeding range have been reduced to <50% of the counts observed in the late 1950's.

To assist recovery of the Hawaiian monk seal population, female pups weaned prematurely or at low body weight and which have a greatly decreased chance of survival, have been collected at French Frigate Shoals for rehabilitation at facilities in Honolulu. When of sufficient size, these seals have been taken to Kure Atoll where the breeding population is extremely low. Before release to the wild, the pups are maintained in a large enclosure at Kure Atoll until they are able to catch live fish. Construction of this enclosure and the method used to stock it with live fish for the seals are described in Gilmartin et al. (1986).

To protect against the potential transfer of disease between island populations, certain genetic studies, cultures and examinations for disease agents, and selected blood tests were performed on animals involved in the rehabilitation program. The information derived from the hematologic and serum chemistry studies reported in this paper, will also prove useful as reference ranges for the clinical evaluation and the diagnosis and treatment of disease in captive or wild Hawaiian monk seals. Results of the other screening studies will be reported elsewhere. Expectations of increased handling of these animals in captive situations and planned research to be conducted in the field have prompted this summary report.

MATERIALS AND METHODS

The animals examined consisted of three groups of Hawaiian monk seal pups. The first trio of seals (Y290, Y299, Y319) were low weaning weight females recovered from French Frigate Shoals during summer 1984. Two seals (Y406, Y420) were recovered from the same atoll in the summer of 1985. These five seals were maintained in Honolulu at the Waikiki Aquarium for the early portion of their time in captivity and at the National Marine Fisheries Service (NMFS), Kewalo Research Facility for the latter part. At the aquarium, they were primarily fed a diet of frozen herring at (5-8% of their body weight) supplemented with multivitamins. They were also occasionally fed live-caught reef fish (as availability permitted). At the NMFS facility their diet consisted of frozen herring and smelt with multivitamins. The seals were housed in enclosures which allowed swimming and haul-out activities. The three other seals (K515, K516, K517) were born at Kure Atoll in

1985 and maintained in the protective enclosure mentioned above for several months postweaning. This 65 x 30 m shoreline enclosure was stocked daily with locally caught live prey. Additional relevant background information on the individuals in this study can be found in Table 1.

Dates of blood sampling were largely dependent on logistics. Most animals were sampled only once; Y420 and Y406 were sampled twice. Blood was obtained via the extradural vein, in the caudal lumbar area. The non-anesthetized animals were manually restrained with the assistance of nets, holding troughs, or strap boards as necessary and were cooled with water. The length of the intervertebral space was maximized by flexing the caudal portion of the animal downwards over the edge of a padded platform. The maximum angle existed at the venipuncture site, 3-4 cm craniad to the wings of the ilium and at the midline. An 18-gauge 4 in. (10 cm) spinal needle and multiple 35 cc plastic syringes were used to draw 80-90 cc of whole blood. To minimize hemolysis in handling, silicone treated glass collection tubes were used for serum samples. Tubes containing Na-EDTA were used for hemogram studies. All samples from which serum was to be obtained, including those taken in the field, were centrifuged and separated within an 2 h of collection. Serum samples held longer than 24 h before analysis were frozen; other samples were refrigerated. Hemograms from seals in the field were read within 24 h of collection.

All hematology and serum chemistry tests were performed by a commercial laboratory and all serum chemistry analysis was carried out by Accupath, a Smith-Kline Bioscience Laboratory in Honolulu, Hawaii. Tests - methods included: Total protein - Biuret; albumin - Bromcresol Green; globulin - calculated; A/G ratio - calculated; glucose - Hexokinase; blood urea nitrogen - UV endpoint; creatinine - Jaffe; uric acid - physiological saline; bilirubin - Jendrassik-Grof (modified); alkaline phosphatase - alpha-napthal phospate; aspartate aminotransferase-IFCC; alanine amino-tranferase - IFCC; lactate dehydrogenase-Wacker (modified); gamma-glutamyltransferase - Szasz & Persign; cholesterol and triglyceride - enzymatic; sodium and potassium - flame photometry; chloride - Schoenfeld & Lewellen (modified); calcium - Cresolphthelein complexone; phosphorus - Phosphomolybdate.

Some of the automated methods to determine hematologic and serum chemistry parameters are calibrated to optimally evaluate human blood. In a few cases, reported values for monk seal blood may be inaccurate. Though absolutely inaccurate, such values accepted as a normal range, will be clinically useful on a relative basis. All monk seal handling facilities in Hawaii will be submitting blood samples to commercial laboratories for analysis conducted likewise by automated equipment, calibrated to human standards. Values reported for electrolytes, glucose, uric acid, cholesterol, and blood urea nitrogen may be low in an absolute sense. Hemoglobin, hematocrit, and red blood cell counts may be reported slightly lower and white blood cell counts slightly higher than is actual.

Table 1.--Historical data: Hawaiian monk seals sampled to establish normal blood values. (FFS = French Frigate Shoals, KRF = Kewalo Research Facility, KA = Kure Atoll, and WA = Waikiki Aquarium.)

Seal	Y290	Y299	Y319	Y406	Y420	K515	K516	K517
Birth place	FFS	FFS	FFS	FFS	FFS	KA	KA	KA
Date of collection	6/1/84	5/31/84	6/21/84	6/6/85	6/29/85	5/13/85	6/1/85	8/28/85
Date of release	8/27/85	8/27/85	8/27/85	5/21/86	6/18/86	9/20/85	9/20/85	9/20/85
Age at collection (days)	[1]60	[1]64	40	[1]35	42	39	42	41
Size at collection								
Length (cm)	116	117	122.5	127	119	130	135	123
Girth (cm)	88	94	89.5	89.5	85.5	110	135	123
Weight (kg)						78	107	68
Location of temporary captivity[2]	KRF Pen at KA	KRF Pen at KA	KRF Pen at KS	WA KRF Pen at KA	WA KRF Pen at KA	Pen at KA	Pen at KA	Pen at KA

[1]Estimated ages based on one or several of the following factors: date of weaning, date of first sighting, size at weaning or first sighting, size at date of capture.

[2]All animals, from all islands are released at Kure Atoll after a transitional adaptation period in pen at Kure Atoll.

RESULTS AND DISCUSSION

Hematology

Hematologic data on the individual seals are presented to facilitate clinical evaluation in Table 2. The mean, range, and standard deviation are also calculated for each parameter.

Mean values of some hematologic parameters obtained during a study of 17 young Hawaiian monk seals on Laysan Island in 1978 are also listed in Table 2 (Gilmartin et al. 1980). The similarities in means should be noted. The means of all hematologic values are within expected ranges as compared to other phocids (Ridgway et al. 1970; Greenwood et al. 1971; Ridgway 1972) and the ranges are sufficiently narrow to believe that they accurately represent normals for this species in this age range.

As is characteristic of other marine mammals, the parameters reflecting the oxygen carrying capacity of the blood of the Hawaiian monk seal are high relative to terrestrial mammals. Such hematologic parameters reflect physiologic adaptations to their environment and activity patterns; in particular, to diving (Ridgway and Johnston 1966; Lenfant 1969). Total leukocyte numbers averaged $8.91 \times 10^3/mm^3$. Differential analysis indicated neutrophils and lymphocytes to make up a similar proportion of the white blood cell count, 47 and 43%, respectively. The ratio is consistent with other phocid species (Englehardt 1979). The remainder of the leukocytes were composed of monocytes, eosinophils, and basophils (in order of prevalence). Relatively high eosinophilias have been reported in other marine mammal species (Ridgway 1965; Tomlin 1967) and the cause of this difference between marine and terrestrial mammals is not fully understood as it does not always seem to be associated with parasitic infestation.

Two animals (Y406, Y420) exhibited abnormal clinical pictures which should be noted. The 7 November 1985 blood sample taken from Y406 revealed an elevated total protein, red blood cell count, and hematocrit, most likely indicative of a mild state of dehydration. Signs of dehydration such as decrease in skin turgor and sunken eyes were not observed and may be difficult to assess in monk seals.

Seal Y420 evidenced a blood picture consistent with a mild iron deficiency anemia on initial sampling (12 September 1985). She had pale mucous membranes and a history of an extended period of low food intake. Poor competition with her tank mate had been observed and could have caused this degree of anemia. Her growth rate relative to her age was slow and supported this diagnosis.

A second blood sample was taken from Y420 on 24 October 1985 subsequent to medical and environmental management for anemia. It showed a normal red blood cell picture and the residual evidence of a neutrophilia and monocytosis consistent with an abscess that had occurred 1 mo earlier. The leukogram data from this sample were not included in statistical determinations.

Table 2.--Hematologic values of the Hawaiian monk seal.[1]

	Seals										Laysan[2] mean			
	Y290	Y299	Y319	Y406	Y406	Y420	Y420	Y517	Y516	Y515	1978	Mean	SD	Range
Sampling date	5/3/85	4/17/85	4/17/85	9/12/84	11/7/85	9/12/85	10/24/85	9/7/85	9/6/85	9/6/85				
RBC ($x10^6/\mu l$)	3.26	3.83	3.55	3.73	4.17	3.19	3.71	3.45	--	--		3.61	0.32	3.19/4.17
Hgb (g/dl)	17.7	20.3	19.3	19.5	22.6	15.9	18.9	17.3	--	--		18.94	6.0	15.9/22.6
Hct (%)	52.2	61.2	58.9	56.8	66.1	47.0	55.8	51.7	--	--	57.1	56.21	2.04	47.0/66.1
MCV (fl)	161	161	167	152	158	147	150	150	--	--		155.75	7.01	147/167
MCH (pg)	54.4	53.0	54.2	52.5	54.4	50.1	51.1	50.3	--	--		52.5	1.81	50.1/54.4
MCHC (g/dl)	33.9	33.1	32.6	34.1	34.4	33.7	33.8	33.8	--	--		33.61	0.58	32.6/34.4
Platlets	adeq.	--	--	adeq.	incr.	adeq.	incr.	adeq.	--	--				
NRBC (/100 WBC)					Occasional Howell jolly body		1							
Total protein (g/dl)	7.0	7.2	7.1	--	8.5	6.7	6.3	8.1	6.6	6.8	7.32	7.1	0.72	6.3/8.5
Total WBC ($x10^3/\mu l$)	9.4	10.0	10.1	7.8	7.9	6.8	11.3	10.4	--	--	9.75	8.91	1.40	6.8/10.4
Segmented neutrophils (%)	5358 (57)	4100 (41)	4242 (42)	3666 (47)	3002 (38)	4080 (60)	5989 (53)	4784 (46)	--	--		4176 (47)	755.5 (8.3)	3002/5358 (38/60)
Bands (%)	0 (0)	200 (2)	505 (5)	0 (0)	0 (0)	0 (0)	0 (0)	416 (4)	--	--				
Lymphocytes (%)	3102 (33)	4600 (46)	4646 (46)	3510 (45)	4661 (59)	2448 (36)	4294 (38)	4056 (39)	--	--		3860 (43)	869.5 (8.6)	3102/4661 (33/46)
Monocytes (%)	564 (6)	500 (5)	505 (5)	312 (4)	79 (1)	272 (4)	678 (6)	728 (7)	--	--		42.3 (4.6)	215.6 (1.9)	79/728 (1/7)
Eosinophils (%)	282 (3)	600 (6)	202 (2)	234 (3)	158 (2)	0 (0)	339 (3)	416 (4)	--	--		270.3 (2.9)	192.2 (1.9)	0/600 (0/6)
Basophils (%)	94 (1)	0 (0)	0 (0)	78 (1)	0 (0)	0 (0)	0 (0)	0 (0)	--	--		24.6 (0.28)	42.2 (0.49)	0/94 (0/1)

Key:
RBC = Red blood cells
Hb = Hemoglobin
Hct = Hematocrit
MCV = Mean corpuscular volume
MCH = Mean corpuscular hemoglobin
MCHC = Mean corpuscular hemoglobin concentration
NRBC = Nucleated red blood cells
WBC = White blood cells
adeq. = adequate
incr. = increase

[1]All hematologic analysis carried out by Accupath, a SmithKline Bioscience Laboratory, Honolulu, Hawaii.

[2]From Gilmartin et al. 1980.

The seals Y406 and Y420 were the only animals sampled twice. Both had increased platelets and occasional immature red blood cells on the second sampling. This is most likely a regenerative response to blood sampling where volumes as large as 80 cc may be collected.

Serum Chemistry

Table 3 presents mean values, standard deviations, and ranges for 21 biochemical parameters of the eight seals tested. Nine samples were evaluated, since one animal was available for a second sampling.

In evaluating the results listed in Table 3, we must consider the effects of hemolysis on sodium, potassium, lactate dehydrogenase, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Several samples were slightly or moderately hemolytic. The lactate, hemoglobin and potassium rich; sodium poor cytoplasm of the red blood cells could be expected to affect results whereby the true mean serum sodium would be slightly higher than reported values and potassium, lactate dehydrogenase, and hemoglobin slightly lower.

Other values which hemolysis might affect in serum chemistry include small increases in creatinine concentrations (Dorner et al. 1981) and total protein. Studies on mammalian blood indicate that these changes are small or even insignificant and attempts to correct for such are therefore not necessary. For all hemolyzed samples, values which can be altered by hemolysis were compared to nonhemolyzed samples and although sample numbers are not large, no consistent parameter alterations were found in the few hemolyzed samples.

In the fasted animals, glucose values were not low. This is not surprising since the diet is low in carbohydrate and energy is largely dependent on gluconeogenesis. The mean blood glucose value was 111.1 mg/dl. Glucose values are within the range of other marine mammals and are slightly above the normal range for young domestic mammals (Ridgway et al. 1970; Duncan and Prasse 1981). A relatively high blood glucose may be a physiologic adaptation of diving mammals (Ridgway 1972).

The mean blood urea nitrogen of these animals, 29.4 mg/dl, is high relative to domestic animals. A high protein diet and highly gluconeogenic energy balance can account for this. The high blood urea nitrogen is of advantage in allowing the formation of hyperosmolar urine is typical of other phocids (Ridgway 1972).

Cholesterol content is fairly high as compared to domestic animals and to other marine mammal species, the range being from 184 to 568 mg/dl. Biosynthesis may account for the greatest portion of blood cholesterol since dietary fat is primarily composed of the unsaturated type (Ridgway 1972).

The lactate dehydrogenase isoenzymes associated with skeletal muscle have been observed to be high relative to domestic animals in several species of marine mammals (Ridgway 1972; Duncan and Prasse 1981). This

Table 3.--Serum chemistry of the Hawaiian monk seal; nine values from eight seals.[1]

Test	Mean	SD	Range
Total protein (g/dl)	7.1	0.72	6.3-8.5
Albumin (g/dl)	3.7	0.37	3.1-4.5
Globulin (g/dl)	3.4	0.49	3.0-4.4
Albumin/globulin ratio	1.1	0.15	0.8-1.3
Glucose (mg/dl)	111.1	17.1	88-133
Blood urea nitrogen (mg/dl)	29.4	10.2	9-43
Creatinine (mg/dl)	1.18	0.23	0.9-1.7
Uric acid (mg/dl)	1.67	0.4	1.1-2.3
Total bilirubin (mg/dl)	0.36	0.05	0.3-0.4
Cholesterol (mg/dl)	316.7	109.7	184-568
Triglycerides (mg/dl)	59.7	23.8	38-116
Alkaline phosphatase U/L	235.8	111.5	50-380
Aspartate aminotransferase U/L	65.8	56.8	10-158
Alanine aminotransferase U/L	53.4	44.0	5-115
Lactate dehydrogenase U/L	928.5	371.8	492-1544
Gamma glutamyl transferase U/L	7.7	4.6	2-16
Sodium (m q/L)	155.9	4.8	150-166
Potassium (m q/L)	5.7	0.67	4.8-6.9
Chloride (m q/L)	106	3.1	104-114
Calcium (mg/dl)	11.1	1.2	9.6-13.6
Phosphorous (mg/dl)	7.6	16.6	5.9-9.2

[1]All serum chemistry analysis carried out by Accupath, a Smith-Kline Bioscience Laboratory, Honolulu, Hawaii. Tests - methods included: Total protein - Biuret; Albumin -Bromcresol Green; Globulin - calculated; A/G ratio - calculated; glucose - Hexokinase; blood urea nitrogen - UV endpoint; creatinine - Jaffe; uric acid - physiological saline; bilirubin - Jendrassik-Grof (modified); alkaline phosphatase - Alpha-napthal phospate; aspartate aminotransferase - IFCC; alanine aminotranferase - IFCC; lactate dehydrogenase - Wacker (modified); gamma-glutamyltransferase - Szasz & Persign; cholesterol and triglyceride - enzymatic; sodium and potassium - flame photometry; chloride - Schoenfeld & Lewellen (modified); calcium - Cresolphthelein complexone; phosphorus - Phosphomolybdate.

enzyme is useful in the evaluation of myopathy and the authors have seen it become highly elevated in stress myopathy in the monk seal (unpubl. data). Hemolysis can falsely elevate lactate dehydrogenase and may occur in the absence of other serum enzyme abnormalities (Cargill et al. 1979).

Of all animals sampled for serum chemistry determination, only one (K517) exhibited values outside of what would appear to be the normal ranges. In this animal, the BUN was low at 9 mg/dl. Values that were slightly elevated include: total protein, 8.1 g/dl; globulins, 4.4 g/dl; creatinine, 1.7 mg/dl; uric acid, 2.3 mg/dl; cholesterol, 568 mg/dl; and, LDH, 1544 U/L. All other serum chemistry values fell within expected ranges. Clinically, the animal appeared to be normal. Because of the low sample numbers used to determine the normal range, it is difficult to say with certainty which, if any, of these values are abnormal. The disparity between certain related chemical parameters, such as the low blood urea nitrogen and slightly high uric acid and creatinine in conjunction with normal albumin, alanine amino transferase, and alkaline phosphatase activities, create an inconclusive picture. Therefore, values determined on this animal were included in all statistical calculations. Due to logistics, K517 could not be resampled to verify laboratory test results or establish possible ongoing trends.

All of the study seals have been released at Kure Atoll between August 1985 and June 1986 (Table 1). Resightings since their release have not indicated any apparent disease problems.

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