

Blood Values in Young Gray Seals

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THE IMPORTANCE of hematologic studies in the veterinary medical care of marine mammals, with particular reference to cetaceans, has recently been emphasized.¹⁷ This emphasis is equally applicable to pinnipeds. In general, understanding of symptomatology and diagnosis of disease in marine animals is poor, and the clinician needs to make all possible use of laboratory methods to counteract this deficiency. Various investigators have determined blood values in seals and sea lions,^{2-7,9,11,13,15,21-23} but references to the gray seal are few.^{4,21}

During the establishment of 6 young gray seals of the Eastern Atlantic race¹⁴ (from Iceland) at Cambridge for experimental studies, it was considered necessary to examine their blood in some detail. In choosing the measurements to be done and the methods to be employed, emphasis was placed on clinical value and on a combination of speed and accuracy in technique. That these values would provide a basis for further physiologic studies of the blood of the gray seal was also a consideration.

Four of the seals were maintained in a seawater pool throughout the study, while 2 were kept in a freshwater tank. All seals (3 males and 3 females) were fed on the same diet of mixed frozen fish, including mackerel, herring, and sprats. Multiple vitamin capsules were given as supplements. Blood samples were taken soon after arrival from Iceland, and again after a period of adjustment to the new environment and diet.

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Materials and Methods

Samples were taken at varying times after feeding, to give a useful range, and with as much speed as was compatible with adequate restraint. Site of venipuncture was the extradural intravertebral vein¹ in the lumbar region or one of its tributaries lateral to the vertebral column. Needles (5 cm., 19 gauge) and disposable plastic syringes were heparinized before use. The seals were restrained manually or with a net for sampling. Average time between the initiation of capture and venipuncture was about 1 minute.

Blood was collected into ethylenediamine-tetraacetic acid (EDTA) for cell and hemoglobin (Hb.) determinations, into fluoride for glucose, and into heparinized or plain bottles for plasma or serum biochemistry.

Red blood cell (RBC) counts were taken in a hemacytometer, using Strong's diluting fluid. (Trial estimations with a cell counter² calibrated for domestic animals were uniformly higher than manual estimations, whereas lower values are usually expected.^{18,21} This method has been abandoned, therefore, until more information about the size of the RBC becomes available.)

Packed cell volume (PCV) was measured by the microhematocrit method, employing a Hawkesley centrifuge.³

Hemoglobin was converted to cyanmethemoglobin with Dakin's fluid and measured in a spectrophotometer.

The Wintrobe erythrocytic indexes—mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)—were calculated from the RBC, PCV, and Hb. values.

Total white blood cell (WBC) counts were taken in a hemacytometer, using a diluting fluid of 0.4% glacial acetic acid and crystal violet.

² Coulter Electronics, Inc., Chicago, Ill.

³ Hawkesley, England.

TABLE 1—Hematologic Values from 6 Young Gray Seals

| | RED BLOOD CELL MEASUREMENTS | | | | | |
|-----------------|--|-------------------------------------|------------------------------------|--|---|--|
| | RBC ($\times 10^6/\text{cmm.}$) | PCV (%) | Hb. (Gm./100 ml.) | MCV (μ^3) | MCH ($\mu\text{g.}$) | MCHC (%) |
| Mean \pm s.d. | 5.04 \pm 0.41 | 57.2 \pm 5.8 | 19.1 \pm 2.2 | 113 \pm 10.3 | 38.0 \pm 4 | 33.4 \pm 2.5 |
| Range | 4.36-5.90 | 46.0-66.5 | 14.4-21.9 | 92-124 | 31-42 | 28-37 |
| | WHITE BLOOD CELL MEASUREMENTS | | | | | |
| | Total wbc | Neutrophils (%) | Eosinophils (%) | Lymphocytes (%) | | |
| Mean \pm s.d. | 10,663 \pm 3,063 | 65 \pm 8 | 2.8 \pm 2.7 | 19 \pm 8 | | |
| Range | 6,560-16,600 | 52-76 | 0-10 | 9-38 | | |
| | PLASMA ELECTROLYTES AND SERUM PROTEINS | | | | | |
| | Sodium ^a (mEq./L.) | Potassium ^a (mEq./L.) | Chloride ^a (mEq./L.) | Calcium ^a (mg./100 ml.) | Magnesium ^a (mg./100 ml.) | Serum proteins |
| Mean \pm s.d. | 159 \pm 5 | 4.5 \pm 0.2 | 108 \pm 3.6 | 11.2 \pm 1.0 | 3.3 \pm 0.4 | 8.0 \pm 0.8 |
| Range | 153-168 | 4.2-4.8 | 106-114 | 9.9-12.4 | 3.0-3.9 | 6.8-9.3 |
| | BLOOD CHEMISTRY VALUES | | | | | |
| | Blood urea** (mg./100 ml.) | Serum GOT (mu./ml.) | Serum GPT (mu./ml.) | Whole blood glucose ^c (mg./100 ml.) | Plasma glucose ^c (mg./100 ml.) | Packed cell glucose ^c (mg./100 ml.) |
| Mean \pm s.d. | 74 \pm 21 | 43 \pm 11 | 16 \pm 5 | 84 \pm 11 | 104 \pm 10 | 7.7 \pm 1.1 |
| Range | 32-108 | 24-59 | 11-30 | 70-94 | 90-112 | 6.2-8.7 |

* Mean, standard deviation (s.d.) and range based on measurements made at 4½ months of age. Measurements for all other determinations made at 2 and 4½ months of age. ** Blood urea nitrogen was recorded as blood urea.

Blood smears were stained with May-Grünwald/Giemsa stain, and 200 cells were counted in the differential analyses.

Sodium and potassium were measured by flame photometry. Calcium and magnesium were measured by the method of Kovacs and Tarnoky,⁸ and chloride by the method of Schales and Schales.⁹

Blood urea (BU) content was determined from heparinized whole blood by the urease nesslerization method.

Serum proteins were measured in a refractometer.⁷ (The accuracy of this instrument has been compared favorably with that of the biuret method for measurement of total protein.²⁰)

Blood glucose values were determined by the glucose oxidase method. The packed cells were obtained by centrifugation of heparinized blood, washed twice with 0.85% NaCl, and lysed in an equal volume of distilled water.

Serum glutamic pyruvic transaminase (GPT) activities were determined with a system that measures the change in optical density, with time, of a solution of serum and a prepared substrate.⁴

Discussion

The results presented here must be regarded as preliminary because of the small number of animals and samplings involved. However, ranges of the values recorded (Table 1) are of the same order as those usually offered in tables of "normal" values for domestic mammals.^{1,20} Throughout and following the study the seal pups have been clinically healthy, as judged by the criteria of general appearance, behavior, food intake, growth, and lack of any recognizable signs of disease. It is hoped, therefore, that these data will provide a working basis for the recognition and analysis of significantly abnormal hemograms.

Previous blood studies have been made of 8 of the 20 species of seals recognized as Phocidae¹⁴ in varying degrees of detail, using both captive and free-living animals.^{2-7,9,11,13,18,21-23} Most of this work has been directed toward an understanding of the physiologic adaptations of seal blood to a submarine existence, and current knowledge on this aspect has recently been summarized.^{10,16} Suffice it

^c ATAGO Optical Works Company, Ltd., Japan.

^d Boehringer Mannheim, Mannheim, Germany.

to say, the gray seals in this study can be seen to have the general pattern of phocid adaptations (e.g., high PCV, high Hb., and large red blood cell volume).

Hematologic measurements have been made on young harp seals (*Pagophilus groenlandicus*),¹⁸ on the harbor seal (*Phoca vitulina*),^{5,16} and on the northern elephant seal (*Mirounga angustirostris*).^{5,16,22} One group¹⁸ concluded that a total WBC count in excess of 12,000/cmm. invariably indicated an abnormal condition, and another investigator⁵ stated that a WBC in excess of 10,000/cmm. in any pinniped was considered to be abnormal. In view of our high mean value and range extending up to 16,600/cmm., we are not in agreement with these considerations, at least as regards the young gray seal. Furthermore, none of the high WBC counts found here were particularly associated with neutrophilia or left shift; indeed, lymphocyte values seemed to follow the total count more closely than did neutrophils. Of particular interest is the high mean monocyte value found in the gray seals—considerably higher than was found by others in the harbor seal.^{5,9} We have noticed in making smears for differential analysis that seal monocytes appear to be particularly fragile, and great care is needed to assure an accurate count. In the dog, monocytosis is considered to reflect stress,²⁰ as well as chronic inflammation, and it might have been that a stress effect was operating here. Certainly a mean monocyte level of 13% is higher than normally recognized for any domestic mammal.²⁰

Electrolyte concentrations for the gray seal tend to the high part of the range for terrestrial carnivores, although only sodium values are greatly increased. The range of sodium values in the gray seal extends somewhat higher than the ranges for the harbor seal and some otariids.⁵

Comparatively high BU and blood glucose values are commonly found in marine mammals^{15,16} and do not reflect a pathologic state. They are most probably related to a diet consisting almost entirely of protein and fat, and reflect

an energy metabolism dependent to a large extent on gluconeogenesis. Those values from samples taken 1 to 2½ hours after feeding were generally higher than the remaining values obtained from fasting seals. The exact role of glucose and the details of carbohydrate utilization in the metabolism of marine mammals remain to be elucidated. Some preliminary studies on glucose tolerance, insulin response, and carbohydrate metabolism have been reported recently.¹⁶ Our values were determined to allow comparison with methods that measure plasma glucose (e.g., autoanalyzers) and indicate mean whole blood values 23% lower.

Little is known of the clinical information to be obtained from serum enzyme activity estimations in marine mammals. In recent years they have attained considerable importance in the assessment of liver disease in domestic animals, and necropsy work has indicated this to be an important pathologic change in young pinnipeds.

In conclusion, we make a plea for standardization and accurate detailed recording methods used in clinical studies of marine mammals. It is clear that any attempt to utilize figures obtained from any one species for clinical assessment of another would be dangerous. Only if similar methods are used can the important physiologic and pathologic significance of different results be clearly interpreted.

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