

from these initial observations that the comparison of fluorescent banded karyotypes will significantly enhance the potential contribution which karyotypic analyses can make to the resolution of the phyletic interrelationships of the modern Cetacea.

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MFR PAPER 1052

Some Physiological Parameters of the Blood of the California Gray Whale

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ABSTRACT

Hematocrit, a O₂-Hb dissociation curve, and blood volume have been determined for a California gray whale, Eschrichtius robustus, and the results are compared to some physiological blood properties of other cetaceans. The E. robustus has a blood volume that is similar to values estimated for large whales by other authors. This is the first time isotopic techniques have been used to determine a large cetacean's blood volume.

Large cetaceans do not appear to follow the trend of most terrestrial mammals when the body size and P₅₀ are compared. The P₅₀ for the E. robustus was 36.5 mm Hg and is the highest reported for any cetacean.

The determination of the physiological properties of the blood of large cetaceans has been confined primarily to animals that are stranded or have been dead for many hours before blood samples can be drawn. Lenfant (1969) has summarized most of the data available on marine mammals. The capture and maintenance of Gigi, a California gray whale, *Eschrichtius robustus*, has given us the

opportunity, for the first time, to study a large cetacean under definable conditions and to determine its blood volume and oxygen-hemoglobin dissociation curves.

METHODS

On two separate occasions the whale was given 10 μ Ci of radioiodin-

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ated human serum albumin (Risa)¹. The labeled compound was administered to the animal in one of the brachial vessels in the right pectoral fin. Two blood specimens were taken following each determination to insure that mixing was complete and the albumin was not being eliminated rapidly from the serum. In the first test (27 December 1971) blood volume determination samples were taken at 14 and 20 minute intervals and in the second test (6 March 1972) were collected at 10 and 18 minute intervals after administration of the labeled compound. The blood samples removed for counting were taken from one of the brachial vessels of the left pectoral fin and put into well-heparinized tubes. Three ml of the heparinized whole blood was added to 3 ml of 1

¹ Abbott Laboratories, Chicago, Illinois. Use of trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

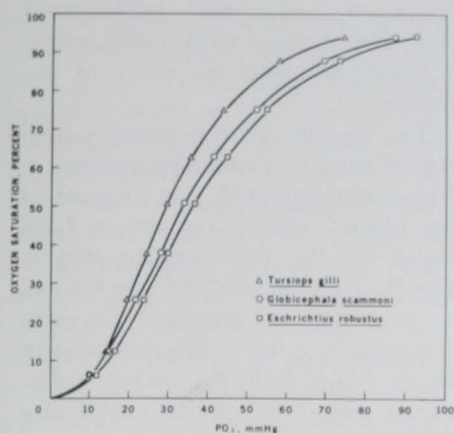


Figure 1.—Oxyhemoglobin dissociation curves for three cetaceans (*T. gilli*, *G. scammoni*, *E. robustus*). All curves have been corrected for pH = 7.4 and determined at 37°C.

percent acetic acid to lyse the red cells to permit counting of a uniform suspension without the problems associated with cells settling while being counted. A standard solution was prepared by adding a fraction of a milliliter of the same solution injected into the animal to saline in a total volume of 1 liter. The standard was prepared for counting by the same procedure as the blood specimens.

All samples were counted for 100 minutes in a 3-inch well TII crystal attached to a Packard Model 2001 Spectrometer Scaler-timer. Counts of the paired blood specimens were very close, within 5 percent in December and 3 percent in March. The reported blood volumes are the mean values of the respective paired samples. The oxyhemoglobin dissociation curve was determined on 21 January 1972. A 20 ml blood sample was secured from a puncture of a distal brachial vein the the pectoral fin. The blood was immediately placed into well-heparinized (250 units heparin per 10 ml blood) plastic test tubes, inverted 4 or 5 times, and then placed in an ice bath.

Less than 4 hours after collection the dissociation curve was completed by a Dissociation Curve Analyzer (DCA-1, Radiometer, Copenhagen). Duvelleroy et al. (1970) have explained in detail the methodology involved in the operation and construc-

tion of the O₂-Hb dissociation curves by the DCA-1. Slight changes in pH were monitored for every point on the dissociation curve. Because P_{O₂} changes with pH variation, corrections were made to a pH of 7.4 with the equation

$$\frac{\Delta \log P_{O_2}}{\Delta \text{pH}} = \text{Bohr effect.}$$

The value for the Bohr effect was obtained from Lenfant (1969).

The hematocrit (Hct) was obtained in the usual manner. A Clay-Adams Autocrit Centrifuge was the instrument used.

RESULTS

The hematocrit, blood volume, and P₅₀ of the *E. robustus* as well as certain physiological blood parameters of other cetaceans are presented in Table 1. The blood volume was determined on two occasions and the oxygen binding capacity was determined with one blood sample. Typical sigmoid O₂-Hb dissociation curves are shown in Figure 1. Curves for two other cetaceans, *Globicephala scammoni* and *Tursiops gilli*, determined by the same methods (Antonelis, 1972)² are included.

DISCUSSION

The hematocrit of Gigi is not unlike that measured in other mammals. While Lenfant (1969) asserts that this is true for all marine mammals, Ridgway and Johnston (1966), Horvath et al. (1968), and Ridgway et al. (1970), have demonstrated an increased packed cell volume in some of the small cetacea. Lenfant (1969) attributes such results to differences in technique or in physical condition. However, the animals who showed high hematocrits were maintained in captivity. Had they followed the normal pat-

tern of captive animals, the values would have been even higher if sampled in their natural environment. It has been amply demonstrated that animals brought into a captive situation soon show a reduction in both hematocrit and hemoglobin content (Gilmartin and Ridgway, 1969,³ Lenfant, 1969).

The first blood volume for a large cetacean using isotopic methods is reported. Although I¹³¹ labeled human serum albumin was used in the analysis, the similarity of the paired blood specimens taken at each test date indicates not only that mixing was complete, but also that this foreign protein was not being eliminated so rapidly that a meaningful blood volume determination could not be made. Unpublished data on the killer whale are included also. Both animals have blood volumes (*E. robustus*: 6.1 and 8.1 percent; *O. orca*: 8.2 percent) within the range reported for other species of large cetaceans; Laurie (1933) reported a large blue whale's blood volume as 6.6 percent. Smith and Pace (1971) estimate that the blood and body fluids of large cetaceans to be between 10 and 15 percent of the body mass.

Lawson (1962) and Sjöstrand (1953, 1962) have reviewed the many factors which affect blood volume and one should be aware of them when evaluating blood volume data. Since the animal is placed under highly stressful conditions as well as the imposition of unaccustomed gravitational forces as a result of removal from the water, the picture for marine mammals is complicated.

Nutrition and electrolyte balance also affect blood volume. To our knowledge neither the freezing point depression nor the osmolality of the urine were determined. Osmolality can be calculated, however, using the formulas of Wolf (1958). Gigi's exclusive squid diet must have produced a urine whose minimum osmotic con-

² Antonelis, G. A. 1972. O₂-Hb dissociation curves of the pilot whale, *Globicephala scammoni*, and Pacific bottlenose porpoise, *Tursiops gilli*. (Unpubl. manusc.)

³ Gilmartin, W. G., and S. H. Ridgway. 1969. Some physiological properties of the blood of the killer whale, *Orcinus orca*. (Unpubl. manusc.)

Table 1.—Some physiological properties of the blood of cetaceans.

Species	Sex and reference	Body weight (kg)	Hematoctrit (%)	Hemoglobin (g/100 ml)	Mean cell Hb content (%)	Derived O ₂ capacity (ml/100 ml)(8)	Derived O ₂ capacity (ml/kg)	Blood volume (ml/kg)	Measured P ₅₀ (mm Hg)	Calculated P ₅₀ (mm Hg)(9)	Bohr effect
Gray Whale <i>Eschrichtius robustus</i>	Immature ♀ (1)	(614-609)	40.0	14.1	30.5	19.2	1,553	61	36.5	21.8	0.456
	Adult (2)	(715-364)	42.6	12.8	33.3	17.4		81	30.0		0.538
	Fetus (2)		44.6	13.9	32.1	18.9			19.0		
Pilot whale <i>Globicephala scammoni</i>	Immature ♀ (1)		39.7	15.1	38.0	20.5			34.0		0.622
	(2)								31.2		
Killer whale <i>Orcinus orca</i>	Immature ♂ (5)	2,525	48.0	17.8	37.0	24.2	1,985	82	30.7	22.7	0.738
	(2)		43.9	16.0	36.4	22.5					
Sperm whale <i>Physeter catodon</i>	(2)		42.0	14.7	35.0	20.0			26.5		0.478
	(1)										
Pacific bottlenosed dolphin <i>Tursiops truncatus gilli</i>	(1)		50.5	18.2	36.0	24.8			29.8		0.712
	(2)								26.0		
Atlantic bottlenosed dolphin <i>Tursiops truncatus</i>	(2)		43.0	15.9	37.0	21.6		71	26.8		0.664
	(3)		45.0	14.4	32.0	19.6	1,390		24.6	26.4	
	(4)	(10) 159	41.7						21.6	28.3	
	(4)	(10) 43	46.7								
Common dolphin <i>Delphinus delphis</i>	(2)		51.2	18.7	36.5	25.4			24.8		0.717
	(3)		53.0	17.0	32.0	23.1	2,497	108	20.2	27.2	
Pacific white sided dolphin <i>Lagenorhynchus obliquidens</i>	(4)	(10) 88	48.9								
	(3)		57.0	20.3	35.6	27.6	3,948	143	19.1	26.6	
Dall porpoise <i>Phocoenoides dalli</i>	(4)	(10) 133	53.6								
	(3)										

- (1) Antonelis (1972).
 (2) Lenfant (1970).
 (3) Ridgway and Johnston (1966).
 (4) Horvath, et al. (1968).
 (5) Gilmartin and Ridgway (1969).
 (6) Weight on 12/28/71.
 (7) Weight on 3/6/72.
 (8) Derived by Hb concentration X 1.36, Bernhart and Steggs (1943).
 (9) Calculated by: $P_{50} = 50.34W^{-0.04}$, Schmidt-Neilsen.
 (10) Average.

centration was 1,670 milliOsmols/liter. The effect of such a diet, which is isoosmotic with sea water, is unknown.

The P_{50} , the partial pressure (mm Hg) of oxygen at which hemoglobin is 50 percent saturated, is a measure of the blood's affinity for oxygen—the higher the P_{50} , the lower the affinity. The P_{50} for Gigi was 36.5 mm Hg and is the highest reported for any cetacean. In a list of fifty mammalian species in which the P_{50} has been determined, only four animals exhibited a higher P_{50} (Bartels, 1971).

Horvath et al. (1968) compared the dissociation curves of several small cetacea and found that a shift to the left, or increased affinity for oxygen, relates to individual species behavior and feeding habits. Apparently this pattern does not hold for the larger cetaceans.

In looking at such parameters as hematocrit, oxygen capacity, and particularly the P_{50} , a pattern emerges and one is tempted to ascribe this to some behavioral characteristic of the animal. However, one should proceed with caution.

Oxygen dissociation curves are determined by several methods. The method used by Horvath et al. (1968) was to treat the blood with varying levels of oxygen and sufficient CO₂ to maintain a pH of 7.4. Lenfant (1969) and Schmidt-Neilsen and Larimer (1958) maintained a P_{CO_2} of 40 mm Hg in determination of their curves. Schmidt-Neilsen and Larimer (1958) observed that in terrestrial animals the blood of the larger animals has the higher affinity for oxygen (low P_{50}). Lenfant (1969) pointed out that this does not hold for marine mammals and the calculated P_{50} 's using their formula supports this.

Steen (1971) has pointed out that a P_{CO_2} of 40 mm Hg may not represent the true arterial P_{CO_2} of the animal, e.g., a cat is about 28 mm Hg. Since the P_{CO_2} has a profound effect on the P_{50} and the magnitude of the Bohr shift, one is hard put to make meaningful comparisons in marine

mammals when different methods are used. To complicate the picture further, Riggs (1960), using a buffered system at a pH of 7.4, observed that animals of varying size had identical P_{50} 's at that pH.

In order to make meaningful evaluations of the dissociation curves in marine mammals, the *in vivo* P_{CO_2} and pH need to be determined. Rieu and Hamar (1968) point out the difficulties of drawing a representative arterial blood sample although these arterial data have been collected from one species, *Tursiops truncatus*, by Ridgway (1968). In short, more work needs to be done.

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MFR PAPER 1053

Feeding of a Captive Gray Whale, *Eschrichtius robustus*

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ABSTRACT

The feeding of a captive yearling female Eschrichtius robustus was observed while diving with her as well as from the surface. She sucked food off the bottom while swimming tipped over about 120° so that her cheek was nearly parallel to the bottom. An increase in mouth volume is apparently caused by action of the tongue, resulting in strong suction, during which the lower lip is opened and food enters the mouth. How food is separated from water and mud or detritus is not known. The observed behavior is probably natural and illuminates earlier records of stomach contents, external markings, and asymmetrical baleen. Clearly, much needs to be learned about the mechanism of feeding of baleen whales. This species' feeding habits may be unique among them.

INTRODUCTION

Observations on the food of *Eschrichtius robustus* (Lilljeborg, 1861), the gray whale, have been summarized by Zimushko and Lenskaya (1970) and Rice and Wolman (1971), indicating that the diet consists predom-

inantly of benthic animals, mostly amphipods and a few other crustaceans; incidental items include polychaete worm tubes, shells, gastropod opercula, feathers, kelp, bits of wood, sand, mud, and gravel. Tomilin (1957, p. 346-347) suggests that *Eschrichtius*