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Physiological and ecological implications of hemorheological variations in marine and terrestrial mammals

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PHYSIOLOGICAL AND ECOLOGICAL IMPLICATIONS OF HEMORHEOLOGICAL VARIATIONS IN MARINE AND TERRESTRIAL MAMMALS

A

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PHYSIOLOGICAL AND ECOLOGICAL IMPLICATIONS OF HEMORHEOLOGICAL VARIATIONS IN MARINE AND TERRESTRIAL MAMMALS

by

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ABSTRACT

The possible significance of variations in interspecific hemorheological properties related to diving behavior was studied in eight species of marine mammals with humans and pigs as terrestrial controls. Diving duration was positively correlated with elevated blood hemoglobin, oxygen capacity and viscosity among animals of the same class. No acclimatization response to activity was evident from studies of blood drawn from newly-captured northern elephant seals and sea otters and those in captivity for extended periods which justified the use of captive animals for rheological studies. Adaptations of marine mammals to diving were evident from comparisons of phocid seal and pig hemorheology. Seals had increased oxygen storage (six times) with less viscosity-dependent reductions in oxygen transport (-22%) when compared to pigs at equal packed cell volume. Phocid seal blood samples were compared with those of pigs and humans for erythrocyte aggregation and blood viscoelasticity to study the mechanics of viscometric variations. Viscous and elastic components of seal blood viscosity were 20 to 73% lower than those of pigs due to decreased aggregation extent and rate ($P<0.05$). Lower

plasma fibrinogen and increased erythrocyte electrophoretic mobility are believed to contribute to lowered seal blood aggregation.

Comparisons of the *in vivo* effects of blood viscosity on whole body and myocardial oxygen consumption by manipulation of whole body hematocrit in seals and pigs revealed that optimal hematocrit ranges for seals were shifted to the right of those from pigs (SEALS: 25%-55%; PIGS: 25%-45%; $P<0.05$). Seals showed significantly less viscosity-dependence in total body oxygen transport and oxygen consumption than did pigs. Myocardial oxygen consumption data were variable and showed no statistically significant differences among seals and pigs. The seals' lower erythrocyte aggregation, decreased low-shear viscosity and a greater ability to compensate for viscosity changes may represent adaptations to reduce the stress necessary to reinitiate flow in stagnant venous sinuses thereby reducing blood-flow resistance during dive-recovery. These adaptations may help maintain circulatory perfusion to vital organs, while flow is restricted to less oxygen-dependent tissues during underwater submergence without sacrificing the advantage of increased blood oxygen storage.

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CHAPTER I.
GENERAL INTRODUCTION

THE PROBLEM

The adaptations made by marine mammals to their oceanic existence have been studied and documented many times since the pioneer studies of Irving and Scholander in the 1930s and 1940s (Irving, et al., 1942; Scholander, et al., 1942). Many of these studies revealed the increased oxygen carrying capacity of marine mammals which enables them to accomplish long forays underwater (Lenfant, 1969; Lenfant, et al., 1970) while others investigated general cardiovascular function (Elsner, 1969) and architecture (Harrison and Tomlinson, 1956; Elsner, et al., 1964 and 1971; Rhode, et al., 1986). Many marine mammalogists have sought to relate the physiology of the animals to their ecology in terms of diving behavior and feeding strategies (Medway and Geraci, 1964; Ridgeway, et al., 1970; Le Boeuf, et al., 1986). Hemoglobin concentrations are the major determinants for oxygen-carrying capacity of the blood and have been shown to increase with the degree of activity in three delphinids: the coastal bottle-nosed dolphin, Tursiops truncatus, the more pelagic Pacific white-sided dolphin, Lagenorhyncus obliquidens, and the

fast-swimming, deep-diving Dall porpoise, Phocoenoides dalli (Ridgeway and Johnston, 1966). Additionally, differences in hemoglobin values, hematocrit (HCT) and red cell counts have been shown to distinguish coastal vs. offshore ecotypes of Tursiops, the latter having higher values for all three variables (Duffield, et al., 1983).

Seals of the family Phocidae are considered the most well-adapted members of the Pinnipedia in terms of diving physiology (Harrison and Kooyman, 1968). Many phocid seals have extremely high HCTs of 55 to 65% (Lenfant, 1969) which, in addition to their large blood volumes (Bryden and Lim, 1969), gives them the ability to dive for extended time periods (Kooyman, et al., 1972; Le Beouf, et al., 1986). However, Ashwell-Erickson found that the aerobic scope (the number of times an animal can raise its oxygen consumption above the basal level) of harbor seals trained to tread water while carrying weights was small, approximately 3 to 4 (Ashwell-Erickson, 1981; Elsner and Ashwell-Erickson, 1982).

Further investigation revealed a large anaerobic component to the total metabolic scope of these seals (Elsner, 1986) and the capability of the seal heart to function anaerobically (Kjekshus, et al., 1982). However, in most situations, it is believed that aerobic

metabolism is used because of its greater energetic efficiency (Kooyman, et al., 1980). Why then is the aerobic capacity of harbor seals so low?

It has been shown that the physical characteristics of the blood such as hematocrit, red cell size and shape affect its flow properties (Stone, et al., 1968). Extremely high HCTs such as those exhibited by phocid seals reach the range where, in humans and terrestrial animals, further increases in the blood's viscosity have been shown to decrease blood flow to tissues, resulting in reduced tissue metabolism as measured by oxygen consumption (Messmer, et al., 1972; Fan, et al., 1980). This led to the hypothesis that the low aerobic scope of seals is due to the high percentage of erythrocytes in their blood which increases its viscosity and hence, reduces the flow rate of blood to the tissues resulting in lowered oxygen consumption.

Are marine mammals qualitatively or quantitatively different from terrestrial mammals in terms of blood flow behavior (hemorheology)? The high hematocrits possessed by harbor seals, Phoca vitulina, make them ideal models for such investigations. Domestic pigs, Sus domesticus, were chosen as terrestrial mammalian models due to their similarity to humans in terms of cardiovascular architecture (White, et al., 1986), their popularity in

clinical research (Mersmann, 1986; Bloor, et al., 1986) and their overall tractability (McKirnan, et al., 1986).

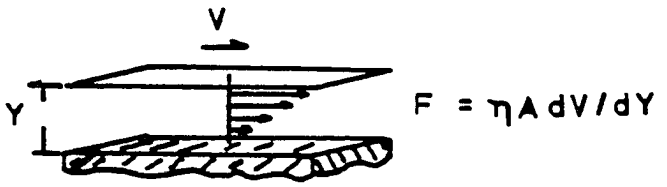
Studies of marine mammal hemorheology are few and incomplete, having been concerned more with adaptations to temperature extremes than with the physical effects of hematologic variables and comparisons among different species (Guard and Murrish, 1975).

Although hematocrit values and other hematologic variables had been studied for many marine mammal species, an investigation of the direct effects of these characteristics on blood viscosity had not been undertaken. The size and habits of most marine mammals make them difficult to study in the wild. However, viscosity measurements and corresponding hematologic characteristics from marine mammals with differing lifestyles and during different stages of development and comparisons between newly-captured animals and those maintained in captivity for extended time-periods was considered a reasonable approach. The hypotheses that the ecology of different marine mammal species may be reflected in their hematology and hemorheology and that the physiological constraints of captivity may be apparent from hematologic and hemorheologic data from comparisons of free-ranging and captive animals of the same species were examined.

RHEOLOGY OF BLOOD

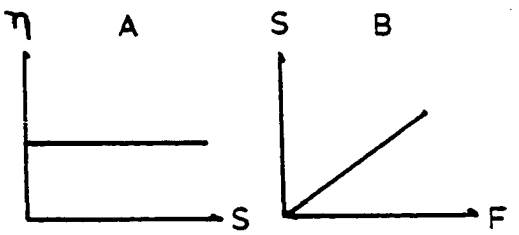
The flow of most fluids through tubes increases in direct proportion to the pressure exerted upon them; such fluids are said to be Newtonian (Figures 1.1 and 1.2).

Viscosity is the ratio of the shearing force to the shear rate. Blood is a non-Newtonian fluid as it is most viscous at low flow rates (Haynes and Burton, 1959). Blood rheology or "hemorheology" involves studying the seemingly opposing forces of different variables upon blood flow in small tubes due to changes in its viscosity (Table 1.1). Additionally, blood behaves differently in glass tubes than in blood vessels, as in vivo values for blood viscosity are considerably reduced (Djojosugito, et al., 1970). Increases in viscosity at low rates of shear are due mainly to the interactions among erythrocytes and their interactions with plasma proteins (Merrill, et al., 1963a and b; Gregersen, et al., 1965; Merrill, et al., 1965; Merrill and Pelletier, 1967; Chien, et al., 1966; Chien, et al., 1970), while at high shear rates the deformation, orientation and internal viscosity of erythrocytes are the major determinants (Scott-Blair, 1958; Seaman and Swank, 1967; Dintenfass, 1968; Goldsmith and Beitel, 1970; Schmid-Schönbein, et al.,



F = Force in Dynes
η = Absolute Viscosity, Poise
A = Surface Area, Sq. Cm.
V = Velocity, Cm/Sec
Y = Plate Separation, Cm
dV/dY = Rate of Shear, Inv. Sec.

Figures 1.1. Schematic representation of shear generated within a fluid during flow and the definition of viscosity (adapted from Brookfield, 1959).



Newtonian Fluid

Figure 1.2. Plots of viscosity (η) versus shear rate (S) and of shear rate versus shear stress (F) for a Newtonian fluid (adapted from Brookfield, 1959).

1973). Increased shear rates in arterioles and venules reduce the effect of viscosity. However, in capillaries, blood flow is reduced and the effects of viscosity are most influential (Merrill, et al., 1963b). Increased shearing force is necessary to accomplish blood flow in capillaries since energy must be expended to deform erythrocytes and force them through diameters smaller than their own (Stone, et al., 1968). The wall shear stress is also greater in the microcirculation (LeVeen, et al., 1980). Thus, the shearing force required to circulate blood through the capillaries should be directly proportional to the concentration of red blood cells.

Apparent viscosity decreases as flow velocity increases regardless of tube size (Haynes, 1960). This anomalous flow behavior is due, in part, to the axial migration of erythrocytes, leaving an almost cell-free layer of plasma next to the vessel or tube wall (Thomas, 1967; Goldsmith and Beitel, 1971). The erythrocytes deform and act as fluid drops with "tank-treading" membranes, orienting themselves so that their flattened surfaces are parallel to the vessel wall which increases their rate of axial flow (Schmid-Schönbein, et al., 1971). The surface condition of the tube (or vessel) is thought to influence this effect which is often referred

to as the Copley-Scott Blair phenomenon (Copley and Scott-Blair, 1961). There are additional anomalies resulting from various rheological variables (Table 1.1). Fibrinogen induces changes in the electrostatic charge of red blood cell membranes which causes aggregation (Merrill, 1969) and a corresponding increase in blood viscosity. The Fahraeus-Lindquist effect (Fahraeus and Lindquist, 1931) involves the reduction in viscosity of blood in tubes and vessels with a diameter less than 500μm. This phenomenon has been explained by the existence of the plasma layer next to the vessel wall and a finite thickness of shearing laminae in the blood which changes with changing hematocrit, the so-called "sigma effect" (Scott-Blair, 1958). Additionally, there is a cell-screening effect or "Fahraeus Effect" with decreasing capillary radius whereby the hematocrit of the blood entering a tube of reduced diameter is greater than that of the blood leaving the tube (Fahraeus, 1929; Gaeghtens, et al., 1978). Dintenfass (1968) found an inversion of the Fahraeus-Lindquist phenomenon in blood flow through tubes of diameter less than 0.3mm.

Table 1.1. Factors affecting blood rheology.

VARIABLE	EFFECT	CONSEQUENCE	REFERENCE
temperature decrease	decreased fluidity	increased viscosity	Barbee (1973)
increased hematocrit	increased internal friction	increased viscosity	Merrill (1963)
RBC-Protein interactions	red cell aggregation	rouleaux-formation and 3-dimensional rouleaux structure, increase in blood viscosity near stasis (non-Newtonian), elastic properties of blood in stasis	Chein, et al. (1966), Schmid-Schönbein, et al. (1968, 1973), Murata (1976)
RBC-fluidity: internal viscosity, membrane flexibility, biconcave shape (surface area/volume not minimized), ATP content, oxygen content, blood osmotic pressure, salinity	deformation of RBCs in flow, axial migration, ability to pass narrow channels (diapedesis)	decrease in blood viscosity at high shear rates (non-Newtonian flow behavior)	Dintenfass (1968), Meiselman (1981), Schmid-Schönbein, et al., (1971), Goldsmith (1971), Volger, et al.(1973)
plasma proteins (fibrinogen, albumins, globulins)	colloid osmotic pressure of plasma	determines viscosity of plasma, may cause thixotropy	Chein, et al. (1970), Zweifach and Intaglietta (1971)
RBC geometry	changes cell rheology	variable changes in viscosity	Gregersen, et al. (1965), Stone, et al. (1968)
RBC specific gravity	sedimentation during stasis	non-homogenous blood viscosity, two-phase flow	Fahraeus (1929), Schmid-Schönbein (1981)
HCT change after flow from large bore to small bore	"Fahraeus Effect" "screening effect"	decreased viscosity in small vessels (less than 120um)	Fahraeus (1929), Gaehtge et al. (1978)
plasma layer next to vessel wall	"Fahraeus-Lindquist Effect" "sigma effect", occurs where	decreased viscosity in small tubes or vessels (less than 500um)	Fahraeus and Lindquist (1931)
wall surface condition	"Copley-Scott Blair Phenomenon"	reduces *in vivo* viscosity relative to *in vitro*	Copley and Scott-Blair (1961), Djojosugito, et al. (1970)
RBC electrostatic charge	RBC interactions	increased negativity causes repulsion, reduced charge enhances RBC aggregation resulting in changes in blood viscosity	Seaman and Swank (1967), Schmid-Schönbein, et al. (1973, 1975)

VISCOMETRY

In the 1920s an American physicist and chemist named Charles Bingham became interested in the flow properties of materials like paint, ink, paste and clay, and drew attention to the importance of the science of deformation and flow of materials (Oka, 1981). Shortly thereafter, in 1929, the American Society of Rheology was established. Bingham called the science of the deformation and flow of materials rheology - "rheo" means flow in Greek. Flow is defined as deformation proceeding irreversibly with time.

Although classical theories for deformation and fluid mechanics already existed, rheology was developed because many classical theories such as Hooke's Law (i.e. strain is proportional to stress) and those of fluid mechanics were based upon Newtonian flow behavior which many fluids do not obey. Rheology was developed to overcome these problems and to present a more comprehensive viewpoint reflecting the variety and individuality of materials. To rheologists, the relationships between mechanical properties and their structures are most important.

Rheology originated in industrial manufacturing and is useful in the production of many products of the petroleum industry: rubber, plastics, as well as ceramics, food, cosmetics, paint. The consistency of

nearly every fluid product on the market is tested using viscometry before it is put on a shelf in the store for the consumer.

Rheology is an interdisciplinary science having close associations with physics and fluid dynamics, as well as mathematics and chemistry. This has led to the interest among scientists in such fields as oceanography, biology, chemistry, engineering and medicine. The application of rheology to biological sciences led to the distinction of Biorheology coined by Copley in 1952. Biorheology deals with rheological phenomena in living organisms and the flow behavior of materials such as blood, lymph, cerebrospinal fluid, synovial fluid, sputum, saliva, cervical mucous, protoplasmic streaming, deformation of cells (erythrocytes, embryos), deformation of soft tissues such as blood vessels, skeletal muscle, myocardium, bladder, mesenteries and connective tissues (cartilage, bone, eye lens) and body solutions (nucleic acids, polysaccharides). The most well-studied subfield is hemorheology - the study of deformation and flow in blood and blood vessels. Instrumentation has been developed for use in bio- and hemorheology. However, the same instruments may be implemented in a broad spectrum of industrial and scientific studies (Oka, 1981).

An instrument which measures viscosity is called a

viscometer. The first viscometers consisted of hollow tubes. Poiseuille (1841 and 1842) studied the rheology of fluids in tubes and formulated the following equation:

$$F = (p_1 - p_2)\ (r^4 / 8 \eta\ l)$$

where F equals the flow ($cm.sec^{-1}$), $p_1 - p_2$ equals the driving pressure or pressure difference between the ends of the tube ($dynes.cm^{-1}$), r is the tube radius (cm), l is the tube length (cm) and η is the viscosity of the fluid in poise (see Figure 1.3). Blood is added to the reservoir to a height (h) while flow is retarded by a stopcock at the outflow tube. The stopcock is then released and the time it takes for the meniscus on the reservoir to move is measured by stopwatch. Viscosity (η), shear stress (τ) and shear rate ($\dot{\gamma}$) are calculated as follows:

$$\eta = \frac{(\rho)(g)(h)r^4\ \pi\ t}{8\ L} \quad \text{(poise)}$$

$$\tau = \frac{P\ r}{4L} \quad \text{(where } P = (\rho)(g)(h)\text{)} \quad (dynes\text{-}cm^{-1})$$

$$\dot{\gamma} = \frac{\tau}{\eta} \quad (sec^{-1})$$

where (ρ) equals the specific gravity (g/cm^3), g is gravitational acceleration (980 cm/s^2), h is the average height of the fluid column during the time-period measured (cm), r is the capillary radius (cm), π is

equal to 3.142, t is the time (s), and L is the length of the capillary (cm). Blood viscosity is additionally dependent upon packed red cell volume (hematocrit), temperature and tube diameter (Table 1.1).

Capillary viscometers or tube viscometers have been popular since the time of Poiseuille for hemorheologic work and a modified capillary viscometer was used during this study. A generalized schematic for the capillary viscometer made after the work of Haynes and Burton (1959) is shown in Figure 1.3. Capillary viscometers are inexpensive and easy to make and therefore, a practical means of studying flow characteristics of materials. The flow in this type of apparatus more closely approximates that in vessels than non-tubular types. Turbulence is also negligible if the tube length is 175 times that of the tube radius (Haynes and Burton, 1959). Additionally, tubes of different bore can be utilized to approximate differing blood vessel sizes. A set of equations derived from that of Poiseuille can be used to determine rheologic variables (Figure 1.3). The complete apparatus can be encased in a watertight chamber and fitted with an input and output valve for a circulating waterbath to allow temperature regulation. One disadvantage of this type of viscometer is the limited range of shear rates obtained. Shear rate and the hydrostatic pressure are

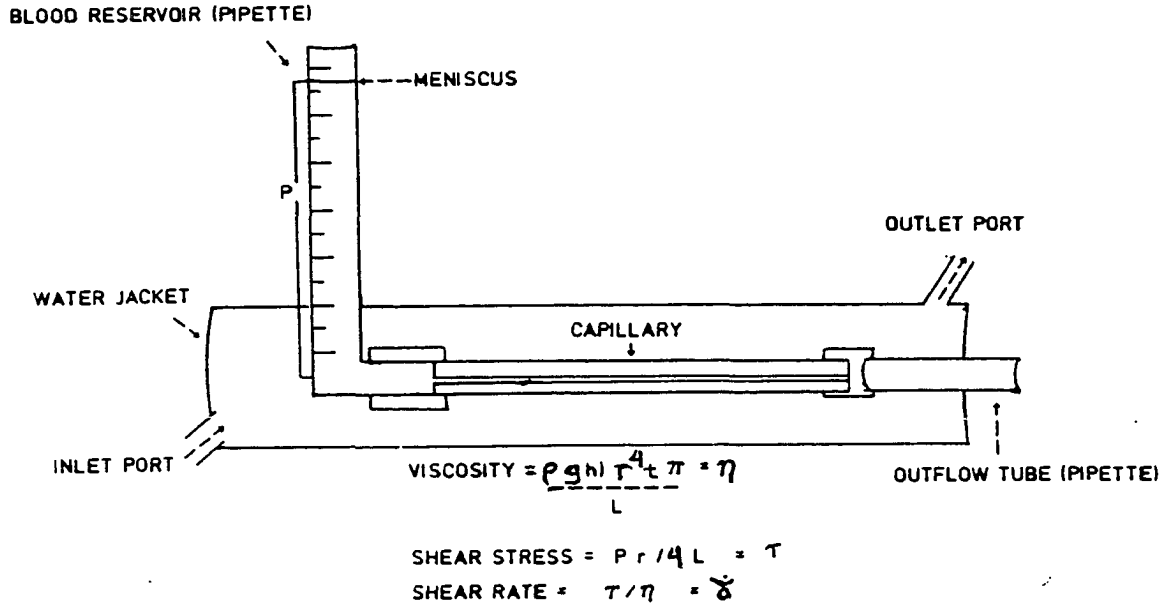


Figure 1.3. Generalized plan of the capillary viscometer used in this study. ρ is the fluid specific gravity, g is gravitational acceleration, h is the average height of the fluid meniscus during measurement, r is the capillary diameter, t is the time required for the meniscus to travel a fixed distance, π is equal to 3.142, and L is the capillary length. Temperature is held constant by water circulating from the outlet port to a constant temperature bath and back to the water jacket through the inlet port.

dependent variables as shear rate depends upon the hydrostatic pressure, the fluid's viscosity and tube size while the hydrostatic pressure is determined by the specific gravity and the volume of fluid in the reservoir. The time necessary for capillary experiments is increased by the absolute necessity for thorough cleaning of the instrument between runs. The limited time available for running each sample precluded the use of more than one tube size. Therefore a capillary of 500μm radius was chosen for the range of shear rates it produced. However, sample volumes can be reduced by adjustment of the reservoir size, alleviating the necessity of large volumes of blood usually needed for the operation of the Ostwald type of viscometer. Accuracy is reported at 1-2% and calibration with water or oils of known viscosity is possible.

A second-type of viscometer is the cone-plate (Brookfield Engineering - see Figure 1.4). This viscometer is used for many different fields of rheology. Differing spindle and cup sizes are available to accomodate different viscosity ranges. The cone-plate viscometer utilizes a rotating cone and a stationary plate (the bottom of the sample cup) to set up internal friction within the sample fluid (Figure 1.5). The resistance to rotation of the cone due to the viscosity

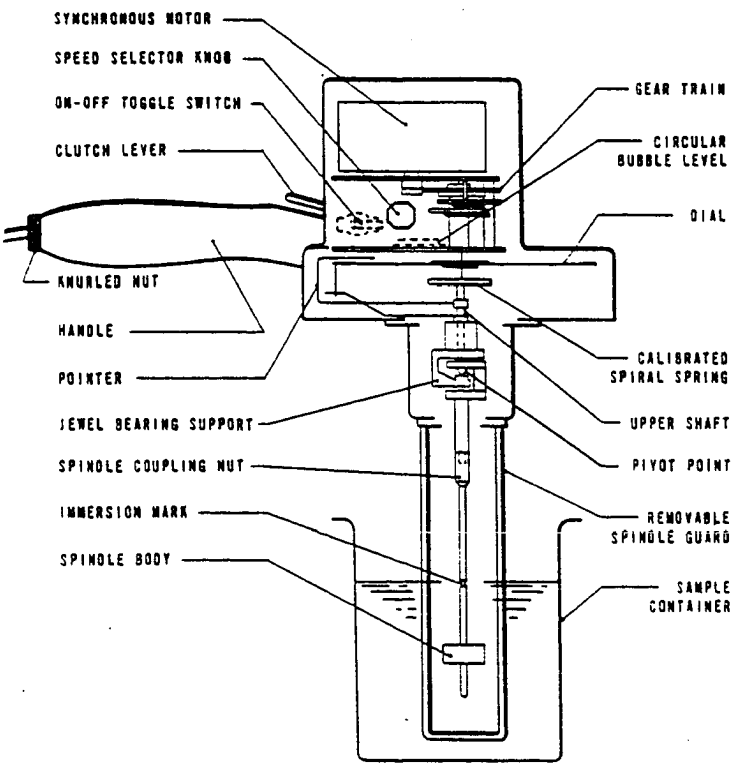
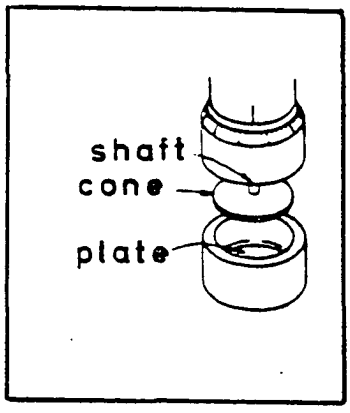
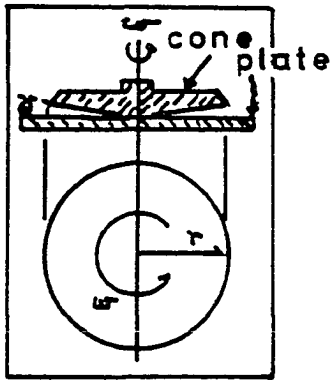


Figure 1.4. Schematic representation of the Wells-Brookfield (LVT) synchro-lectric viscometer. (From Brookfield Engineering, 1959).





$$\text{SHEAR STRESS} = \tau = \frac{T}{2/3\,\pi r^3}\ (\text{dynes/cm}^2)$$

$$\text{SHEAR RATE} = \dot{\gamma} = \frac{\omega}{\text{Sine}\,\theta}\ (\text{Sec}^{-1})$$

$$\text{VISCOSITY} = \eta = \frac{\tau}{\dot{\gamma}}\ (\text{poise})$$

Figure 1.5. Illustration of the mechanism of viscosity measurement used in a Wells-Brookfield (LVT) cone-plate viscometer (from Brookfield Engineering, 1959). T is equal to percentage of full-scale torque (dyne-cm), π is 3.142, r is cone radius (cm), ω is cone speed (rad/sec) and θ is the cone angle in degrees.

of the fluid creates torque in a calibrated spring within the spindle mechanism. The percentage of full-scale torque (T) is indicated by a dial on the anterior portion of the instrument. This percentage can be used to calculate the shear stress (τ), the dependent variable, using equations provided by the manufacturer.

The geometry of the apparatus consists of a conical vertex perpendicular to and in point contact with a flat plate. The cone angle (θ) is very obtuse (1.5°) and cone rotation speed (ω) is constant. Viscosity (η) is the ratio of shear stress (τ) to shear rate ($\dot{\gamma}$). Shear stress is the summation of torque (T) over the conical surface in dyne-cm. Shear rate is proportional to the cone rotational speed (ω) in rad/sec and the gap width (c) in cm at any radial distance (r) in cm from the center of rotation of the cone. The ratio of (ω.r) and (c) is a constant for any value of (r), and since (c) is a maximum at cone radius (r), the shear rate is proportional to (ω) and sine (θ). The mathematical relationships are:

$$\text{Shear Stress } (\tau) \text{ (dynes.cm}^{2}\text{)} = \frac{T}{2/3\ \pi\ (r^{3})} \qquad \text{where } \pi = 3.142$$

$$\text{Shear Rate } (\dot{\gamma}) \text{ (sec}^{-1}\text{)} = \frac{\omega}{\text{sine } (\theta)}$$

$$\text{Viscosity } (\eta) \text{ (centipoise = mPa.s)} = \frac{\tau \times 100}{\dot{\gamma}}$$

Shear rate ($\dot{\gamma}$) is the independent variable and can be varied by a toggle-switch connected to a drive-train mechanism. Therefore, the viscosities of fluids can be determined at a great range of shear rates and can be regulated with a cone-plate viscometer. The apparatus has an inlet and outlet port on the hollow sample cup so that temperature regulation is also possible. The manufacturer provides calibration oils so that precision and accuracy can be checked and maintained. Accuracy is reported to fall within 1% and reproducibility to 0.2% (Brookfield Engineering, 1959). A small sample size (0.5 - 2.0 ml) makes this type of viscometer particularly popular among hemorheologists (Wells, et al., 1961). However, viscosity measurements made under 11.5 sec^{-1} may be questionable due to the large effects of artifacts on viscosity at low shear (Schmid-Schönbein, 1980).

In order to accomplish a thorough investigation of hemorheology and its implications on biological systems, both capillary and cone-plate viscometry are important. Similar viscometers such as the Ostwald tube and the falling-ball types produce less information and may require large sample volumes. Data obtained from capillary and cone-plate viscometry can be used,

comprehensively, to plot viscosity versus shear rate to examine flow characteristics. Differing hematocrits and temperatures can then be utilized to elucidate the influences of these variables upon the flow behavior of the suspension. It was for these reasons that both types of viscometers were employed during the course of this study.

CHAPTER II.

COMPARATIVE MARINE MAMMALIAN HEMATOLOGY AND HEMORHEOLOGY

INTRODUCTION

There is considerable variation in hematologic characteristics such as erythrocyte size, shape and number among members of the animal kingdom and within the class Mammalia. However, for many variables such as hemoglobin concentration, Hb (g/dl), and mean corpuscular hemoglobin concentration (MCHC), very little difference exists among terrestrial mammals. Few investigations have involved studies on the effects of species differences in hematology on blood rheology (Stone, et al., 1968; Usami, et al., 1969; Chien, et al., 1971; Snyder and Weathers, 1977; Weathers and Snyder, 1977).

It has been known for some time that many marine mammals have increased their oxygen carrying capacity by virtue of increased blood volume (Bryden and Lim, 1969; Lenfant, 1969; Simpson, et al., 1970), packed cell volume (HCT) and MCHC (Irving, et al., 1935; Lenfant, et al., 1970). The importance of the latter mechanism has not been recognized until recently. Additionally, many of these same studies, as well as others, revealed differences in RBC size as represented by mean corpuscular volume (MCV) (Englehardt, 1979) and shape

(Lenfant, 1969). However, viscometric studies of marine mammal blood have been rare (Guard and Murrish, 1975) and have not addressed comparative differences related to hematology.

The objectives of the present study were to accumulate hematologic data for a wide variety of marine mammals species, and to determine their effects on blood flow behavior and the possible adaptive significance of hemorheological differences among species in terms of ecology and diving behavior.

MATERIALS AND METHODS

Blood samples from 5 Northern elephant seals, Mirounga angustirostris, 7 harbor seals, Phoca vitulina, 3 California sea lions, Zalophus californianus, one walrus, Odobenus rosmarus, 11 sea otters, Enhydra lutris, 2 false killer whales, Pseudorca crassidens, and 9 bottlenosed dolphins, Tursiops truncatus (one of which was T. truncatus gilli) in captivity at Sea World of San Diego and 2 ringed seals, Phoca hispida which were housed at the University of Alaska, Fairbanks were drawn from the extradural intravertebral vein as described by Harrison and Tomlinson (1956), the plantar aspect of the hindflipper as described by Geraci (1971), or the tail fluke (Cornell, 1983). All animals were conscious.

RBC counts ($10^6/mm^3$) and Hb (g/dl) measurements were made using a Coulter-counter, hemocytometer and Coulter-hemoglobinometer techniques. Samples were centrifuged for five minutes in a microfuge (Clay-Adams) and the resulting HCTs used to calculate MCV and MCHC using the following equations: MCV = HCT/RBC; MCHC = Hb/HCT. The resulting plasma was analysed for total plasma proteins using a clinical refractometer (Shuco Model 5711-2020).

Absolute viscosity measurements were made at various shear rates from 11.5 to 230.4 sec^{-1} on a Wells-Brookfield (Model LVT) cone-plate viscometer. Apparent viscosity measurements were made using a capillary viscometer (radius = 500μm) similar to that used by Haynes and Burton (1959). Both viscometers were calibrated with fluids of known viscosity (Brookfield Viscosity Standard, 5.4 cP and water) and operated at 37^oC. Plots of viscosity (cP) versus shear rate (sec^{-1}) resulted in flow curves based on rational (cubic and parametric) spline interpolation and linear regressions using a "least squares" algorithm and comparisons were made among the different species.

Statistical comparisons were made using paired and mean "t" tests. Comparisons of $P<0.05$ were considered significant. Correlations were made using Pearson correlation coefficients.

RESULTS

Age estimates and hematologic data for the 8 different species of marine mammals are summarized in Table 2.1. Hemoglobin levels (Hb), mean corpuscular hemoglobin concentration (MCHC) and packed cell volumes (HCT) were highest among the phocid seals and lowest for the two cetacean species while erythrocyte size (MCV) and plasma protein concentrations were variable and showed no systematic trend.

Rheologic behavior of bloods from all species showed the typical mammalian dependence of viscosity upon shear rate and HCT. Blood viscosity was highest for elephant seals and lowest for bottle-nosed dolphins (Figure 2.1). Although the species with the higher hematocrits generally had highest viscosities, there were some exceptions. Walrus which had the lowest HCT had higher viscosity than many species with higher HCTs such as sea otters, false killer whales and sea lions. The viscosity at low shear rates was more a reflection of HCT differences while high shear viscosity was significantly influenced by plasma protein concentration (Table 2.1 and Figure 2.2). Viscosity was independent of MCV. Whole blood viscosity was reduced in both cetacean species relative to blood viscosity of the pinnipeds and sea otters.

Table 2.1. Age and hematologic characteristics of blood samples from captive marine mammals at Sea World of San Diego and the University of Alaska, Fairbanks. Hemoglobin concentration (Hb), hematocrit (HCT), RBC counts, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are from Coulter-counter, hemocytometer and Coulter-hemoglobinometer techniques. Plasma protein concentrations (PP) are from a clinical refractometer. Values are Means (S.E.).

SPECIES	# Animals	AGE* (months)	Hb (g/dl)	HCT (%)	RBC ($10^6/mm^3$)	MCV (μ^3)	MCHC (%)	PP (g/dl)
Mirounga angustirostris	5	25.6 (1.6)	24.6 (0.5)	57.4 (2.7)	3.19 (0.07)	176 (2.2)	44.1 (1.2)	8.2 (0.4)
Phoca vitulina	7	22.8 (7.3)	21.0 (0.5)	53.2 (1.6)	5.11 (0.16)	105 (2.3)	40.3 (0.3)	7.7 (0.4)
Phoca hipida	2	36.3 (2.4)	21.0 (0.4)	57.2 (1.8)	4.47 (0.15)	129 (3.8)	37.1 (2.7)	7.2 (0.05)
Zalophus californianus	3	22.3 (8.2)	17.4 (1.0)	48.8 (2.18)	4.59 (0.36)	100 (1.8)	38.2 (2.3)	8.6 (0.4)
Odobenus rosmarus	1	48.0	15.6	41.0	3.11	140	35.5	8.0
Enhydra lutris	11	12.0 (0.0)	18.0 (0.6)	52.4 (1.8)	4.51 (0.17)	113 (1.7)	34.8 (0.1)	6.4 (0.3)
Pseudorca crassidens	2	43.2 (29)	15.3 (0.2)	43.4 (0.7)	3.58 (0.12)	123 (4.4)	34.7 (0.3)	7.9 (0.3)
Tursiops truncatus	9	56.0 (7.0)	14.0 (0.2)	41.4 (0.4)	3.55 (0.04)	118 (1.6)	33.4 (0.3)	7.6 (0.2)

* Age estimates from Sea World, Inc.

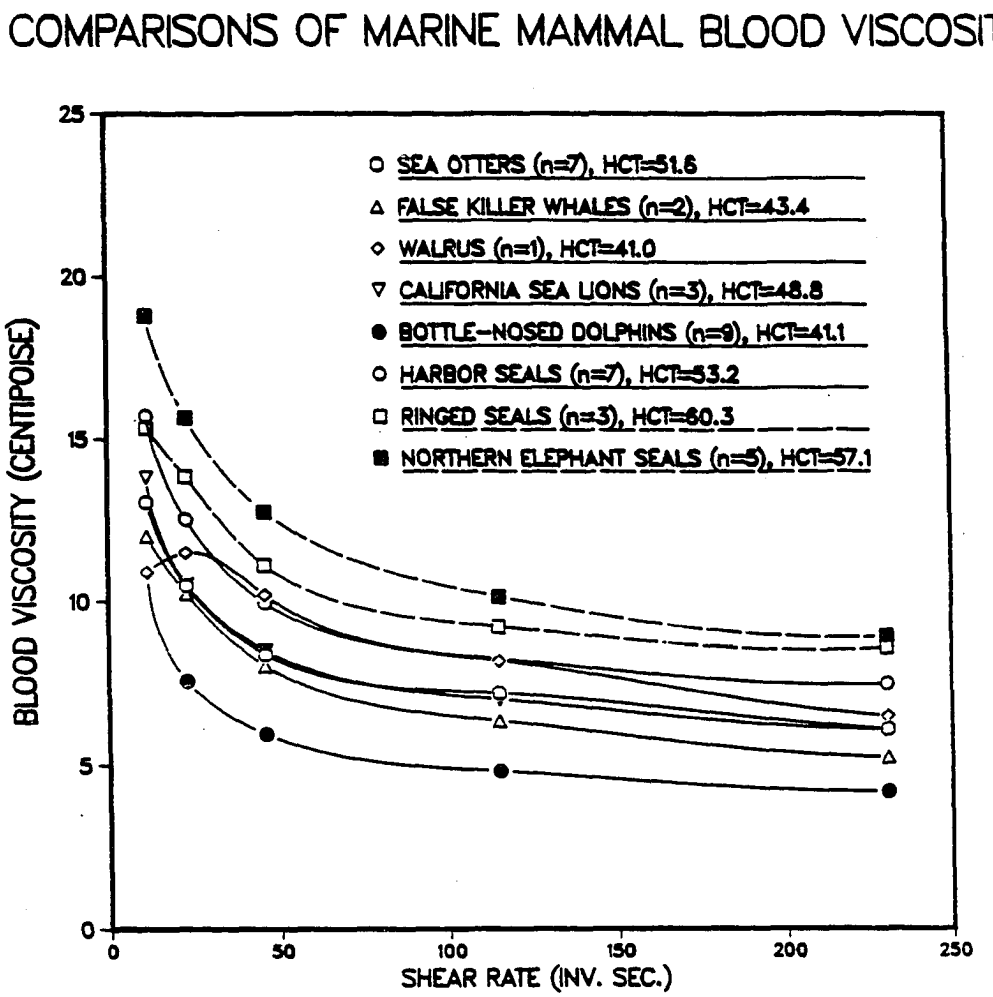


Figure 2.1. Plots of absolute viscosity (cP) versus shear rate (sec^{-1}) for sea otters, false killer whales, walrus, California sea lions, bottlenosed dolphins, harbor seals, ringed seals and Northern elephant seals obtained from measurements made in a Wells-Brookfield (Model LVT) cone-plate viscometer at 37°C. Hematocrits (HCT) are shown to the right of each species.

Apparent viscosities of blood of all marine mammal species obtained from capillary viscometry were comparable to values from the cone-plate at low rates of shear. However, blood viscosity was considerably reduced at shear rates above 100 sec^{-1} (Figures 2.2-2.9). Due to the small samples sizes available from some animals, the flow curves from capillary viscometry were incomplete - usually in the low-shear zone (Figures 2.3, 2.4 and 2.5). There was no correlation between hematology and hemorheology and diving behavior or activity levels found in the literature (see Discussion), age or sex ($P>0.05$; 95% confidence).

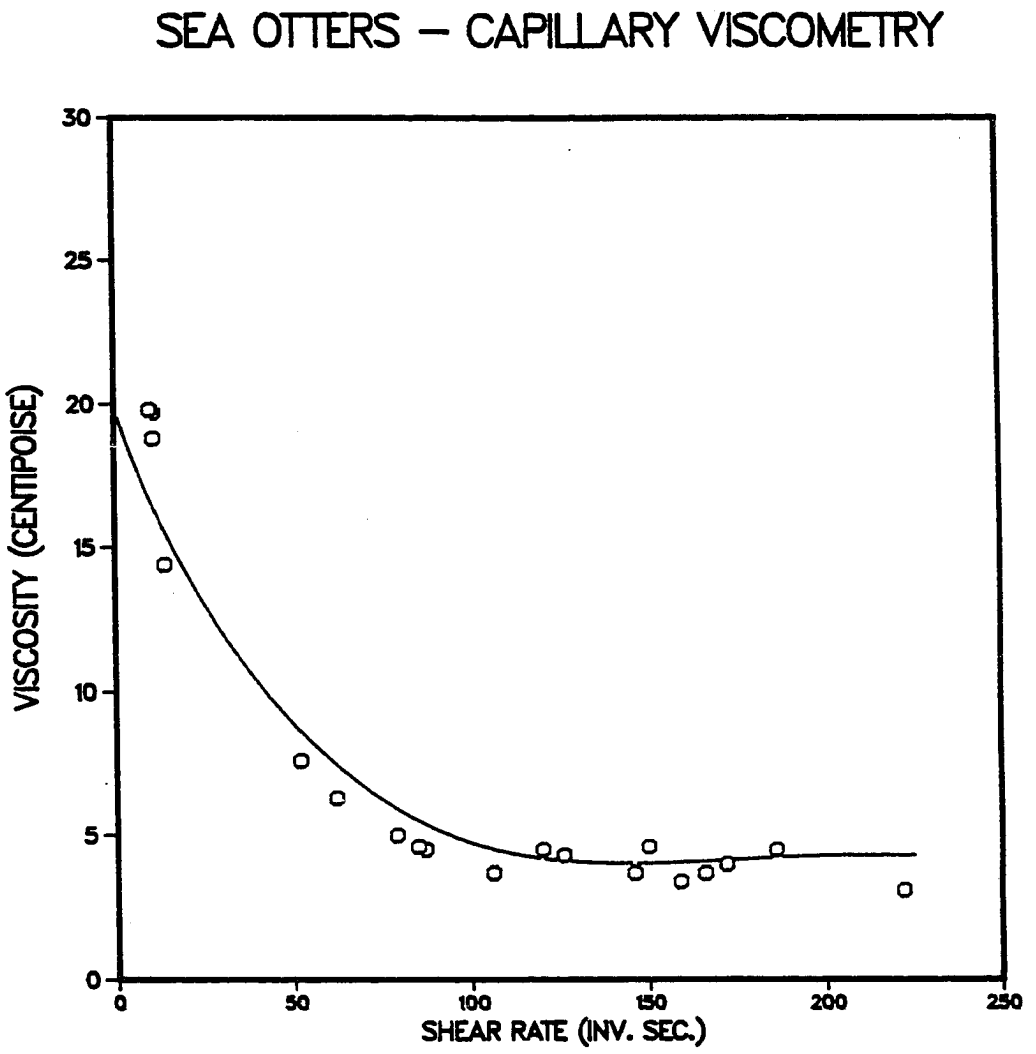


Figure 2.2. Plot of apparent viscosity (cP) versus shear rate for whole blood (HCT = 52.6 [2.24], mean [S.D.]) from 11 captive sea otters from Sea World of San Diego obtained from measurements made in a capillary viscometer (r = 500μm) at 37°C.

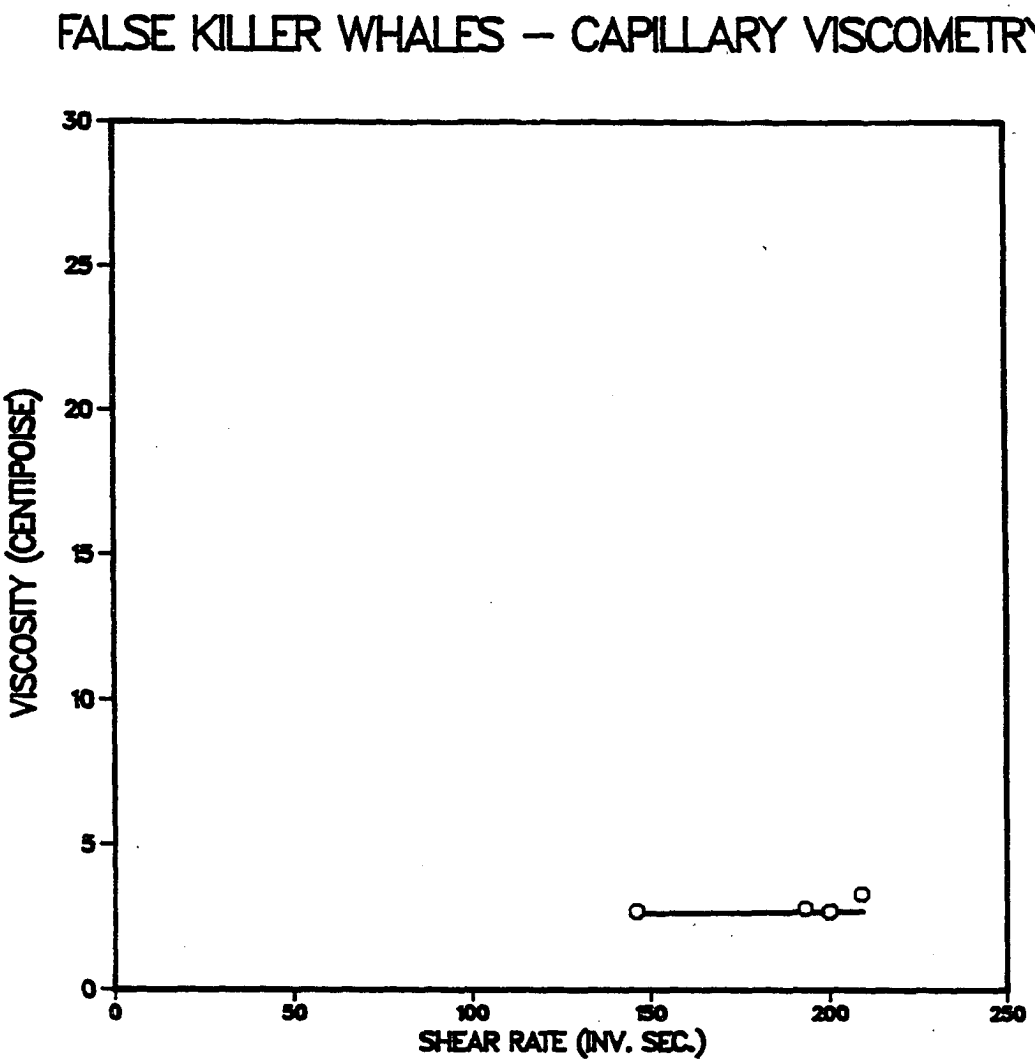


Figure 2.3. Plot of apparent viscosity (cP) versus shear rate (sec^{-1}) of whole blood (HCT = 48.5 [4.60], mean [SD]) for two captive false killer whales from Sea World of San Diego obtained from capillary viscometry (r=500μm) at 37°C.

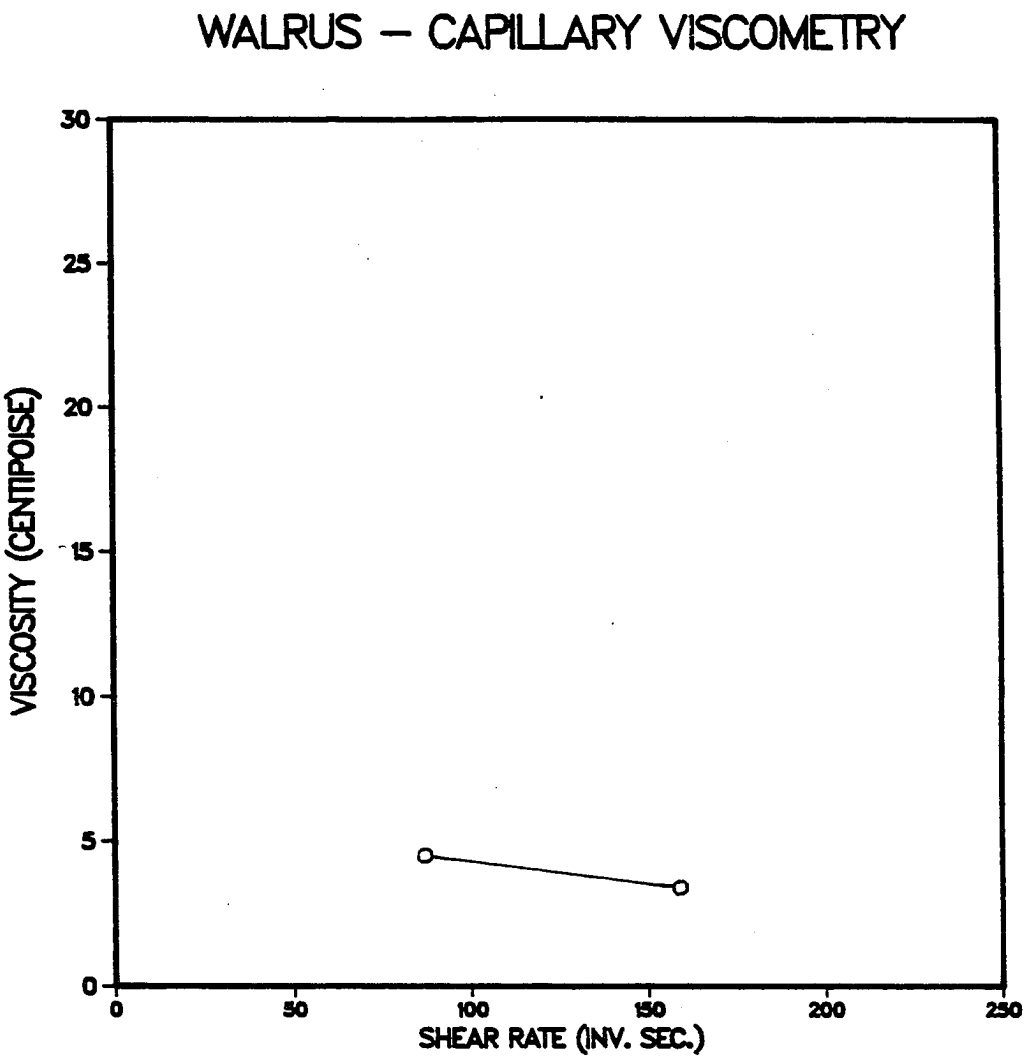


Figure 2.4. Plot of apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood (HCT = 41.0) from one walrus from Sea World of San Diego obtained from capillary viscometry (r=500μm) at 37°C.

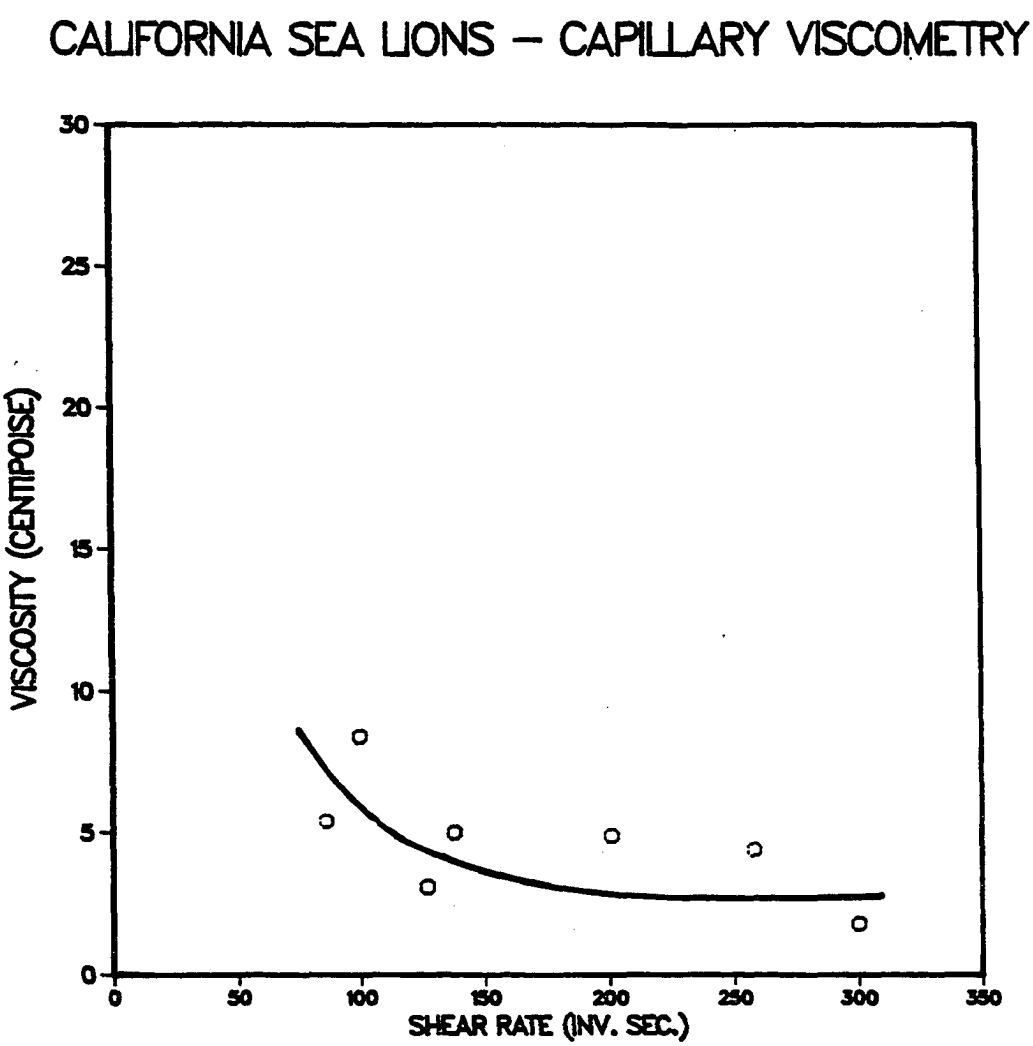


Figure 2.5. Plot of apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood (HCT = 49.4 [4.66], mean [SD]) from three California sea lions from Sea World of San Diego obtained from capillary viscometry (r=500μm) at 37^{o}C.

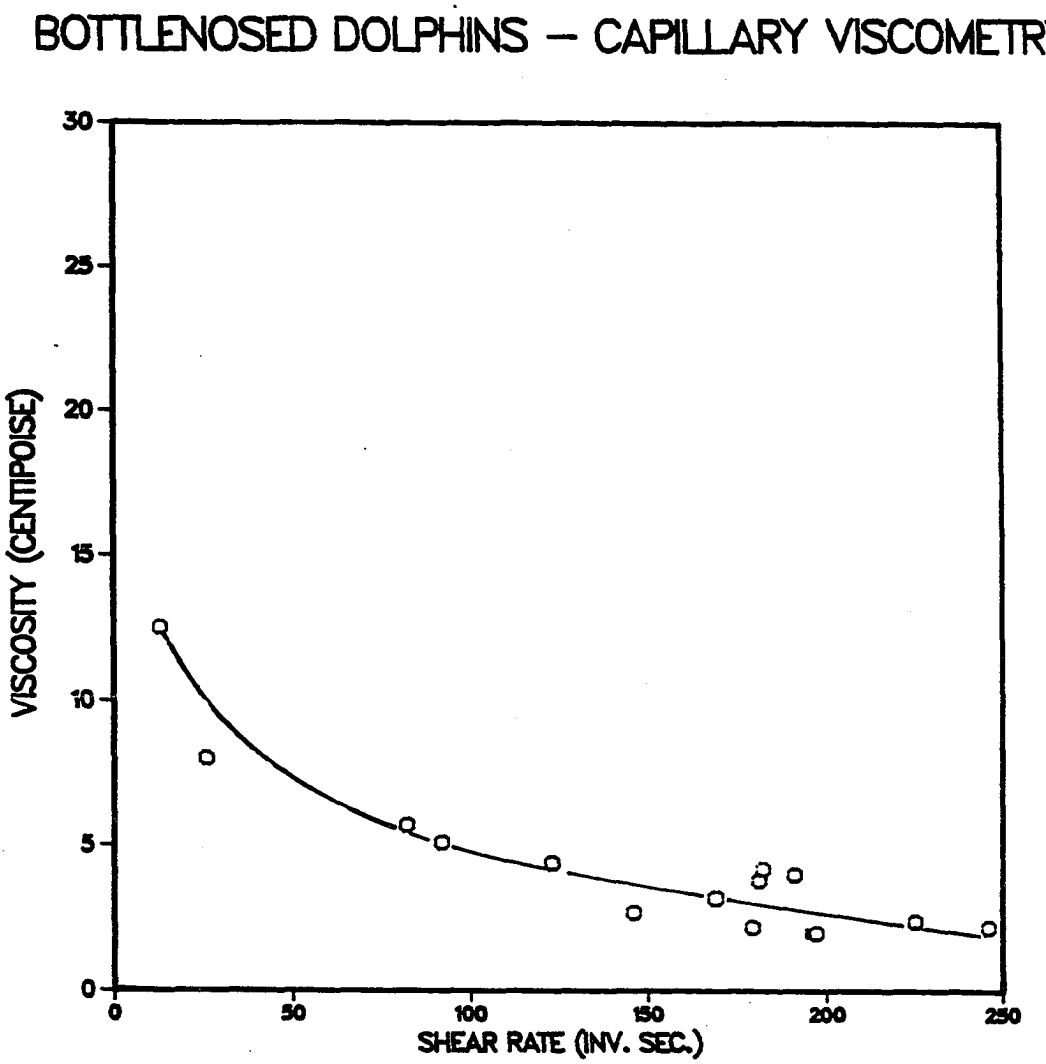


Figure 2.6. Apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood (HCT = 41.1 [1.65], mean [SD]) from nine bottlenosed dolphins from Sea World of San Diego obtained using capillary viscometry (r=500μm) at 37°C.

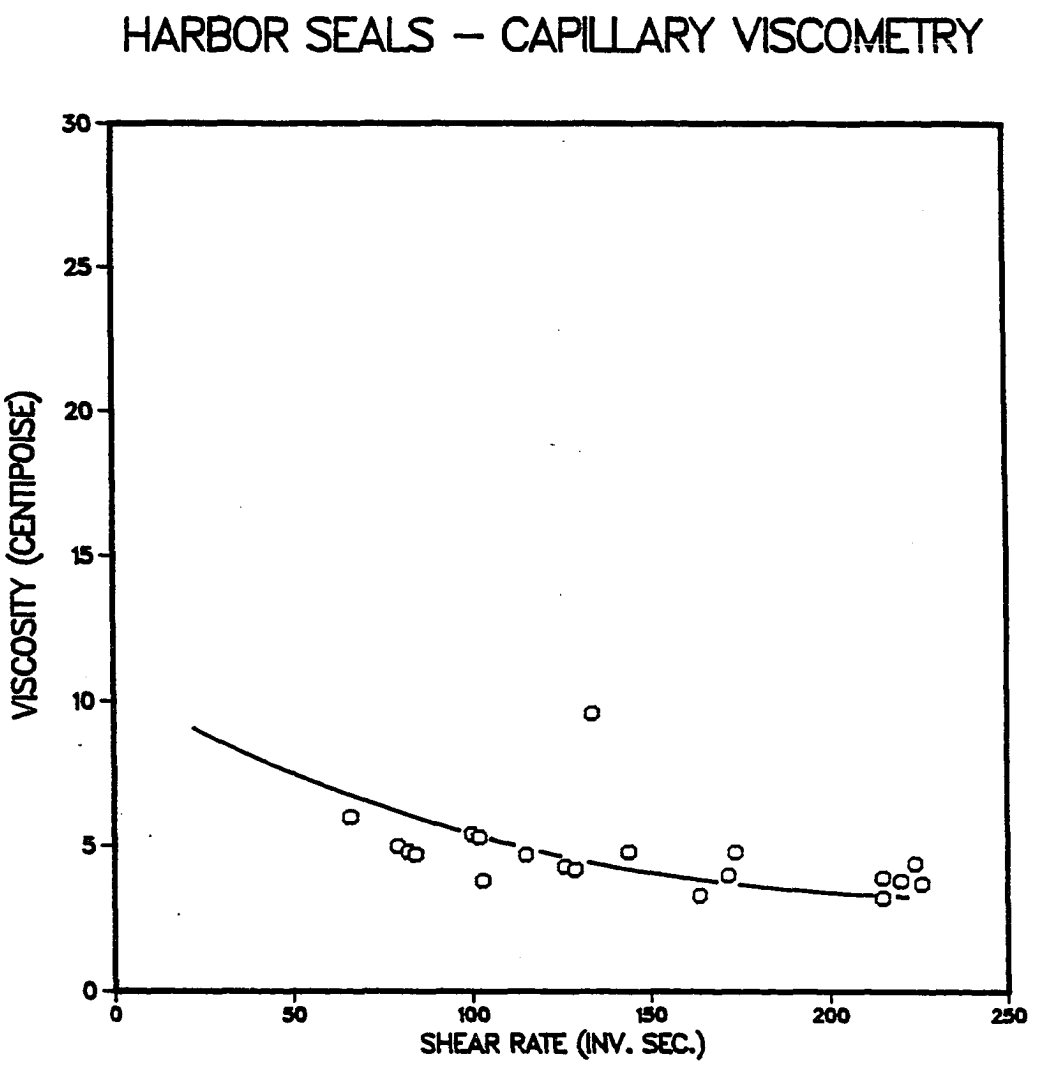


Figure 2.7. Apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood (HCT = 54.4 [4.52], mean [SD]) from seven captive harbor seals at Sea World of San Diego from measurements made in a capillary viscometer (r=500μm) at 37°C.

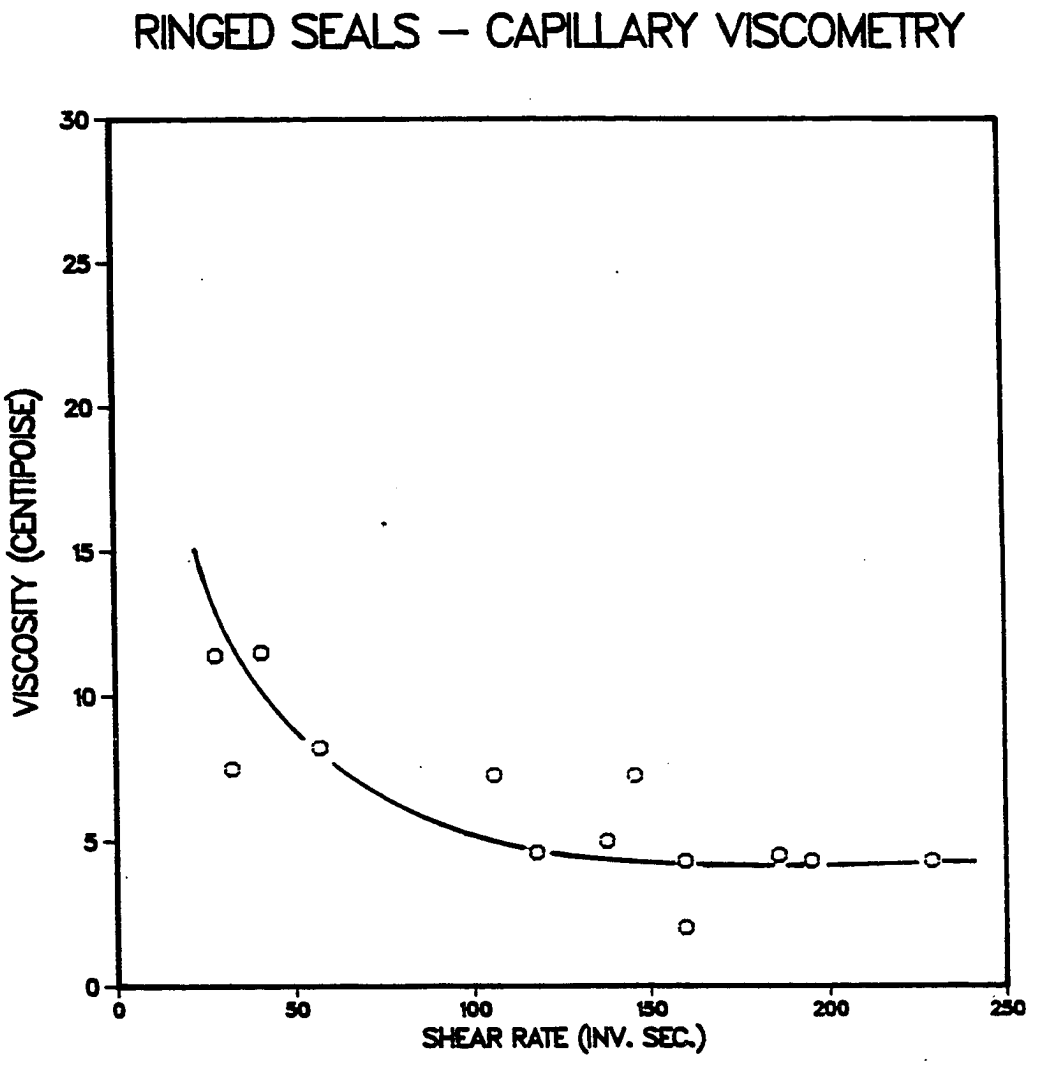


Figure 2.8. Apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood (HCT = 52.4 [15.17], mean [SD]) from two captive ringed seals from Alaska obtained from measurements made by capillary viscometry (r=500μm) at 37°C.

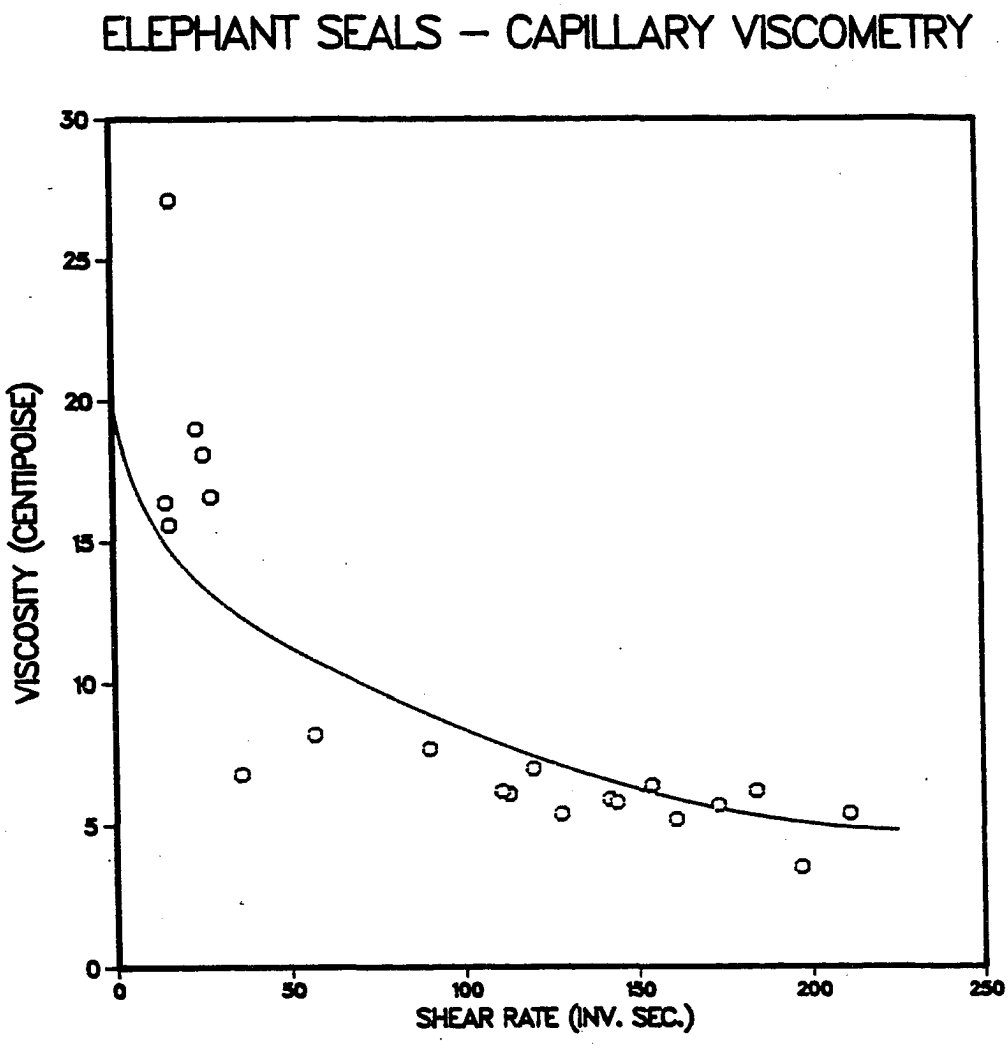


Figure 2.9. Plot of apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood (HCT = 58.5 [13.58], mean [SD]) from five captive northern elephant seals at Sea World of San Diego obtained from measurements made by capillary viscometry (r=500μm) at 37°C.

DISCUSSION

Some studies have shown relationships between ecology and hematology of different marine mammal species (Ridgeway and Johnston, 1966; Ridgeway et al., 1970) and among members of the same species (Duffield, et al., 1983). In the present study the ecology and diving behavior of the 8 species of marine mammals are not generally reflected in their hematology and hemorheology. The phocid seals were the highest in terms of HCT, Hb and MCHC and therefore oxygen carrying capacity, a result similar to those of previous studies (Bryden and Lim, 1969; Lenfant, 1969; Lenfant, et al., 1970). Values for the two cetacean species were also comparable to those reported earlier in the literature (Ridgeway and Johnston, 1966; Lenfant, 1969) and were the lowest of the 8 species of marine mammals studied. However, the viscometric behavior of different species seems to be dependent on the synergistic effects of several hematologic factors. Blood viscosity increased with HCT with the exception of the walrus blood (Figures 2.1-2.9). This discrepancy may be due to the increased plasma protein concentration in walrus blood relative to the sea otters and cetacean species. Increased plasma protein concentrations cause increased viscosity of the plasma and whole blood.

Indeed, the absolute viscosity of blood at low shear rates (< 50 sec^{-1}) shows a direct relationship with HCT while at high shear rates the effects of plasma protein concentrations can be seen in both walrus and sea lion blood viscosity (Figure 2.1 and Table 2.1). Sea lion blood viscosity was consistently higher than sea otter blood viscosity presumably due to the high concentration of plasma proteins in sea lion blood even though the sea lions had lower HCTs than sea otters, (Table 2.1). High shear viscosity of walrus blood was increased relative to other species independent of hematocrit. The results of investigations aimed at evaluating the influence of erythrocyte geometry on blood flow behavior have been contradictory (Gregersen, et al., 1965; Stone, et al., 1968; Usami, et al., 1969; Chien, et al., 1971). Likewise, the viscosity of marine mammal blood reported here fits no generalization relative to erythrocyte size as represented by MCV.

Correlations with maximum and "average" dive length and depth (natural and simulated) reported in the literature for harbor seals (Carl, 1964; Harrison and Tomlinson, 1960; Harrison and Kooyman, 1968; Kooyman, et al., 1972), ringed seals (Ferren and Elsner, 1979), northern elephant seals (Le Beouf, et al., 1986), California sea lions (Hobson, 1966), walrus (Buckley,

1958), sea otters (Kenyon, 1982), false killer whales (Norris, et al., 1965) and bottlenosed dolphins (Ridgeway and Johnston, 1966) also revealed no obvious relationship between the diving behavior and ecology of the animals and their hematologic or rheologic profiles as a group ($r^2 < .85$). However, if each of the two major classes of marine mammals are examined separately, some trends become evident (Figure 2.1).

In the Pinnipedia the elephant seals, which are the deepest divers, have increased Hb, MCHC, HCT and whole blood viscosity while harbor and ringed seals both of which are similar to each other in diving behavior also closely resemble one another in terms of hematology and hemorheology (Table 2.1, Figures 2.1, 2.7, 2.8, and 2.9). Walrus and sea lions which are generally considered more shallow divers possess lower values for both sets of variables (Table 2.1, Figures 2.1, 2.4, and 2.5). The sea otters closely resemble sea lions in viscometric behavior (Figures 2.1, 2.2 and 2.5). Inspection of the data for the two cetacean species again shows increases in Hb, MCHC, HCT and whole blood viscosity for the deeper-diving false killer whales relative to that for the bottlenosed dolphins (Table 2.1 and Figure 2.1).

These generalized trends may appear simplistic. This may be due to the lack of comprehensive and accurate data

on diving behavior for some species. Due to their pelagic lifestyle, it is often difficult to obtain accurate records of behavior for marine mammals. The use of time-depth recorders (Kooyman, 1966; Le Boeuf, et al., 1986) has done much to overcome these problems and to present a more complete picture of diving behavior. However, many species have not been studied using this technique and we must rely upon observational data that may not be truly representative of actual activity levels.

In conclusion, the ecology and diving behavior of the eight marine mammal species studied does not seem to be reflected in the hematologic characteristics or rheologic behavior of their blood as evidenced by low r-squared values. However, upon closer inspection, it is apparent that the effects of the hematologic characteristics upon the flow behavior of the blood are extremely complex and that interactions among these variables may be responsible for the differences among different species and perhaps among individuals. A study involving manipulation of the blood in terms of suspending medium and RBC volume concentration could provide a better understanding of these interactions and their effects on marine mammalian hemorheology.

CHAPTER III.

HEMATOLOGIC AND RHEOLOGIC EFFECTS OF CAPTIVITY: ACCLIMATIZATION?

INTRODUCTION

The successful maintenance of marine mammals, as well as any other exotic species in captivity is variable. Captivity imposes several environmental constraints upon animals and is also thought to confer both physical and "emotional" stresses upon non-domesticated species by virtue of changes in diet, disease, training or experimental regimes (Englehardt, 1979; McConnell and Vaughan, 1983). Most marine zoological parks which house marine mammals routinely monitor hematologic variables in order to establish "captive norms" and to anticipate the onset of infection or disease.

Several studies have been aimed at evaluating the effects of environment on the physiology of marine mammals. Ridgeway and Johnston (1966) found significant differences in hematologic characteristics of three species of Delphinids. They hypothesized that these differences in hematology were a result of the diving styles and activity levels exhibited by each species (e.g. those species which "normally" dive deeper or longer and possessing higher activity levels having

higher oxygen carrying capacity by virtue of increased hemoglobin levels in the blood). Certainly, the results of many investigations on both cetaceans and pinnipeds support this contention (Medway and Geraci, 1964; Ridgeway, et al., 1970; Lenfant, 1969; Lenfant, et al., 1970). Additionally, Duffield et al. (1983) have separated coastal and offshore ecotypes of the same species (Tursiops) of dolphin based on hemoglobin levels, packed cell volumes and red blood cell (RBC) counts, the offshore form having the higher values for all three variables. They also suggest a genetic basis for these differences based upon captive-bred crosses of the two forms which were intermediary in hematologic profiles. Similarly, Kodama, et al. (1977) suggested that although there is variation in hematology in response to environment and diving activity, the major determinant of blood oxygen capacity was genetic.

There have been few viscometric studies on marine mammals (Guard and Murrish, 1975; Wickham, et al., 1985; Hedrick, et al., 1986) and none have been aimed at evaluating the possible hemorheological effects of captivity. Is there an acclimatization response to captivity in terms of hematology and hemorheology in marine mammals? Captive northern elephant seals (Mirounga angustirostris) and sea otters (Enhydra lutris)

from Sea World in San Diego, California and free-ranging seals from Año Nuevo Island and the mainland colony near Santa Cruz, California and newly-captured sea otters from Prince William Sound, Alaska were studied in order to answer this question.

MATERIALS AND METHODS

Blood samples from 5 northern elephant seals (2 females, 1 subadult, 1 juvenile and 3 males, all juvenile) in captivity at Sea World of San Diego and 11 sea otters (all male, all juvenile) newly-captured from Prince William Sound, Alaska were drawn from the extradural intravertebral vein as described by Harrison and Tomlinson (1956) or from the plantar aspect of the hindflipper as described by Geraci (1971) into syringes and placed in heparinized Vacutainer tubes. The sea otters were sampled again after three weeks of captivity. Blood sampling procedures were similar for 12 wild northern elephant seals (6 males: 4 weanlings and 2 yearlings; 6 females: 2 adults, 2 yearlings and 2 weanlings) from Año Nuevo Island and the mainland colony near Santa Cruz, California. All animals were conscious except for two adult female northern elephant seals which were anesthetized with Ketamine.

RBC counts ($10^6/mm^3$), white blood cell (WBC)

counts (10^3/mm^3) and Hb (g/dl) measurements were made using a Coulter-counter, hemacytometer and Coulter hemoglobinometer techniques. Samples were centrifuged for five minutes in a microfuge (Clay-Adams) and the resulting HCTs used to calculate mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) using the following equations: MCV = HCT/RBC; MCHC = Hb/HCT. The resulting plasma was analysed for total plasma proteins using a clinical refractometer (Shuco Model 5711-2020).

Viscosity measurements were made at various shear rates from 11.5 to 230.4 sec^{-1} on a Wells-Brookfield (Model LVT) cone-plate viscometer. A capillary viscometer (radius = 500µm) similar to that employed by Haynes and Burton (1959) provided additional viscometric data. Both viscometers were calibrated with fluids of known viscosity (Brookfield Viscosity Standard, 5.4 cP and water) and operated at 37 ^{o}C. Curves of viscosity versus shear rate were constructed based on rational (cubic and parametric) spline interpolation and linear regression using a "least squares" algorithm and comparisons made among captive and free-ranging animals. Statistical comparisons were made using paired and mean "t" tests. Comparisons of $P<0.05$ were considered significant. Correlations were made using Pearson

correlation coefficients.

RESULTS

Hematologic variables for captive and free-ranging northern elephant seals and sea otters are listed in Table 3.1. Age estimates, RBC counts, hemoglobin levels (Hb), and MCHC showed no statistical differences between captive and wild elephant seals ($P>0.05$; 95% confidence). However, white blood cells were elevated 41% in captive seals relative to wild seals while MCV was elevated 12% and total plasma protein were 16% lower ($P<0.05$, see Figure 3.1 and Table 3.1). Cone-plate and capillary viscometry (Figures 3.2 and 3.3, respectively) show a slight elevation in whole blood and plasma viscosity of wild seals compared to captive seals. However, this difference is not statistically significant ($P>0.05$; 95% confidence). Capillary viscometry data are comparable to that from the cone-plate but apparent viscosity values are lower at high shear rates (above 100 sec^{-1}) for captive seals and elevated for wild seals (Figure 3.3).

Similarly, hematologic characteristics for newly-captured sea otters and those in captivity for three weeks show no statistical differences save MCHC which is 2.9% higher in the "acclimatized" otters ($P=0.03$; 95%

confidence). (Table 3.1). Cone-plate (Figure 3.4) and capillary (Figure 3.5) viscometry also show no significant ($P>0.05$; 95% confidence) differences in flow behavior among the captive and free-ranging sea otters, although blood from captive otters was slightly more viscous (Figure 3.4). Capillary data for sea otter blood viscosity are similar to that of the elephant seals in that they are comparable to the cone-plate data at low shear rates but they decreased at shear rates above approximately 100 sec^{-1}. There was no correlation of hematologic or rheologic variables with age or sex (all variables $r^2 < 0.85$).

Table 3.1. Hematologic characteristics: hemoglobin concentrations (Hb), hematocrits (HCT), red blood cell counts (RBC), mean corpuscular volumes (MCV), mean corpuscular hemoglobin concentrations (MCHC), plasma protein concentrations (PP) and white blood cell counts (WBC) of blood samples from captive and free-ranging northern elephant seals and sea otters. Captive animals are from Sea World of San Diego. Wild elephant seals are from Ano Nuevo Island and the mainland population near Santa Cruz, California. Newly-captured sea otters from Prince William Sound, Alaska were flown to Sea World of San Diego where sampling took place. Values are means (S.E.). For P-values see Appendix.

SPECIES	# Animals	AGE* (months)	Hb (g/dl)	HCT (%)	RBC (10^6/mm^3)	MCV (μ^3)	MCHC (%)	PP (g/dl)	WBC (#/mm)
Mirounga angustirostris									
Captive Animals	5	25.6 (1.6)	24.6 (0.5)	57.4 (2.7)	3.19 (0.07)	195 (2.2)	44.1 (1.2)	8.2 (0.4)	20860 (1721)
Free-ranging Animals	12	19.6 (6.4)	22.8 (0.8)	58.6 (2.0)	3.38 (0.15)	174 (4.6)	39.2 (0.9)	9.7 (0.4)	14766 (768)
Enhydra lutris									
Three weeks captivity	11	12.0 (0.0)	19.2 (0.2)	52.7 (1.0)	4.75 (0.06)	112 (1.0)	35.8 (0.4)	6.8 (0.2)	---
Newly-captured	11	12.0 (0.0)	18.0 (0.6)	52.4 (1.8)	4.51 (0.17)	113 (1.7)	34.8 (0.1)	6.4 (0.3)	---

* Age estimates from Sea World, Inc.

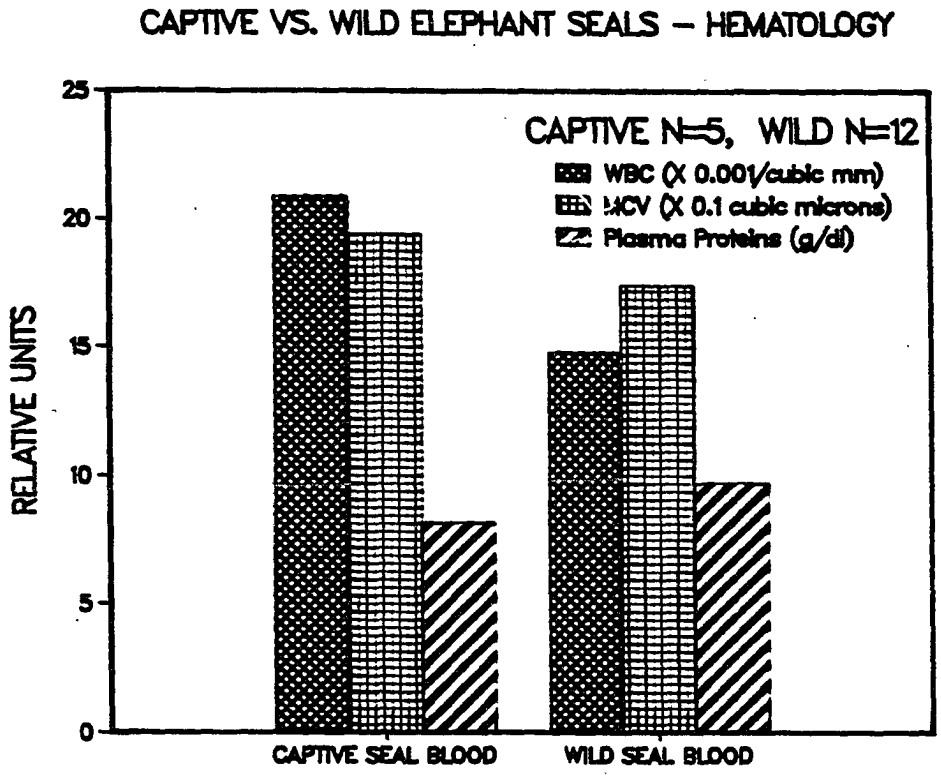


Figure 3.1. Hematologic differences among captive and free-ranging northern elephant seals. White blood cell counts (WBC) are 41% higher for captive seals. Mean corpuscular volume (MCV) and plasma protein concentrations are +12% and -16% for captive seals relative to wild seals. All differences are statistically significant ($P<0.05$; 95% confidence, see Appendix).

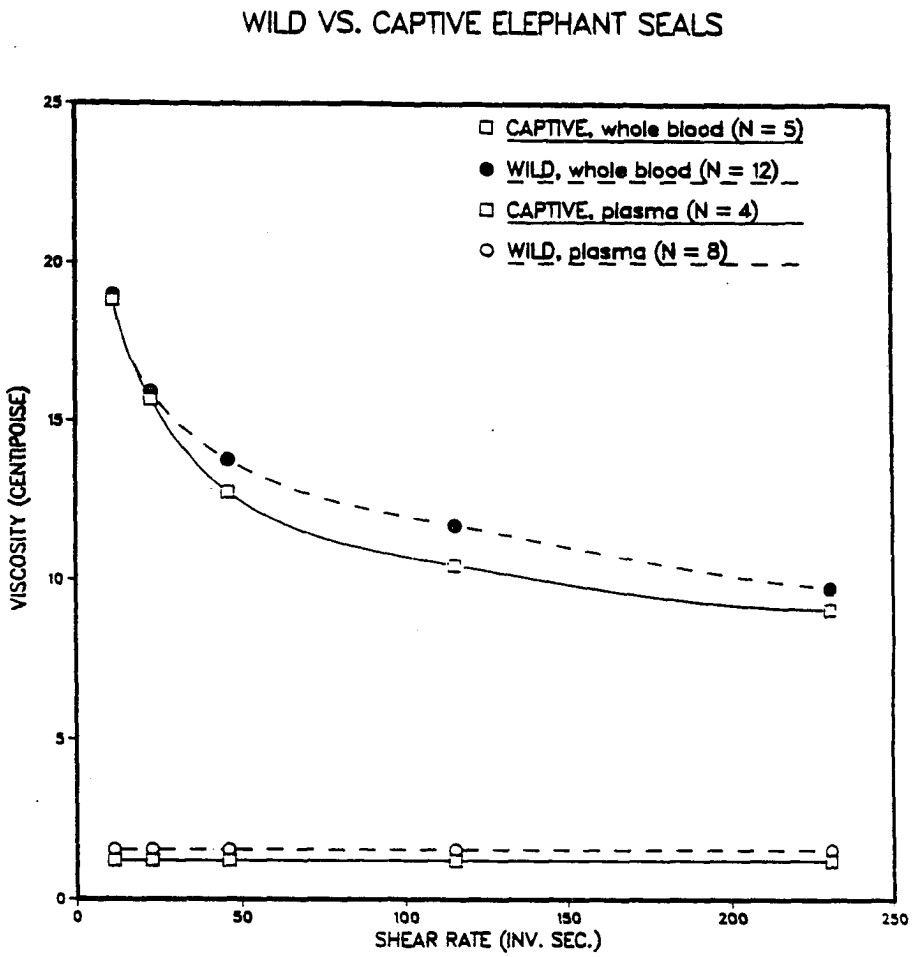


Figure 3.2. Whole blood and plasma viscosity measurements for captive (open squares) and free-ranging northern elephant seals (closed and open circles) made using a Wells-Brookfield (Model LVT) cone-plate viscometer over a range of shear rates from 11.5 to 230 sec^{-1} at 37°C. Values are not statistically different (P>0.05; 95% confidence, see Appendix).

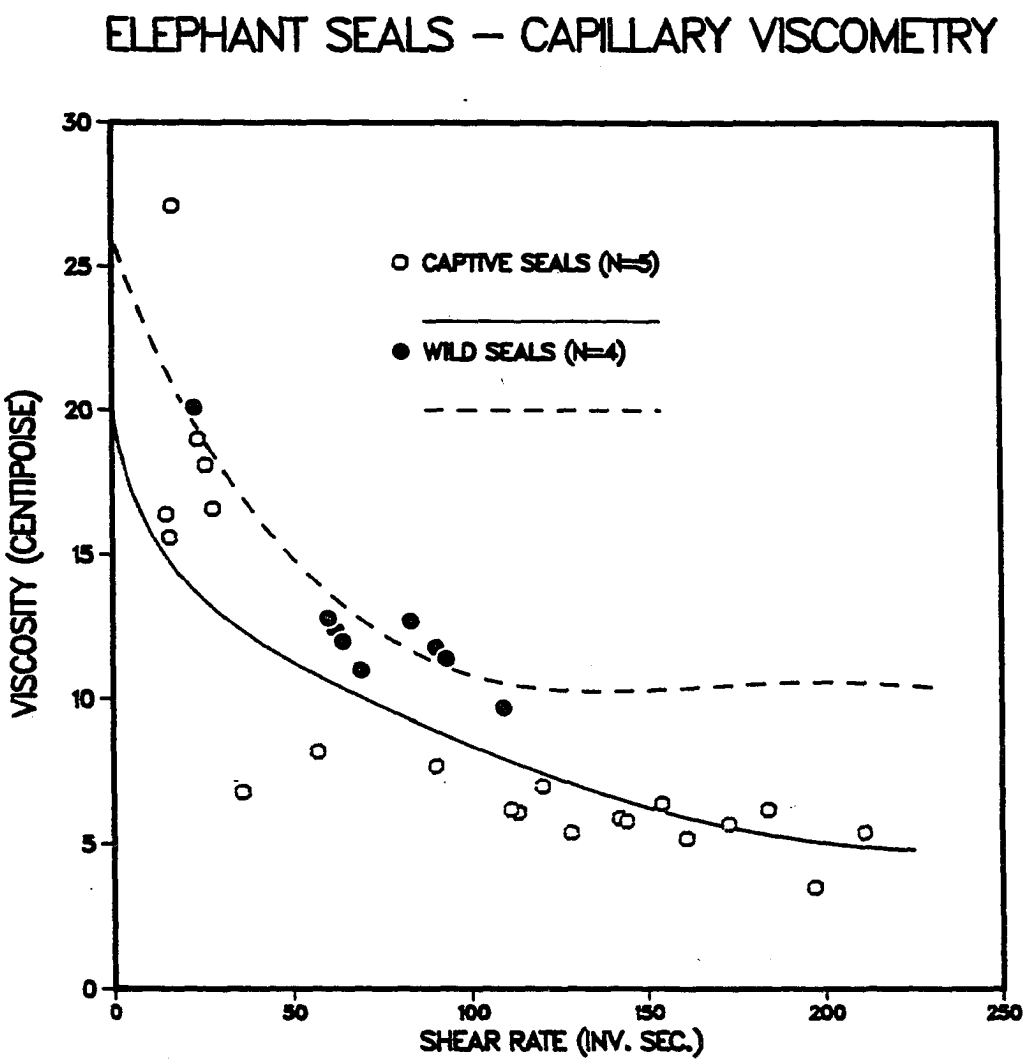


Figure 3.3. Plots of apparent viscosity versus shear rate for whole blood from captive (open circles) and free-ranging (closed circles) northern elephant seals obtained from capillary viscometry (r=500μm) at 37°C. Captive seals HCT = 58.5 (13.58); wild seal HCT = 58.7 (3.02). Means (S.D.). Values are not statistically different (P>0.05; 95% confidence).

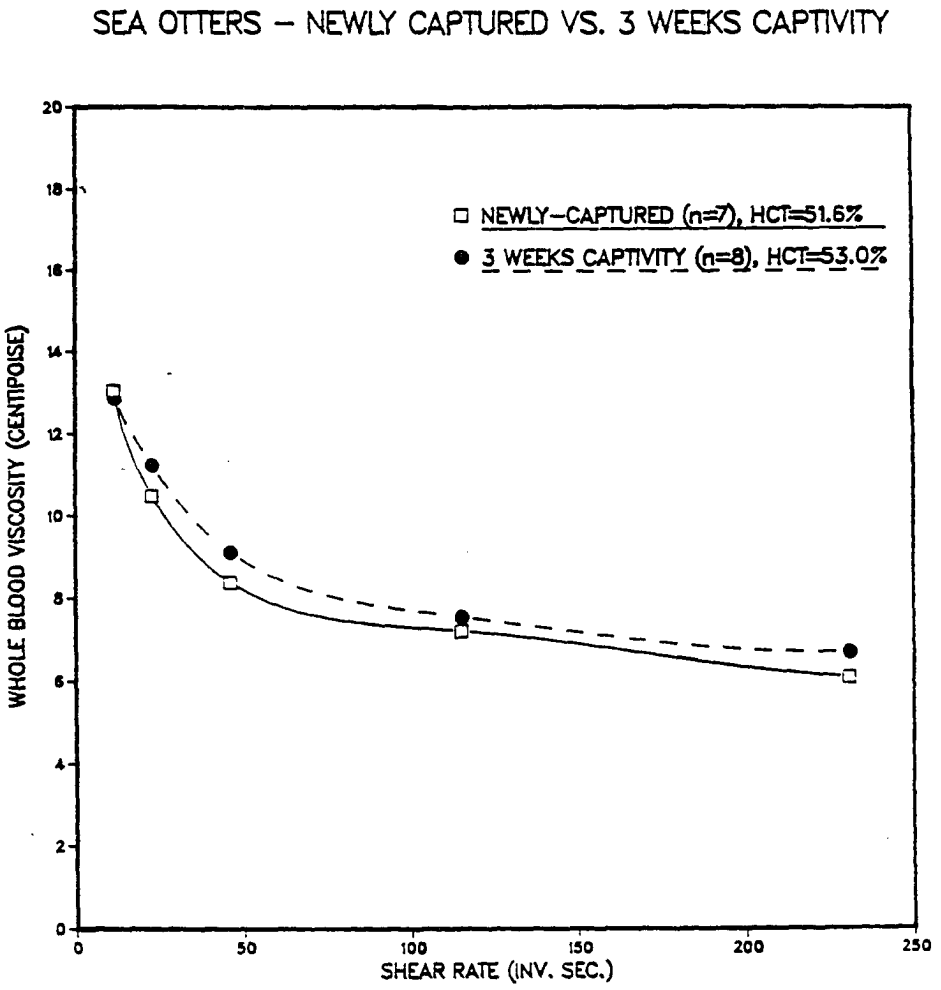


Figure 3.4. Viscosity (cP) versus shear rate (sec^{-1}) for whole blood from 11 newly-captured sea otters from Prince William Sound, Alaska (open squares) and the same animals after three weeks of captivity (closed circles) obtained from measurements made on a Wells-Brookfield (Model LVT) cone-plate viscometer from shear rates of 11.5 to 230.4 sec^{-1} at 37°C. Values are not significantly different ($P>0.05$; 95% confidence, see Appendix).

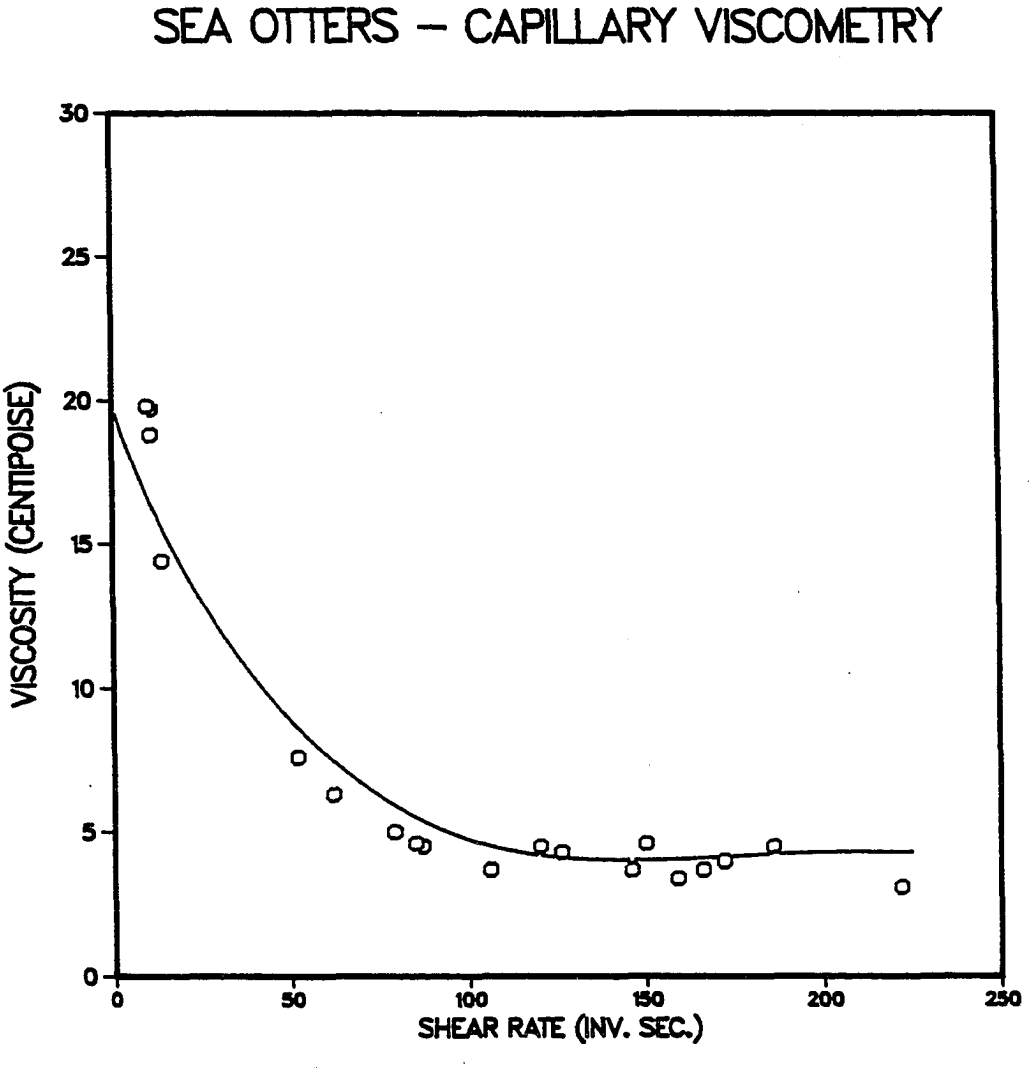


Figure 3.5. Plot of apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood from sea otters (n=11) newly-captured from Prince William Sound, Alaska made using a capillary viscometer (r=500μm) at 37°C. HCT = 52.6 (2.24). Mean (S.E.)

DISCUSSION

Comparisons of hematology and hemorheology of blood from captive and free-ranging northern elephant seals and sea otters revealed very few differences between captive and wild animals contrary to earlier hematologic studies on captive marine mammals (Englehardt, 1979; McConnell and Vaughan, 1983). However, values for hematologic variables in this study (Table 3.1) are within the ranges reported earlier for the same species (Van Citters, et al., 1965; Simpson, et al., 1970; Lenfant, et al., 1970; Castellini, et al., 1986) and closely related species (Bryden and Lim, 1969; Lane, et al., 1972).

Three hematologic characteristics differed among the captive and free-ranging elephant seals: WBC counts were elevated 41% and MCVs 12% higher while plasma protein concentrations were 16% lower for blood from captive animals relative to blood of wild animals (Figure 3.1). The captive elephant seals showed no indications of infection externally or as evidenced by "normal" white cell differentials (L. H. Cornell, D.V.M., pers. comm.). Plasma viscosity was also elevated 5% in wild elephant seals (see Figure 3.2). These differences may be explained by fasting in the free-ranging animals as all blood samples were taken during the spring. At this time the animals neither drink nor eat for several weeks

(three weeks to three months) (Costa and Ortiz, 1982; Le Beouf, 1986). Northern elephant seals spend most of their lives at sea where they procure their food. However, they undergo these prolonged fasts on land throughout their lifecycle in order to accomplish molting, reproduction and parturition (Castellini, et al., 1987). Consequently, blood of many of the animals becomes dehydrated as water leaves the blood to compensate for decreased tissue water which may cause a corresponding increase in hematocrit as plasma volume decreases (Huntley, 1985). Blood dehydration in free-ranging elephant seals would explain the decreased cell volume and increased plasma protein concentrations relative to captive seals. Water loss would increase the concentration of proteins in the plasma and therefore its oncotic pressure which would then result in a net movement of water out of the erythrocytes into the plasma thereby reducing MCV. Additionally, the increased plasma protein concentration causes the viscosity of the suspensing medium (plasma) and also that of whole blood to increase (Figures 3.2 and 3.3). Whole blood viscosity is also highly correlated to HCT (r=.95), the latter also contributing to the increased suspension viscosity of wild seals.

Hematologic (Table 1) and rheologic (Figures 3.4 and

3.5) characteristics of sea otters newly-captured and after three weeks of captivity also revealed no significant "short-term" acclimatization phenomenon. Hematologic variables were within the ranges reported in earlier studies (Lenfant, et al., 1970). Mean corpuscular hemoglobin concentration (MCHC) was significantly lower ($P<0.05$) in newly-captured otters. Packed cell volume (HCT), and hemoglobin concentrations were also higher after three weeks of captivity. These changes are the opposite of what one would hypothesize if the sea otters were acclimatizing to a less-active lifestyle. Plasma protein concentrations also increased with captivity, and may be responsible for the decreased MCV and increased RBC count, as noted earlier for elephant seals, although the differences are not statistically significant.

Viscometric behavior of blood reflects the hematology. There was no statistical difference in blood viscosity measurements in the sea otters after captivity (Figure 3.4). Blood viscosity was slightly elevated after captivity presumably due to the increased HCT and plasma protein concentration. As in the other species viscosity was highly correlated ($r=.95$) with plasma protein concentration and HCT.

Blood flow behavior in the capillary viscometer,

although comparable to that observed for the cone-plate viscometer, revealed some interesting differences. In both elephant seal blood and sea otter blood, the two viscometers produced similar values for viscosity at low shear rates, but apparent viscosity was considerably reduced at high shear (Figures 3.2, 3,3, 3.4 and 3.5). At low shear rates, blood viscosity is mainly determined by interactions among RBCs and between RBCs and the plasma (Chien, et al., 1966), while at high shear rates RBC orientation and deformation are more important (Chien, et al., 1970). The reduced apparent viscosity in the capillary viscometer at high shear rates is probably due to axial-migration of cells towards the center of the tube leaving a "slick" plasma layer next to the wall which has been shown to decrease apparent viscosity in human blood, the so-called "Fahraeus-Lindquist Effect" (Fahraeus and Lindquist, 1931).

In conclusion, there appears to be no acclimatization phenomenon in these marine mammals in terms of hematology and hemorheology on a short-term (sea otters) or long-term (elephant seals) basis. The water balance and redistribution of blood water to tissues and the ensuing blood dehydration is believed to be a physiological response to fasting in elephant seals. It would be interesting to study non-fasting elephant seals for these

variables to better test the hypothesis that hemorheological differences exist among captive and wild elephant seals. However, as such, this study provides justification for the use of captive marine mammals in physiological investigations of cardiovascular adaptations of this type.

CHAPTER IV.

TERRESTRIAL VS. MARINE MAMMALIAN HEMORHEOLOGY: Seals vs. Pigs

INTRODUCTION

Due to their aquatic lifestyle, some seal species frequently experience long periods of apnea during dives. Seals of the family Phocidae have several adaptations which increase their oxygen storage, one of which is an increase in the number of red blood cells (RBCs) in whole blood. Phocid seal hematocrits (HCT) are among the highest known to mammals (Lenfant, 1969). Additionally, seals have a high concentration of the oxygen-binding pigment hemoglobin in their RBCs measured as mean corpuscular hemoglobin concentration (MCHC) (Lenfant, et. al., 1970). Elevated blood volume in these marine mammals, compared with other terrestrial species, further contributes to their blood oxygen storage capacity (Lane, et al., 1972). High hematocrits such as those exhibited by phocid seals have been shown to inhibit oxygen delivery to exercising muscles in terrestrial mammals such as cats (Whalen, et al., 1973), humans (Le Veen, et al., 1980), and dogs (Gaeghtgens, et al., 1979). This is due to an increase in the blood's viscosity and a

consequent increased resistance to flow in the tissues that it serves. Therefore, it might be expected that these marine mammals may have elevated blood viscosity and related perfusion impairment.

Blood viscosity is a complex variable and is the resultant of several physical and physiological factors. The non-Newtonian behavior of blood is indicative of the shear rate dependence of viscosity and has been well-established in the literature (Haynes and Burton, 1959). The reduction of viscosity with increasing shear rate, the so-called "shear-thinning" behavior of blood, is a function of the interactions between RBCs, their deformation and the properties of the suspending medium (plasma). Weak bonds between erythrocytes at rest result in loose, rod-like rouleaux formations in normal human blood. Rouleaux in turn may form branching three-dimensional aggregate networks. The reversible breakdown of rouleaux, of loose aggregates and of weak bonds between RBCs with increasing shear rate is one explanation for non-Newtonian behavior, especially at low flow rates (Murata, 1976). However, at high shear rates, cell deformation and orientation are more important (Chien, et al., 1970; Schmid-Schonbein, et al., 1971). The bonds between RBCs are dependent upon cell surface charge, plasma protein interactions and hematocrit

(Chien, et al., 1966). RBC size and shape can vary among species, and have diverse effects on blood rheology. However, the mechanisms by which different geometric variables affect flow behavior are unclear and controversial (Amin and Sirs, 1985; Chien, et al., 1971, Stone, et al., 1968).

The aim of the present study was to characterize the bulk properties of seal and pig blood samples by means of blood viscosity measurements and to determine the effects of hematologic variables which are known to influence viscosity in other mammals. Domestic pigs (Sus domesticus) were used as terrestrial mammalian models because of their similarity to humans in terms of cardiovascular dynamics and architecture (White, et al., 1986). Additionally, pigs are routinely used as experimental models for cardiovascular studies in the author's laboratory and were readily available for blood sampling. Due to the exotic nature of phocid seals, the number of individuals of each species available for physiological experimentation is low. However, seals represent an extraordinary natural model for responses to many physiological constraints. A study of terrestrial versus marine mammal hemorheology was undertaken to elucidate possible adaptations of phocid seals to their diving habit.

MATERIALS AND METHODS

Blood samples from 7 harbor seals and 5 northern elephant seals were drawn from the extradural intravertebral vein (Harrison and Tomlinson, 1956) into heparinized syringes or Vacutainer tubes. Pig blood was drawn from the pulmonary artery by an indwelling catheter flushed with heparinized saline into vacutainer tubes or blood bags. Pulmonary artery catheters had been previously placed in the pulmonary artery during thoracotomy. All seals were conscious. One pig was anesthetized with Ketamine while the remaining two pigs were conscious and chronically catherized for use in experiments unrelated to the present study. Fresh blood smears were observed microscopically to detect any possible morphological abnormalitites and to eliminate such samples from consideration. RBC counts ($10^6/mm^3$), white blood cell (WBC) counts ($10^3/mm^3$) and Hb (g/dl) measurements were made using Coulter-counter, hemocytometer and Coulter hemoglobinometer techniques, respectively. Samples were centrifuged for 5 minutes in a microfuge (Clay-Adams) and the resulting HCTs used to calculate mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) using the following equations: MCV = HCT/RBC ; MCHC = Hb/HCT. The resulting plasma was analysed for total plasma

proteins using a clinical refractometer (Shuco Model 5711-2020) . Pig blood was used as a standard reference for viscosity measurements due to the small volume of blood available from each seal and the large quantities available from pigs. Pig blood was centrifuged and reconstituted to hematocrits from 10% to 80% in 10% increments using autologous plasma. The reconstitution of blood using autologous plasma is an accepted procedure in rheological laboratories and no significant differences in viscosity result from this practice (H. J. Meiselman, pers. comm.). Viscosity measurements were made at various shear rates from 11.5 to 230.4 sec^{-1} on a cone-plate viscometer calibrated with fluids of known viscosity (Brookfield Viscosity Standard, 5.4 centipoises and water) and operated at 37°C. Standard curves of viscosity versus shear rate for pig blood were constructed and compared with those of harbor seals and elephant seals at their normal hematocrits of 53% and 57%, respectively. All curves were based on rational spline interpolation (cubic and parametric). Pooling the viscometric data from both seal species yields curves for a mean hematocrit of 55% (± 5.9%). Therefore, the mean values for pig reconstitution curves of 50% and and 60% were averaged to obtain viscometric data for a hematocrit of 55% suitable for comparison with those of

the seals (see Table 4.1). Statistical comparisons were made using paired and mean "t" tests. Comparisons of $P<0.05$ were considered significant. Correlations were made using Pearson correlation coefficients.

RESULTS

Phocid seals exhibited increased blood oxygen storage capacity in terms of HCT and MCHC compared with similar values from other terrestrial mammals (Table 4.2). Hematocrits of elephant seals and harbor seals averaged 57 $\pm$ 4.68% and 53 $\pm$ 5.40%, respectively, while those of pigs averaged 28 $\pm$ 4.60%. Seal erythrocytes contained more hemoglobin per unit volume. MCHC for harbor seal and elephant seal bloods averaged 38% and 44%, respectively, 29% higher than the 34% MCHC for pig blood. MCHC values for blood from other terrestrial mammals averaged 33-35% (Mayerson, 1930; Wintrobe, 1933; Wintrobe, 1974). Using blood volume values from the literature (Simpson, et al., 1969; Lane, et al., 1972) and the body weights of the animals used in the present study, theoretical blood oxygen storage was computed for elephant seals and pigs (Figure 4.1 and Table 4.2). Higher blood volume, hematocrit and MCHC result in a six-fold increase in blood oxygen capacity in the elephant

Table 4.1. Viscometric data (viscosity values as shear rates indicated) from seals and swine determined on a Wells Brookfield cone plate viscometer at 37°. Data from both seal species were pooled for comparison to pigs at 55% HCT. Means (S.D.). Ratios of seal data relative to pigs are below the dashed line. P-values (95% confidence level) for comparisons among seals and pigs are in parentheses under the ratios.

	VISCOSITY (centipoise)				
	SHEAR RATE (sec^{-1})				
Species	11.5	23.0	46.1	115.2	230.4
Elephant seals (n=5) (HCT=57%)	18.6 (1.65)	15.5 (0.93)	12.6 (0.93)	10.3 (0.79)	8.9 (0.77)
Harbor seals (n=7) (HCT=53%)	15.8 (1.93)	12.7 (1.36)	10.1 (1.06)	8.4 (0.86)	7.6 (0.83)
Pigs (n=4) (HCT=55%)	22.2 (1.32)	15.8 (0.97)	11.9 (0.92)	9.4 (0.77)	8.2 (0.64)
SEALS* (HCT=55%)	17.2 (1.31)	14.1 (1.12)	11.4 (0.82)	9.3 (0.77)	8.3 (0.24)
Harbor seals/Pigs	-28% (P=0.005)	-19% (P=0.000)	-15% (P=0.000)	-10% (P=0.003)	-6.7% (P=0.020)
Elephant seals/Pigs	-16% (P=0.012)	-1.6% (P=0.040)	+5.9% (P=0.110)	+10% (P=0.090)	+9.2% (P=0.090)
SEALS*/Pigs	-22% (P=0.004)	-10% (P=0.000)	-4.6% (P=0.250)	0.0% (P=0.950)	+1.2% (P=0.540)

* arithmetically pooled data from both seal species.

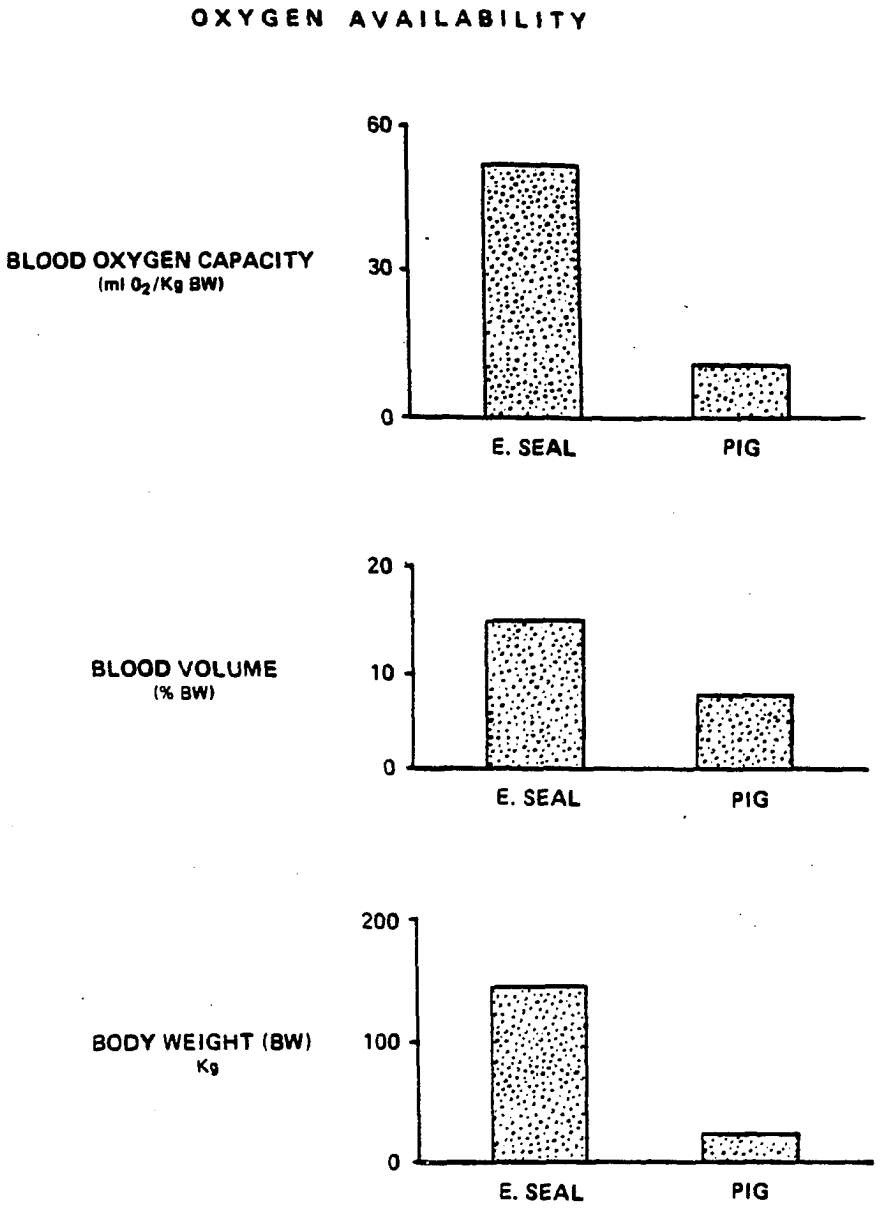


Figure 4.1. Theoretical blood oxygen storage comparison for elephant seal and pig. Body weights approximate those of study animals. Blood volumes were taken from the literature (Simpson, et al., 1969; Lane, et al., 1969). Increased blood volume, hematocrit, and MCHC contribute to a six-fold higher oxygen capacity in the elephant seal compared to the domestic pig ($P<0.05$). Blood oxygen capacity = Hb g/dl X ml O_2 / gHb X Kg BW X Blood Volume (%BW).

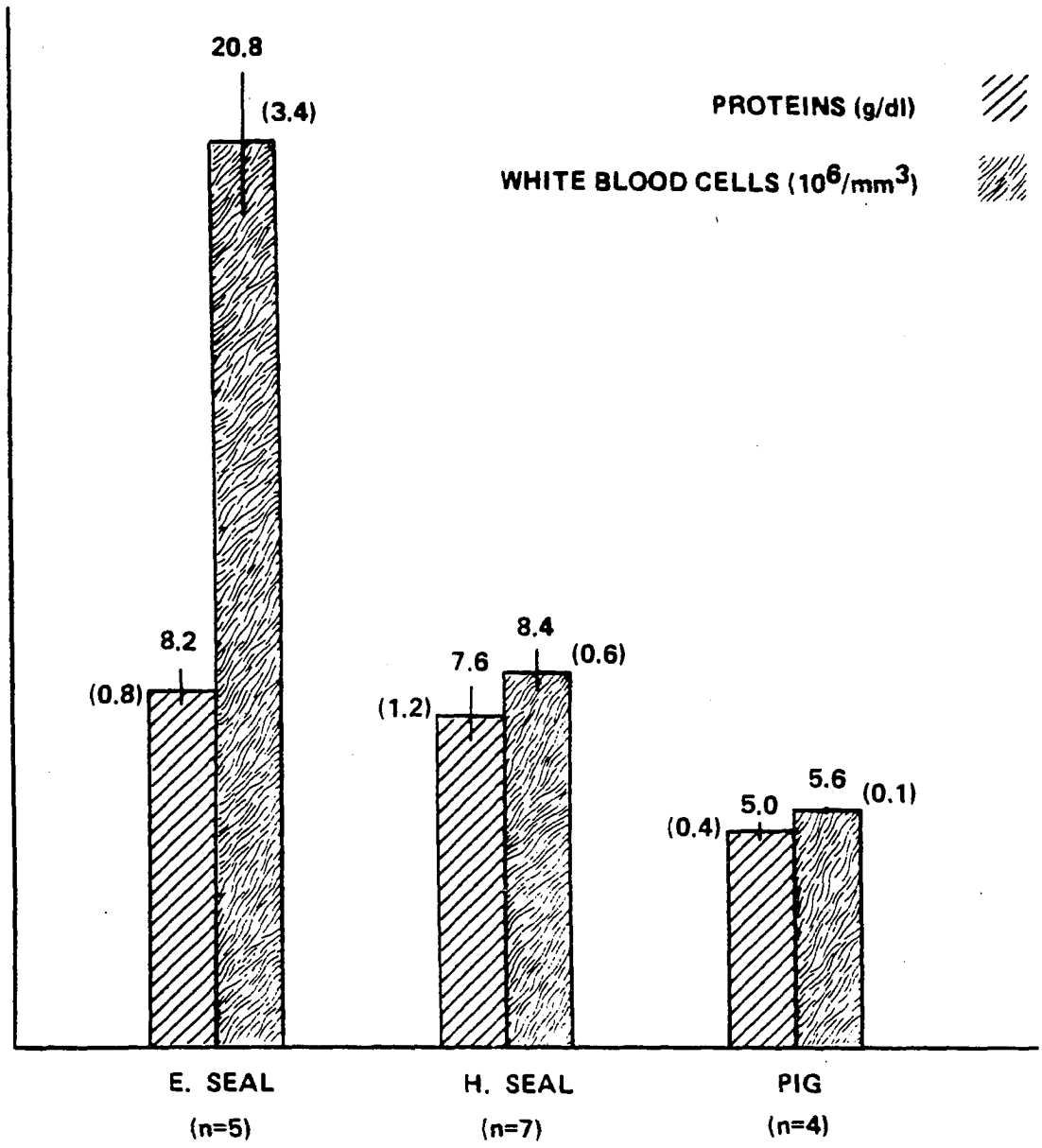


Figure 4.2. Total plasma proteins and white blood cell counts for phocid seals are increased relative to those for pigs ($P<0.05$). Elephant seal plasma protein concentrations are 64% higher while those for harbor seals are 52% higher than those for pigs. WBC counts are 271% and 50% higher for elephant seal and harbor seal blood, respectively, compared to pig blood. Means (S.D.).

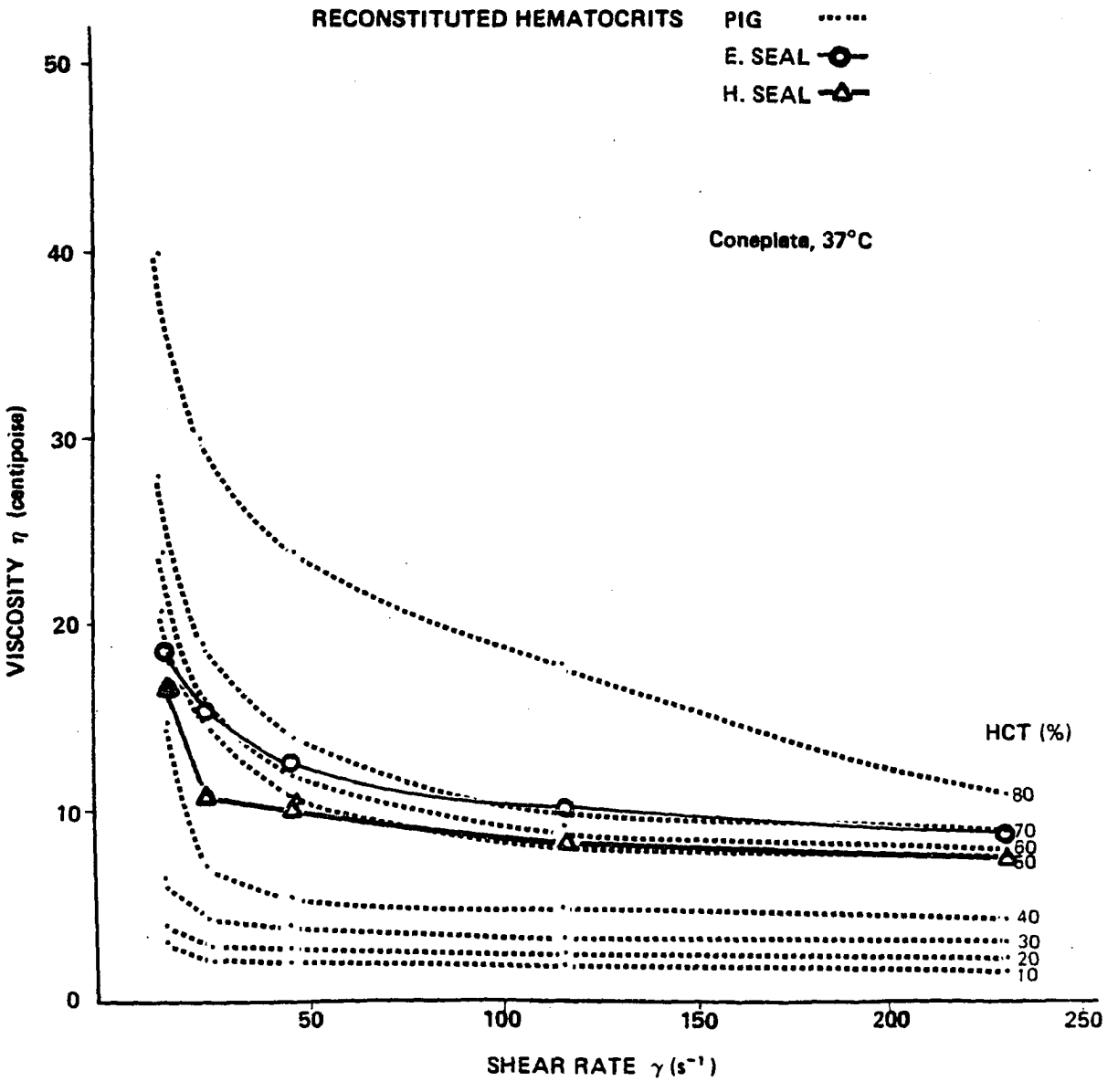


Figure 4.3. Viscometric curves for pig, elephant seal (HCT=57±4.7) and harbor seal (HCT=53±5.4) blood (see Table 4.2). Pig blood (dashed lines) was reconstituted to HCTs from 10% to 80% in 10% increments. Curves for harbor seals (n=7; triangles) and elephant seals (n=5; circles) are superimposed upon standard curves for pig blood. Blood viscosity is up to 22% lower in seals at shear rates below 50 sec^{-1} ($P<0.05$), but slightly elevated (+1.2%, $P>0.05$) at higher shear rates (see Table 4.1). Viscosity measurements were made on a Wells-Brookfield LVT coneplate viscometer at 37°C.

Table 4.2. Hematologic characteristics: mean corpuscular volume (MCV), red blood cell count (RBC), hematocrit (HCT), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of various phocid seal and terrestrial mammal species which affect blood viscosity. Literature values are consistent with those obtained in the present study. Standard deviations are in parentheses. All differences for seals versus terrestrial mammals are statistically significant ($P<0.05$).

SPECIES	MCV (μ^6)	RBC ($10^6/mm^3$)	HCT (%)	Hb (g/dl)	MCH ($\mu\mu g$)	MCHC (%)
Elephant seal (n=5(9))	176 (17)	3.19 (0.14)	57 (4.7)	24.6 (1.1)	77.4 (5.6)	44 (2.6)
Harbor seal (n=7(11))	105 (10)	5.11 (0.56)	53 (5.4)	21.0 (1.8)	40.0 (2.7)	38 (1.4)
Swine (n=3)	54 (0)	4.80 (0.86)	28 (4.6)	8.8 (1.6)	18.2 (0.2)	34 (1.0)
Human*	87	5.40	47	---	29.0	34
Goat*	19	16.00	33	10.5	6.7	34
Dog*	66	6.30	46	14.8	23.0	33
Weddell seal** (n=4)	---	---	61	23.7	---	38
Ribbon seal** (n=5)	150	4.49	67	24.5	55.1	37

* From Wintrobe, 1933; Mayerson, 1930; Wickham, et al., 1987.
** From Lenfant, et al., 1970; Lenfant, et al., 1969.

seal compared with the pig.

Phocid seal erythrocytes were larger than those of terrestrial mammals. Mean corpuscular volume (μ^3) of harbor seal RBCs was almost twice that of swine, while elephant seal RBCs were larger by nearly four-fold (Table 4.2). Consequently, RBC counts of elephant and harbor seal bloods were lower than those of the pig at the same hematocrit (Table 4.2). Pig blood has almost 10 million cells/mm^3 when reconstituted to 55% hematocrit, nearly three-fold higher than elephant seal blood at 3.19 X 10^6 cells/mm^3 (HCT=57%). Harbor seal blood contained approximately 5 million cells/mm^3 at its natural HCT of 53%. Literature values for hematologic variables of other terrestrial and marine mammalian species are consistent with those obtained in the present study (Table 4.2). Plasma protein concentrations (g/dl) and WBC counts for phocid seal bloods were also elevated compared to those for pig bloods (Figure 4.2 and Table 4.2). Plasma protein concentrations for elephant seal, harbor seal and pig bloods were 8.2, 7.6 and 5.0 g/dl, respectively. WBC counts were almost 4 times higher for elephant seal blood (20.8 X $10^3/mm^3$) than for pig blood (5.6 X $10^3/mm^3$). Harbor seal WBC counts averaged 8.4 X $10^3/mm^3$.

All blood samples studied exhibited non-Newtonian

behavior, as blood viscosities showed shear rate and hematocrit dependence. However, at shear rates less than 50 sec^{-1} seal bloods were up to 22% less viscous than reconstituted pig blood ($P < 0.005$) (Table 4.1). Seal blood viscosity showed an elevated trend (+1%), relative to pig blood, at 230 sec^{-1} (Figure 4.3 and Table 4.1). However, this difference was not statistically significant.

DISCUSSION

Comparisons of viscosity values for elephant and harbor seals and domestic pigs over a wide range of shear rates revealed that seal bloods were less viscous at low rates of shear than pig blood despite higher MCV, plasma proteins levels and WBC counts, all of which are known to increase viscosity in human blood (Chien, et al., 1966). Viscosity differences among the three species investigated diminished with increasing shear rate. Thus, if the blood of a pig were packed with RBCs to equal the hematocrit of the seals, it could not deliver as much oxygen as could the seal blood due to its increased viscosity and lower MCHC.

There have been comparative studies of terrestrial mammalian hemorheology (Amin and Sirs, 1985; Chien, et al., 1971) and viscometric studies on other phocid seal

species (Guard and Murrish, 1975; Hedrick, et al., 1986). However, the reduction in seal blood viscosity at low shear rates relative to that of pigs shown here is heretofore undescribed. Although the functional significance of these findings is not clear, it may be useful to speculate about their possible role in physiological adaptations of seals. During long breath-holding dives, a large proportion of the seal's blood volume may be pooled in large venous sinuses, such as the inferior vena cava (Elsner, et al., 1964 and 1971; Van Citters, et al., 1965) in virtual stasis with only brief, episodic circulation. The low viscosity in harbor and elephant seal blood may represent an adaptation for reducing the pressure necessary to restart blood near zero flow during dive recovery. The shear rate-dependence of this phenomenon may be a function of reduced RBC-aggregation at low shear rates (Wickham, et al., 1987).

Increased blood viscosity in seals relative to pigs at high shear rates should be expected due to their increased plasma viscosity (at all shear rates), increased RBC size, and corresponding cell dispersion. Seal blood has elevated oxygen storage due in part to higher MCHC, and the viscometric behavior suggests that they may have an adaptation to prevent related decreases

in tissue perfusion. This may also be an important adaptation to diving recovery to ensure a reinitiation of flow in static venous pools. An understanding of the mechanics involved in this process may provide insights into clinical conditions of rheological abnormalities in humans and such animals, in particular, diseases of increased peripheral resistance due to aggregation near stasis.

CHAPTER V.

RED CELL AGGREGATION AND VISCOELASTICITY OF BLOOD OF SEALS, HUMANS AND PIGS

INTRODUCTION

Blood viscosity is shear rate-dependent. Decreased blood viscosity with increasing shear rate, the so-called "shear-thinning" behavior of blood is an established characteristic of mammalian hemorheology (Haynes and Burton, 1959; Merrill, 1969). The non-Newtonian behavior of blood and RBC suspensions is related to the presence of erythrocytes. Red cells pile up into rod-like formations called rouleaux when at rest. The reversible breakdown of rouleaux, aggregates and weak bonds between erythrocytes with increasing shear rate is considered one cause of non-Newtonian behavior (Murata, 1976), especially at low flow rates (Merrill, et al. 1963). Cell deformation (Chien, et al., 1970) and orientation (Goldsmith and Beitel, 1971) elicit more effects at high rates of shear. Bonds between RBCs are influenced by cell surface charge (Seaman and Swank, 1967), proteins in the plasma such as albumins and fibrinogen, as well as hematocrit (Chien, et al., 1966). Interactions among these factors are responsible for

rheological behavior near stasis. In healthy humans, rouleaux are dispersed in normal blood flow (Whitmore, 1963). However, in some pathological disorders such as polycythemia, intermittent claudication and during infection, increased red cell aggregation may lead to problems of increased peripheral resistance (Repolge and Merrill, 1973). Red cell aggregation can be measured by the microscopic method, the light intensity method, or the rheological method at low shear rates (Sacks, 1977; Schmid-Schönbein, et al., 1975).

Although there have been a few comparative studies of hemorheology (Usami, et al., 1969; Chien, et al., 1971; Amin and Sirs, 1985), marine mammals have been little studied from this perspective (Guard and Murrish, 1975; Wickham, et al., 1985). Many marine mammals normally exhibit elevated hematocrits relative to those of terrestrial mammals. Seals of the family Phocidae (e.g. harbor, elephant and ringed seals) possess some of the highest hematocrits known to mammals (Lenfant, 1969), thereby providing an excellent "natural experiment" for possible adaptations to increased peripheral resistance. Earlier investigations by Wickham, et al. (1985) revealed decreased blood viscosity in seal blood when compared to those of swine at the same hematocrit (see Chapter IV.). The phenomenon was shear rate-dependent as viscosity

differences were more pronounced at low rates of shear and became insignificant at high shear rates. Viscosity was independent of MCV. Serum protein concentrations, MCV, mean corpuscular hemoglobin concentrations (MCHC) and white cell counts were consistently higher in seal blood than in pig blood (see Chapter IV.). These findings suggest an adaptation for increased oxygen storage capacity in seal blood and a less than anticipated viscosity-dependent reduction in oxygen transport.

The rationale behind the present study was to elucidate the factors responsible for the decreased viscosity of seal blood near stasis. The hypothesis that reduced red cell aggregation in seal blood is responsible for this flow behavior was tested using the light intensity method and the rheological method at low shear rates.

MATERIALS AND METHODS

Whole blood was obtained from three pigs that were chronically catheterized (White, et al., 1986) for experiments unrelated to the present study. Pig blood were taken from the pulmonary artery while samples from two harbor seals, four elephant seals and three ringed seals were taken from the extradural intravertebral vein

using a spinal needle or disposable catheter (B-D, 14 gauge) (Geraci and Smith, 1975). These vessels were selected in order to obtain large, well-mixed samples. All animals were conscious during the sampling procedure. At least two samples from each animal were drawn into EDTA-treated vacutainer tubes and transported to the laboratory within three hours. One sample set was flown from the Alaska Zoo in Anchorage, Alaska, to the laboratory in Los Angeles, which took twelve hours. Samples were centrifuged at 6g for 10 minutes. The plasma was removed, centrifuged in a Sorvall benchtop centrifuge for 10 minutes and the remaining white blood cells were removed. Leucocytes were not removed from elephant seal blood. Red blood cells were washed twice in a solution of 30mM phosphate buffer plus 0.1% albumin (bovine) then resuspended in autologous plasma and reconstituted to a hematocrit of 40% as is standard procedure in rheological laboratories with no significant resulting viscosity differences (H. J. Meiselman, pers. comm.). Reconstituted samples were then analysed for sedimentation, aggregation, and viscoelasticity.

Sedimentation rates (ZSR) were determined using a zetafuge and mean corpuscular volume (MCV) was obtained from a celloscope. Aggregation indices (AIs), T_{fast}, T_{slow}, T_{tot}, and $\dot{\gamma}_{min}$ T (Bauersachs, et al.,

1987), were calculated from optical density measurements made on a Myrenne aggregometer (MA-1 Aggregometer, Myrenne GmbH, Roetgen, F.R.G.). The Myrenne system consists of a transparent cone which rotates above a stationary transparent plate. Aggregation of individual erythrocytes into rouleaux or three-dimensional aggregate networks causes gaps to form in the suspending medium (plasma) between cell aggregates causing an increase in light transmission through the blood sample. The latter is measured by an infrared detector above the cone which receives light from an infrared source below the plate (Figure 5.1). A blood sample is introduced to the plate and sheared at 500 sec^{-1} to disperse all aggregates. Aggregation "extent" is determined by running the cone at 500 sec^{-1} and then instantly arresting rotation which causes a characteristic drop in light transmission due to a transient state of random cellular orientation (Schmid-Schonbein, et al., 1975). Light transmission then increases with time at a rate proportional to the formation of cell-free gaps between aggregates. Light transmission is plotted against time and fitted to a second-order polynomial using a multiple linear model with one dependent variable. The AI is equal to the integration of the area beneath the curve for a 10 second time-period (Figure 5.2).

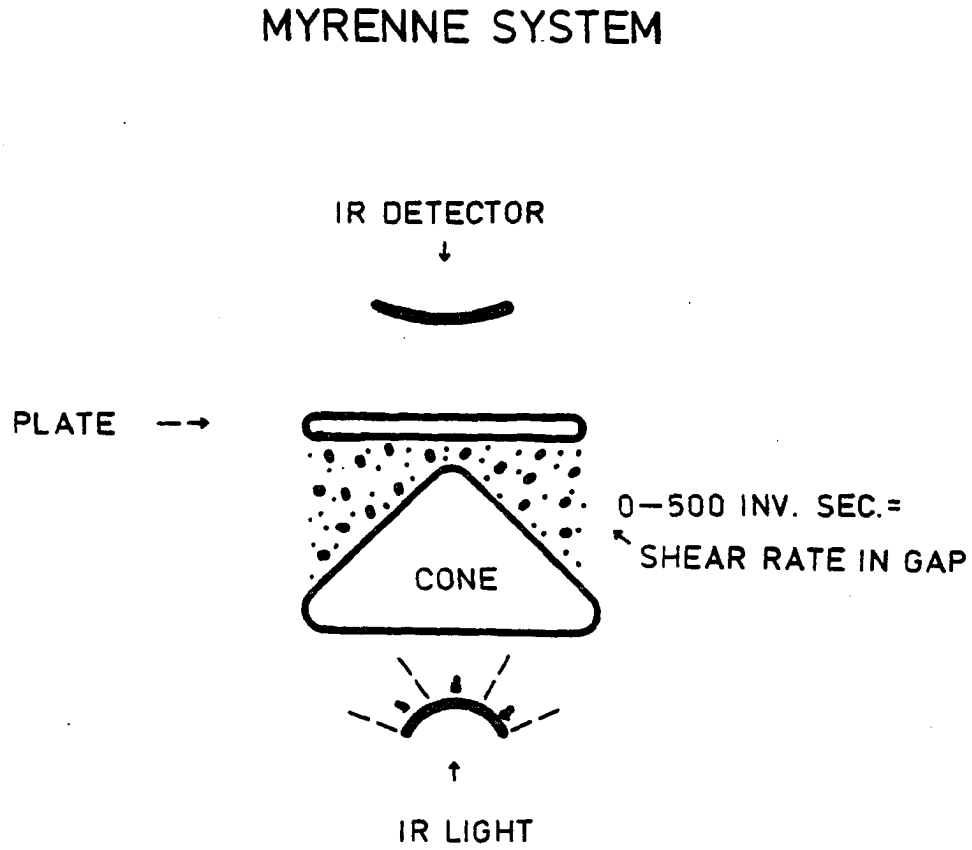


Figure 5.1. Schematic of the Myrenne Aggregometer (GmbH, Roetgen. F. R. G.) used to determine extent, rate and strength of RBC aggregation. The system consists of a transparent cone which rotates below a stationary transparent plate. Aggregation of RBCs causes gaps in the suspending medium and a corresponding increase in light transmission passing through the sample from an infrared source below the cone and detected by an infrared detector above the plate.

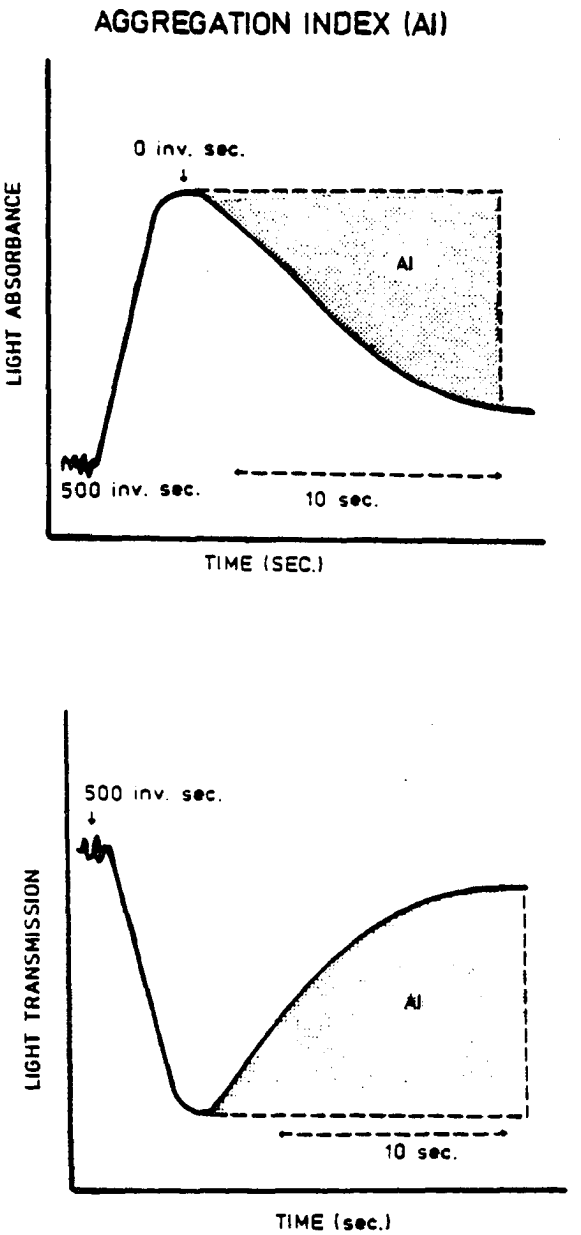


Figure 5.2. Typical plots of light absorbance (top) and light transmission (bottom) versus time obtained from Myrenne aggregometry. The aggregation index (AI) is determined by integrating the area beneath the light transmission curve (equals the area above the light absorbance curve) for a 10 second time-interval.

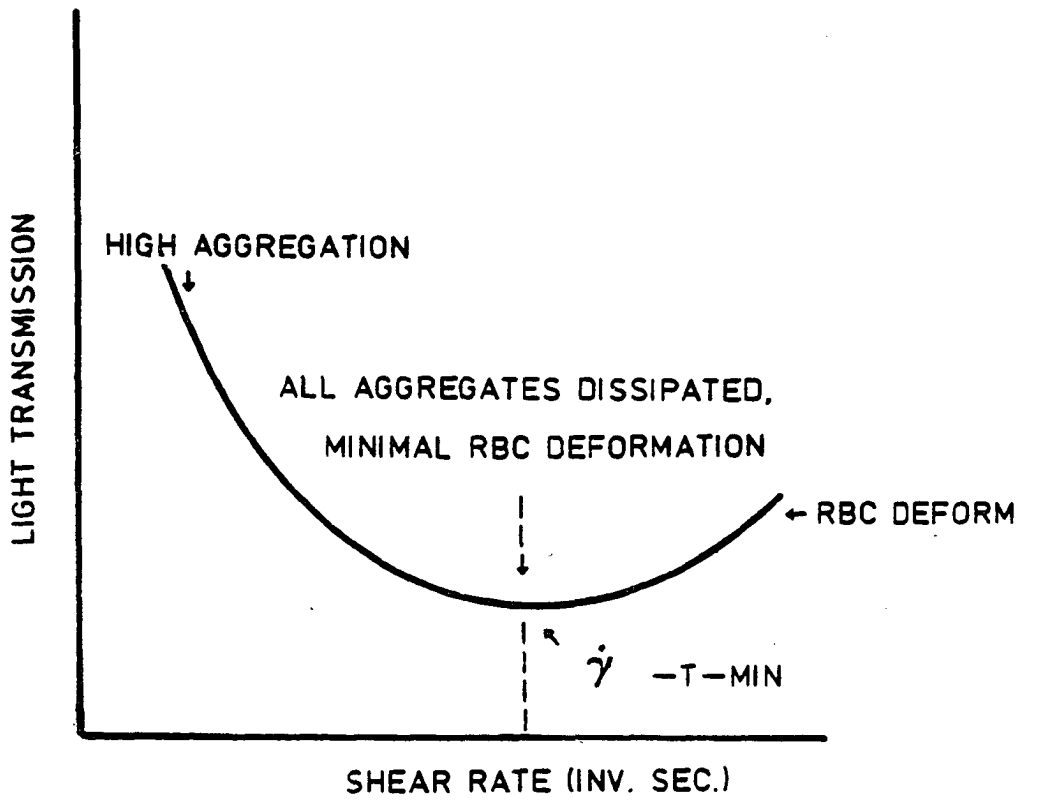


Figure 5.3. Typical plot of light transmission versus shear rate in the gap in the Myrenne aggregometer. The data are fit to a third degree polynomial and the lowest point on the curve represents the shear rate at minimal light transmission ($\dot{\gamma}$ Tmin), the point at which all aggregates are dispersed and minimal erythrocyte deformation.

Analysis of the light transmission curve at stasis via an external computer-controlled analog to digital conversion of the light transmission signal yields the kinetics of aggregation or "rate". T_{fast} is the time constant for the first 6 seconds. T_{slow} is the time constant for the last 15 seconds and T_{tot} is the overall half-time for the aggregation process. Aggregation "strength" ($\dot{\gamma}\,^{T}min$) is determined by running the cone and collecting data in light transmission and shear rate. Plotting light transmission versus shear rate in the gap fitted to a third-order polynomial using a multiple linear model with one dependent variable allows determination of shear rate at minimal light transmission. The lowest point of this curve, $\dot{\gamma}\,^{T}min$, indicates the point at which all aggregates are broken up, but there is minimal RBC deformation (Figure 5.3). Viscosity at $\dot{\gamma}\,^{T}min$ is calculated from interpolation of the apparent viscosity data from a capillary viscometer (OCR-D); $n\,\dot{\gamma}\,^{T}min$ is OCR-D apparent viscosity at $\dot{\gamma}$ = $\dot{\gamma}\,^{T}min$. Shear stress at $\dot{\gamma}\,^{T}min$, $\tau\dot{\gamma}^{T}min$, is equal to the product of $n\dot{\gamma}^{T}min$ and $\dot{\gamma}\,^{T}min$.

Apparent blood viscosity (η') and elasticity (η'') measurements were made using an Oscillating Capillary Rheo-densimeter (OCR-D) at shear rates from 2 to 100 sec^{-1}.

The viscous (η') and elastic (η'') components of

dynamic viscosity were determined from the phase shift in the pressure-flow curves, where the phase of the strain (flow) is always behind that of the stress (pressure). The samples were oscillated at a fixed 2 Hz and the shear varied to obtain the two phases. Dynamic viscosity is equal to the loss modulus divided by the angular frequency, and is thus a ratio of dissipated energy to angular frequency - the component out of phase with the strain. Elasticity is the ratio of the dynamic modulus (shear modulus) to the angular frequency - the component in phase with the strain. For a comprehensive treatise concerning the theoretical basis of these calculations please see Fung (1981).

Plasma viscosity (η_{pl}) was calculated from at least five measurements per sample made using a HAAKE capillary plasma viscometer. Ringed seal blood and pig blood were analysed for viscosity at very low shear rates (0.51 to 94.5 sec^{-1}) on a Contraves Ls-30 viscometer. All viscometers were calibrated with fluids of known viscosity and operated at a constant temperature of 25°C. Electrophoretic mobility (EM) ($\mu m.sec^{-1}v^{-1}.cm$) was determined for red blood cells (n=50) of ringed seals and normal humans (Table 5.1). Erythrocytes were washed, suspended in 30mM phosphate buffer solution, placed in the chamber of the mobility apparatus and a voltage was

applied across the tube. The time required for each cell to pass a fixed distance was measured by stopwatch during microscopic observation.

Total plasma proteins, plasma fibrinogen and sedimentation rates in Westergren tubes (ESRs) were determined for a total of five EDTA-treated blood samples from two ringed seals, one sample each from two northern elephant seals, five harbor seals and five domestic pigs (Table 5.3). Plasma proteins and fibrinogen were obtained using a clinical refractometer (Shuco Model 5711-2020). Fibrinogen was precipitated from the plasma in a hot water bath at 56°C for three minutes, spun down in a benchtop centrifuge for 5 minutes and observed in the refractometer. The resulting reading was subtracted from the total proteins to obtain fibrinogen in mg/dl. The average of two readings from each sample per animal were used for statistical calculations. Two Westegren tubes per sample were observed at one and two hour intervals and the results averaged to obtain ESRs in mm/hr. Comparable data for humans in terms of aggregation dynamics, viscosity, plasma proteins and sedimentation were taken from studies performed in the same laboratory using the same instrumentation by colleagues (Bauersachs, et al., 1987).

Statistical comparisons were made using paired and

mean "t" tests. Comparisons of $P<0.05$ were considered significant.

RESULTS

Aggregation indices (AIs), T-fast, T-slow, T-tot, $\dot{\gamma}^{T}$ min, $\eta\dot{\gamma}^{T}$ min, $\tau\dot{\gamma}^{T}$ min, ZSRs, MCVs, plasma viscosities (η_{pl}) and RBC electrophoretic mobilities (EM) are listed in Tables 5.1A and 5.1B. Ringed seal blood aggregation was too low to measure on the aggregometer. Two of the elephant seal bloods had extremely low aggregation (AI = 1.0, 2.7) while samples from the remaining two animals were considerably higher (A = 11.5, 12.7). Harbor seal blood had elevated AIs (12.7, 23.0) and $\dot{\gamma}^{T}$ mins and shorter half-times, and the shear stress necessary to break-up harbor seal RBC aggregates was over twice that for elephant seal erythrocytes. Blood from elephant and ringed seals showed decreased aggregation in terms of rate, strength and extent as half-times (T-slow, T-fast, T-tot) were longer and $\dot{\gamma}^{T}$ min, AIs, ZSRs, and ESRs were lower when compared to human values (Tables 5.1A and 5.1B). Pig bloods were intermediate in aggregation when compared to the human norm of AI=16 (Bauersachs and Meiselman, 1987). Shear rate at minimum light transmission ($\dot{\gamma}^{T}$ min), T_{fast}, T_{slow}, T_{tot}, $\eta\dot{\gamma}^{T}$ min, and $\tau\dot{\gamma}^{T}$ min reflect the differences in AIs; those samples

Table 5.1A. Aggregation indices (AI), $\dot{\gamma}_{Tmin}$, T-fast, T-slow, T-tot, and $\tau_{\dot{\gamma}_{Tmin}}$ calculated from Myrenne aggregometry of blood samples from seals, pigs and humans reconstituted in autologous plasma to 40% HCT. Means (S.D.). n_2 denotes total sample number per species.
* From (Bauersachs,et al., 1987).

Species	AI	$\dot{\gamma}_{Tmin}$ (sec^{-1})	T_{fast} (sec^{-1})	T_{slow} (sec^{-1})	T_{tot} (sec^{-1})	$\tau_{\dot{\gamma}_{Tmin}}$ (mPa)
Harbor seals (n=2)	17.88 (5.80)	90.75 (49.2)	1.61 (0.08)	13.04 (2.64)	5.40 (1.50)	522 (285)
Elephant seals (n=4) (n_2=8)	6.98 (5.98)	34.80 (8.26)	2.41 (0.49)	18.55 (2.85)	9.81 (0.99)	213 (35)
Ringed seals (n=3) (n_2=7)	0.0	3.35 (0.65)	---	---	---	28 (4.1)
Pigs (n=3) (n_2=6)	7.42 (1.15)	22.67 (8.70)	2.01 (0.25)	14.90 (1.48)	7.53 (1.79)	179 (67.1)
Humans* (n=42)	16.60 (4.20)	58.40 (28.8)	1.63 (0.39)		4.25 (2.44)	275 (94.6)

Table 5.1B. Plasma viscosity (η_{pl}) from a Haake plasma viscometer, zeta sedimentation rate (ZSR) from a zetafuge, mean corpuscular volume (MCV), and RBC electrophoretic mobility (EM) measurements obtained from the same samples as in Table 1A (above). Means (S.D.). (n_2 denotes total samples per species).

Species	η_{pl} (mPa.s)	ZSR (%)	MCV (μ^3)	EM (n=50) ($\mu m.s^{-1}.v^{-1}.cm$)
Harbor seals (n=5)	---	52.35	105* (4.80)	---
Elephant seals (n=4, n_2=8)	---	---	176*	---
Ringed seals (n=3, n_2=7)	1.63 (0.08)	40.50 (0.87)	122 (3.6)	1.369 (0.02)
Pigs (n=3, n_2=6)	1.56	46.00 (5.00)	54*	---
Humans** (n=42)	1.59 (0.08)	49.80 (5.00)	87*	1.092 (0.02)

* From (Wickham, et al., 1985).
** From (Bauersachs, et al., 1987).

with high aggregation indices exhibiting increased $\dot{\gamma}_{T_{min}}$ and $\tau_{\dot{\gamma}_{T_{min}}}$. The viscosities at $\dot{\gamma}_{T_{min}}$ for ringed seal blood were higher than those of the other seal, pig or human blood, corresponding to the extremely low shear rate at which aggregates dissipated in ringed seal blood. Seal plasma viscosities were consistently higher than those for pigs or human norms. Similar to aggregation indices, ZSRs indicated no measureable sedimentation for ringed seal blood (equal to the hematocrit of the sample) while those for harbor seals were considerably higher than those for human blood (Table 5.1). Electrophoretic mobility (EM) was increased 25% in ringed seal RBCs compared to human erythrocytes.

The results of rheological studies at low to high shear rates in the capillary viscometer (OCR-D) are represented in Table 5.2. Apparent viscosity (η') measurements for harbor seal and elephant seal blood were lower than those for pigs at shear rates to 100 sec^{-1}. Ringed seal blood viscosity was also lower up to 50 sec^{-1} of shear, then averaged slightly higher than that of pigs at 100 sec^{-1}. Elasticity (η'') measurements were consistently lower in all seal blood relative to those of pig bloods at shear rates below 100 sec^{-1}. Differences in apparent viscosity data between seals and pigs were statistically significant ($P<0.05$) only at

Table 5.2. Viscoelasticity of seal, pig and human blood at hematocrits of 40%. Data for humans are from Bauersachs and Meiselman (1987). Viscous (η') and elastic (η'') components of dynamic viscosity were determined at shear rates from 2 to 100 inverse seconds using an oscillating capillary rheo-densimeter (OCR-D) at a fixed frequency of 2 Hz. Values are means (S.D.).

	SHEAR RATE (sec^{-1})							
	2		10		50		100	
Species	η'	η''	η'	η''	η'	η''	η'	η''
Harbor seals (n=2)	9.38 (0.28)	2.94 (0.24)	7.84 (0.62)	1.38 (0.36)	5.96 (0.36)	0.11 (0.03)	5.62 (0.32)	0.02 (0.02)
Elephant seals (n=4)	9.36 (0.64)	2.48 (0.34)	5.89 (0.53)	1.12 (0.18)	6.06 (0.41)	0.28 (0.18)	5.88 (0.34)	0.26 (0.21)
Ringed seals (n=3)	8.76 (0.32)	1.57 (0.06)	7.30 (0.16)	0.44 (0.04)	7.15 (0.20)	0.10 (0.02)	6.78 (0.20)	0.02 (0.02)
Pigs (n=3)	11.74 (0.64)	5.74 (0.63)	9.35 (0.75)	2.31 (0.55)	6.88 (0.40)	0.40 (0.10)	6.06 (0.29)	0.00 (0.04)
Humans (n=15)*	9.23 (0.60)	2.90 (0.45)	7.43 (0.56)	1.26 (0.36)	5.66 (0.24)	0.15 (0.05)	5.13 (0.25)	0.03 (0.03)

shear rates below 2 sec^{-1}.

Blood viscosity measurements at very low shear rates obtained from the Contraves (Ls-30) rotational viscometer showed a pronounced reduction in viscosity near stasis (0.51 to 8.1 sec^{-1}) in ringed seal blood compared to pig blood (Figure 5.4). However, at shear rates above 27.7 sec^{-1}, viscosities were slightly higher in ringed seal blood. The differences were not statistically significant presumably due to small sample size.

Although total proteins were higher in the seal blood than in pig blood, fibrinogen levels were considerably reduced (Table 5.3). Sedimentation rate in Westergren tubes was unmeasurable (ESR=0) for ringed seal blood while that of pig blood was 24.40 mm/hr. Harbor seals exhibited relatively low ESRs of approximately 5 mm/hr. In an effort to establish any possible sedimentation reading, ringed seal blood was allowed to sit overnight in the Westergren tubes. After 24 hours, still no sedimentation was observed. Data for pigs were comparable to literature values for humans (Wintrobe, 1933).

Table 5.3. Plasma proteins and sedimentation rates for at least two blood samples each from two ringed seals, one sample each from two northern elephant seals, five harbor seals and five pigs. Sedimentation rates (ESR) are the average of two successive one-hour readings in Westergren tubes from two subsamples. Fibrinogen measurements were made by precipitation at 56°C.

Species	Total Protein (g/dl)	Fibrinogen (mg/dl)	ESR (mm/hr)	HCT (%)
Ringed seals n=2	7.5±0.29	172±2	0.0	53±7.80
Pigs n=5	7.2±0.76	400±100	24.40±14.70	34±1.67
Elephant seals n=2	8.2±0.80**	125±75	---	57±4.70**
Harbor seals n=5	8.1±0.34	102±37	5.5±1.5	53±5.4

** from (Wickham, et al., 1985).

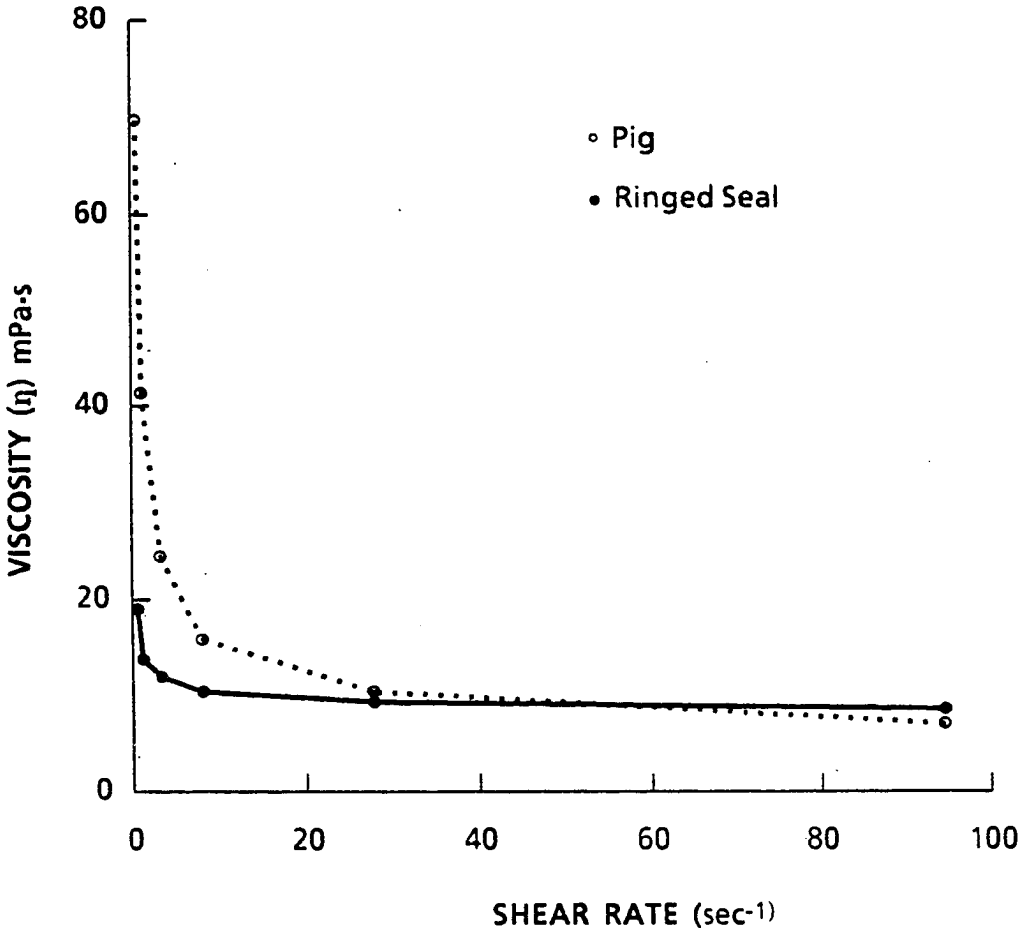


Figure 5.4. Plot of viscosity (mPa.sec = centipoise) versus shear rate (sec^{-1}) for ringed seal blood (solid line) and pig blood (dashed line). Data were obtained using a Contraves Ls-30 rotational viscometer at 25°C.

DISCUSSION

The finding of reduced aggregation in seal blood relative to that of terrestrial mammalian models in this study are suggestive of an adaptation to diving and lend further support to the conclusions of earlier studies (Guard and Murrish, 1975; Wickham, et al., 1985). During long dives seals conserve oxygen by increased peripheral vasoconstriction (Irving, et al., 1942). Some species of phocid seals may experience extremely reduced flow to extremities for as long as an hour before surfacing from a dive restores peripheral flow. During this time blood may be pooled in large venous sinuses (Elsner, et al., 1964 and 1971; Van Citters, et al., 1965). Thus, long periods of blood stasis may be normally experienced by diving seals but not by pigs and other terrestrial mammals. It is well-known that human blood viscosity near stasis approaches infinity and the shear stress necessary to overcome the elastic forces of red cell aggregates (yield stress) becomes large (Merrill, et al., 1963). Decreased red cell aggregation and a concomittent decrease in blood viscosity may represent an adaptation to reduce the force necessary to restart stagnant blood during dive recovery in phocid seals.

It is interesting that even though the seal species

have elevated plasma viscosities and larger MCVs (Table 5.1), which might be expected to increase blood viscosity and peripheral resistance (Chien, et al., 1970; Stone, et al., 1968), these marine mammals exhibit a reduction in blood viscosity when compared to pigs. In harbor and elephant seal bloods this viscosity decrease was evident over all shear rates examined (Table 5.2) even though white cells were present in elephant seal blood. The increased elasticity and rigidity of white cells compared to red cells act to increase viscosity and resistance to flow in human blood (Dintenfass, 1968). Results of investigations aimed at evaluating the influence of erythrocyte shape and size on blood viscosity have been contradictory (Gregersen, et al., 1965; Stone, et al., 1968; Usami, et al., 1969; Chien, et al., 1971) Our results support these anomalous influences as the rheological behavior of seal blood reported here fits no generalization relative to erythrocyte size as represented by MCV.

Aggregation indices and associated light transmission measurements of $\dot{\gamma}^{T}_{min}$, $\eta_{\dot{\gamma}^{T}_{min}}$, and $\tau_{\dot{\gamma}^{T}_{min}}$, however, were not entirely consistent with the reduction in seal blood viscosity. Even though some of the seals (e.g. elephant and harbor seals) had higher AIs, $\dot{\gamma}^{T}_{min}$, and $\tau_{\dot{\gamma}^{T}_{min}}$ than pigs (Table 5.1), apparent

viscosities measured in the OCR-D were still lower at all shear rates (Table 5.2). This leads to the conclusion that a variable(s) other than or in addition to aggregation may have important influences upon this phenomenon. Conversely, the rheological behavior of ringed seal blood supports the contention that reduced aggregation is responsible for lower viscosity in that species at low shear rates. The cause(s) of increased ringed seal blood viscosity at high shear rates is unknown but increased cell size and increased membrane stiffness can be suggested as one possible explanation (G. Schmid-Schonbein, pers. comm.).

The variability in RBC aggregation among seals of the same species is also intriguing. Elephant seal AIs varied by as much as twelve-fold (Table 5.1). The presence of white cells in elephant seal bloods may have contributed to this discrepancy. Additionally, cell surface charge phenomena such as ionic charge differences may account for some of the reduced aggregation in ringed seal bloods. The mean electrophoretic mobility of ringed seal erythrocytes was 1.369 $\mu m\ sec^{-1}.v^{-1}.cm$ compared to 1.092 for human red blood cells (Table 5.1B). This 25% increase in cell surface charge may cause ringed seal erythrocytes to slightly repel one another thereby reducing the interactions responsible for cell

aggregation and sedimentation at low shear rates. The same characteristics may cause the increases in seal blood viscosity relative to pig blood as shear rate and cell dispersion increases.

Another factor which has bearing on the results of this study is the influence of diet upon hemorheology. Omega-3 polyunsaturated fatty acids such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) have been found to decrease blood viscosity (Kobayashi, et al., 1981) and platelet aggregation (Ahmed and Holub, 1984) and increase erythrocyte deformability in human blood (Terano, et al., 1983; Popp-Snjiders, 1985). Due to their high fish diet, the blood of some seals contains high amounts of these fatty acids (Dr. B. J. Holub, pers. comm.). Recently, Puppione, et al. (1987) reported that northern elephant seal platelet response to agonists which normally stimulate platelet aggregation appear to be dissociated. The possible influence of dietary fatty acids upon flow behavior of seal blood warrants further investigation.

The reduced fibrinogen levels in blood from all three seal species and reduced ESRs of ringed seal and harbor seal blood, despite a significantly higher hematocrit (Table 5.3), provide additional evidence for the extraordinary reduction in aggregation and viscosity

in seal blood. Aggregation is known to vary directly with fibrinogen concentration in human blood (Chien, et al., 1966). Thus, lower fibrinogen levels in ringed and elephant seal blood may account for differences in sedimentation and rheology near zero flow.

In conclusion, the reduced viscosity of ringed seal blood when compared to terrestrial mammalian models is a function of erythrocyte aggregation. The cause(s) of viscosity reductions for harbor and elephant seal blood is not clear. However, aggregation rate, extent and strength are much lower for blood samples from both elephant and ringed seals than for humans. Electrophoretic mobility, fibrinogen levels and perhaps diet may be factors responsible for this unusual rheological phenomenon. The flow behavior of seal blood seems to represent an adaptation to reduce problems of increased peripheral resistance upon dive recovery by reducing the stress necessary to re-establish flow in large, stagnant venous sinuses.

CHAPTER VI.

EXPERIMENTAL HEMOCONCENTRATION AND HEMODILUTION AND THE DETERMINATION OF OPTIMAL HEMATOCRIT: Harbor seals vs. Domestic pigs

INTRODUCTION

The formula which links blood pressure (P) and blood flow (F) (or cardiac output) with resistance is well-known (P=RF). Resistance (R) in the preceeding equation is characterized by blood vessel geometry and the effects of internal friction within the flowing blood (blood viscosity). According to Poiseuille's Law (Poiseuille, 1841 and 1842), resistance to flow in a tube (or vessel) is primarily related to its length and inversely related to the fourth power of the radius resulting in a sixteen-fold increase in flow rate for each corresponding increase in vessel radius of one-half. Resistance to flow is highest in the arterioles, capillaries and venules where a large portion of the circulating blood volume makes contact with vessel walls (Burton, 1966). Blood viscosity is a major contributor to vascular resistance in all vessel sizes (Wells, 1964). However, until recently, it had not been considered a variable suitable for manipulation.

A reduction in hematocrit (HCT) will reduce blood

viscosity and increase blood flow, but a decrease in oxygen carrying capacity will result if cardiac output is constant. Conversely, an increase in HCT will increase the oxygen carrying capabilities of the blood, but the expected increase in viscosity may reduce the flow rate and thus, perfusion of tissues (Shepherd and Reidel, 1982; Gaeghtgens, et al.,1979). Somewhere between these extremes lies the optimal or "ideal hematocrit" (Crowell and Smith, 1967). It has been found that within a range of hematocrit from 20% to 40% in humans and dogs that cardiac output increases sufficiently to compensate for the decreased oxygen carrying capacity during experimentally-induced anemia without change of volume concentration (isovolemic hemodilution) (Repolge, et al., 1970; Messmer, et al., 1972; Restorff, et al., 1975). Consequently, the use of hemodilution to reduce vascular resistance has become a popular topic in medicine (Fowler and Holmes, 1975; LeVeen, et al., 1980), especially among researchers interested in myocardial oxygen consumption (Jan and Chien, 1977; Jan, et al., 1980; Baer, et al., 1987; Vergroesen, et al., 1987) and diseases of increased peripheral resistance (Gelin, 1961; Rand, et al., 1964; Hakanson and Oh, 1977; Pruzanski, et al., 1972).

Interest among comparative physiologists has led to

the determination of the optimal hematocrit of several species including amphibians (Weathers, 1976; Hillman, et al., 1985), cats (Whalen, et al., 1973), goats (Vergoesen, et al., 1987), as well as humans (Messmer, et al., 1972) and dogs (Fan, et al., 1980). However, manipulation of whole body hematocrit had never been performed on a marine mammal. Hematocrit values for several species of marine mammals maintained in captivity, as well as values for some newly-captured and free-ranging individuals have been recorded (Lenfant, 1969; McConnell and Vaughan, 1983; Cornell, 1983). However, blood viscosity and flow behavior have been little studied (Guard and Murrish, 1975).

The purpose of this study was to determine the optimal relationship of hematocrit and circulating hemoglobin to plasma volume in harbor seals, Phoca vitulina, and domestic pigs, Sus domesticus. Seals of the family Phocidae have large blood volumes and some of the highest hematocrits known to mammals (55-65%) (Lenfant, 1969). Their hematocrits reach the range where increased viscosity may begin to interfere with oxygen transport to exercising muscles (Gaeghtgens, et al., 1979). Seals show a relatively low maximum oxygen consumption during exercise compared with terrestrial mammals (Ashwell-Erickson, 1981), thus making them an

ideal model for experimental hemodilution. Pigs were selected as models of terrestrial mammalian physiology because they are routinely used in clinical studies of cardiovascular dynamics due to their similarity to humans in coronary architecture and their overall tractability (White, et al., 1986). The questions addressed in this investigation were: Does whole body or myocardial oxygen consumption ever become dependent upon viscosity at arteriolar shear stress or at flow conditions comparable to those of the animals under experimentation? What are the optimal hematocrits for seals and pigs based upon oxygen consumption data and are they different? In seals is the trade-off a high oxygen carrying capacity at the expense of reduced oxygen transport such that the tissue oxygen consumption declines? The manipulation of whole body hematocrit in harbor seals and domestic pigs was used as a model for studying the in vivo effects of hematocrit on blood viscosity and oxygen consumption and to answer these questions.

MATERIALS AND METHODS

Experimental measurements were made using the surgical preparation schematically represented in Figure 1. Ten domestic pigs and four harbor seals weighing between 20 and 30 Kg were instrumented under general anesthesia.

All animals were immature. Ketamine (intramuscular, 25 mg/Kg) and atropine were used initially, followed by surital (intravenous, 25 mg/Kg) for introduction of an endotracheal tube and insertion of peripheral catheters. Halothane (0.5%) was continuously administered in a Harvard ventilator for most of the surgical period. The animals were initially given 300 units/Kg Heparin followed by 100 units/Kg every hour to prevent clotting. Ventilation was adjusted initially to maintain the appropriate arterial P_{CO2} and pH (7.4 ± 0.5) then adjusted minimally during the experiments. On-line blood gas measurements were made with an Instrumentation Laboratories IL-113 blood gas analyzer.

After thoracotomy, an electromagnetic flow probe was placed at the root of the aorta and cardiac outputs (Q_T) were calculated by integration of the tracings for flow vs. time with a planimeter. Aortic, left atrial and coronary (left circumflex coronary artery) blood pressures were registered with inserted tygon catheters attached to calibrated electromanometers (Statham Instruments P23D6). The left atrial catheter was also used for injection of radiolabeled microspheres which were used for coronary flow calibration. Coronary flow was determined using a cuff-type electromagnetic flow transducer. Electrocardiogram (ECG) was monitored as

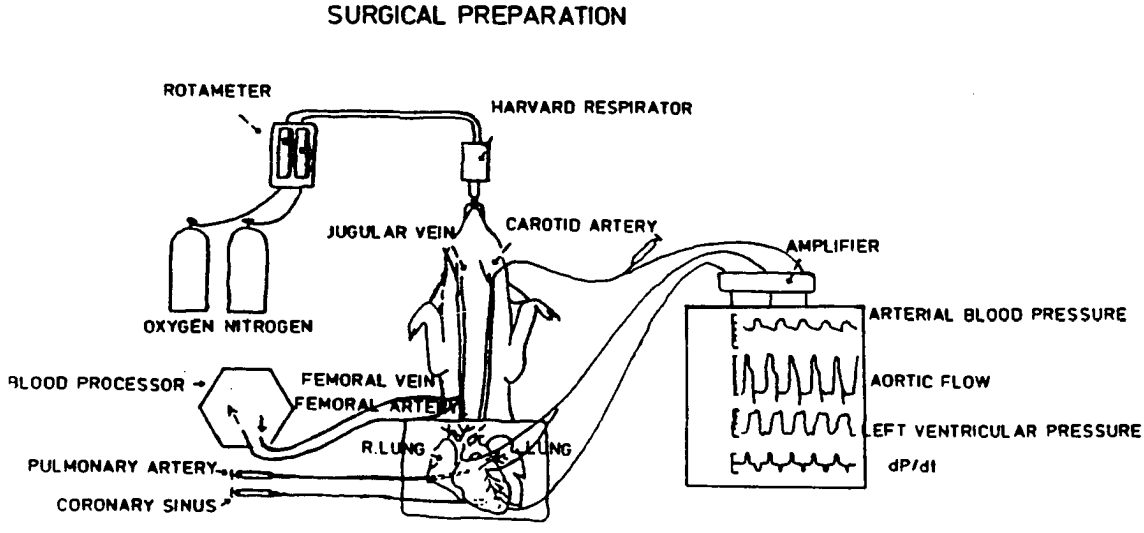


Figure 6.1 Surgical preparation of swine and seals used during experimentation. Animals were anesthetized and were maintained on a Harvard ventilator. A flow probe was inserted in the root of the aorta, ECG monitored as limb lead II, pressure transducers measured arterial, left atrial and coronary blood pressures while organ blood flow was determined using radiolabeled microspheres. Total body hematocrit (HCT) was altered progressively using a Haemonetics (30-S) blood processor. Catheters were placed in the carotid artery, pulmonary artery, coronary sinus and femoral artery. Blood gas and pH values were derived from an Instruments Laboratories IL-813 blood gas analyzer and IL-282 co-oximeter.(redrawn from Willford, 1985).

limb lead II. Left ventricular pressure and its first derivative dP/dT were measured by a high fidelity Konigsberg pressure transducer (P-22) calibrated against the Statham transducer and placed in the left ventricle.

Organ blood flow was determined using radiolabeled microspheres. Seven or eight types of radiolabeled microspheres were used in each animal during each experiment : ^{153}Gd, ^{114}In, ^{141}Ce, ^{51}Cr, ^{113}Sn, ^{103}Ru, ^{95}Nb and ^{46}Sc (New England Nuclear, Boston, Mass, 15 um in diameter) injected into the left artrium during aortic withdrawal (Heymann, et al., 1977). At the end of the experiment, the coronary bed distal to the flow probe was dissected and weighed so that blood flow could be normalized to milliliters per minute per kilogram.

Total body hematocrit was progressively altered during the experiments using a blood processor (Haemonetics, Inc. 30-S) which takes blood from the animal and separates it into RBCs and plasma; the latter is then drained back into the animal along with a solution of Ringer's Lactate + 1.2% albumin or saline and a low molecular weight dextran (<70,000 MW) to replace the original volume withdrawn (i.e. isovolemic hemodilution). Hemoconcentration involved the addition of packed RBCs back to the animal to replace the volume withdrawn.

Catheters for blood sampling were placed in the carotid artery , the pulmonary artery and the coronary sinus in order to collect arterial, mixed venous and venous blood from the heart, respectively. Blood gas and pH values were determined using an Instruments Laboratories IL-813 blood gas analyzer, while blood oxygen saturation and blood oxygen contents were measured using an Instruments Laboratories IL-282 co-oximeter.

Oxygen consumptions were calculated using the Fick Equation :

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2) \qquad \text{(Eq. 6.1)}$$

where $\dot{V}O_2$ represents oxygen consumption in ml/minute/Kg, Q_T is the cardiac output in liters/min/Kg, and CaO_2 and CvO_2 are the arterial and mixed venous oxygen contents, respectively, in ml O_2/dl. Total oxygen transport (TOT) was calculated as:

$$CaO_2 \times Q_T \qquad \text{(Eq. 6.2).}$$

Absolute blood viscosity was calculated from measurements made on a Wells-Brookfield (Model LVT) cone-plate viscometer at shear rates from 11.5 to 230.4 sec^{-1} according to the following equations:

$$\text{shear stress} = \tau = T / 2/3\,\pi r^3 \ (\text{dynes/cm}^2) \qquad \text{(Eq. 6.3)}$$

$$\text{shear rate} = \dot{\gamma} = \omega / \sin\theta \ (\text{sec}^{-1}) \qquad \text{(Eq. 6.4)}$$

$$\text{viscosity} = \eta = \tau / \dot{\gamma} \ \text{(poise)} \qquad \text{(Eq. 6.5)}$$

where T is the percentage of full-scale torque generated

by resistance to rotation of the cone by the fluid (dyne-cm), r is the radius of the cone (cm), ω is the cone speed (rad/sec) and θ is the cone angle (degrees).

Apparent viscosity of blood samples was calculated from measurements made in a capillary viscometer (radius = 500μm) using Poiseulle's Equation:

$$\eta = \frac{\pi r^4 \Delta p}{Q\ 8\ L} \qquad \text{(Eq. 6.6)}$$

where η is viscosity in poise, r is the capillary radius (cm), Δp is the pressure change (dynes), Q is flow (cm/s) and L is the capillary length (cm). Both viscometers were calibrated with fluids of known viscosity (Brookfield Viscosity Standard, 5.4 cP and water) and operated at 37°C.

Arteriolar shear rates were calculated using the blood flow data for each animal at each sampling set using the following equation:

$$\dot{\gamma} = \frac{4V}{R} \qquad \text{(Eq. 6.7)}$$

where V is equal to the mean flow velocity (ml/sec) and R is the vessel radius (mm). Mean arteriolar radius was estimated at 100 um based on typical mammalian histology. This calculation gives only an estimate of shear rate and therefore is taken as a rough indicator of relative rates of shear and not as an actual measurement of shear inside an arteriole.

Statistical comparisons were made using paired and mean "t" tests. Comparisons of $P<0.05$ were considered significant. Multiple linear regression models were used to fit data to second and third order polynomials with one dependent variable to construct curves for $\dot{V}O_2$, $M\dot{V}O_2$, TOT, flow rate, shear rate and viscosity versus hematocrit. Rational spline interpolation (cubic and parametric) was used to construct curves for viscosity versus shear rate.

RESULTS

Oxygen consumption ($\dot{V}O_2$) decreased at hematocrits below 25% and hematocrits above 45% in the pigs (Figure 6.2). The oxygen consumption of swine myocardium increased from hematocrits of 8% to 45%. Oxygen consumption data were not available for hematocrits above 45% making the curve incomplete. Due to the lack of data at high hematocrits, the data did not fit a second degree polynomial and therefore only a linear trend could be established (Figure 6.3) with the highest values of oxygen consumption at hematocrits of 35% to 40%. Whole body total oxygen transport (TOT) increased with hematocrit to approximately 45% HCT, at which point the curve then leveled off (Figure 6.4). The optimal HCT as measured by whole body oxygen consumption in swine was

approximately 35%, which equals the animals natural HCT. The following mathematical relationships were established:

$TOT = -2.00 + 0.837(HCT) - 0.00733(HCT)^2$ (Eq. 6.8);

$\dot{V}O_2 = 0.966 + 0.303(HCT) - 0.0043(HCT)^2$ (Eq. 6.9);

$M_{\dot{V}O2} = 150 - 8.32(HCT) + 0.223(HCT)^2$ (Eq. 6.10);

$Q_T = 2.26 + 0.107(HCT) - 0.00203(HCT)^2$ (Eq. 6.11);

$SHEAR = 48.2 + 2.28(HCT) - 0.433(HCT)^2$ (Eq. 6.12).

Whole body oxygen consumption ($\dot{V}O_2$) in seals decreased at HCTs below 25% and above 55% (Figure 6.5). Myocardial oxygen consumption for seal hearts was highly variable and when fit to a second order polynomial, the optimal range of HCT for seal heart appears to be between 20 to 50% (Figure 6.6). Whole body oxygen transport (TOT) for seals increased from HCTs of 5% to 68% with no apparent decrease in oxygen transport (Figure 6.7). The large broad plateau in oxygen consumption over HCTs from 25% to 65% for seals indicates that their natural HCT of approximately 55% is also their optimal HCT. The following relationships were revealed for seals:

$TOT = 4.99 + 0.129(HCT) + 0.00216(HCT)^2$ (Eq. 6.13);

$\dot{V}O_2 = 2.21 + 0.193(HCT) - 0.00232(HCT)^2$ (Eq. 6.14);

$M_{\dot{V}O2} = 23.9 + 3.39(HCT) - 0.537(HCT)^2$ (Eq. 6.15);

$Q_T = 2.73 + 0.0180(HCT) - 0.000691(HCT)^2$ (Eq. 6.16);

$SHEAR = 58.3 + 0.38(HCT) - 0.0147(HCT)^2$ (Eq. 6.17).

Comparisons among seals and pigs in terms of TOT and $\dot{V}O_2$ (Figures 6.8 and 6.9) show a later onset and less of a decline in total body oxygen consumption in seals when compared with the pigs ($P<0.05$). However, oxygen consumption values for myocardium in the two species are variable with values for seals being lower ($P<0.05$). The data for pigs are incomplete (Figure 6.10). Flow resistance and arteriolar flow resistance calculated from flow data and fitted to a second order polynomial again show a broad flat plateau for seals and an inverted parabola for swine when plotted against HCT (Figures 6.11 and 6.12, $P<0.05$). Apparent viscosity increases with decreasing shear rate in both pigs (Figure 6.13) and the harbor seal (Figure 6.14). The relationship between HCT and absolute blood viscosity at each shear rate (Figure 6.15, 6.16, 6.17, 6.18 and 6.19) and shear rate and absolute blood viscosity for pigs (Figures 6.20) and seals (Figures 6.21, 6.22, 6.23, 6.24, 6.25 and 6.26) are as follows for pigs:

$$\text{VISCOSITY}_{(11.5\ sec^{-1})} = 2.18 + 0.0436(HCT) + 0.00399(HCT)^2 \quad \text{(Eq. 6.18);}$$

$$\text{VISCOSITY}_{(23.0\ sec^{-1})} = 1.74 + 0.052(HCT) + 0.00212(HCT)^2 \quad \text{(Eq. 6.19);}$$

$$\text{VISCOSITY}_{(46.1\ sec^{-1})} = 1.48 + 0.159(HCT) - 0.00121(HCT)^2 \quad \text{(Eq. 6.20);}$$

$$\text{VISCOSITY}_{(115.2\ sec^{-1})} = 1.31 + 0.0713(HCT) + 0.000138(HCT)^2 \quad \text{(Eq. 6.21);}$$

$$\text{VISCOSITY} = 1.31 + 0.0042(\text{HCT}) + 0.00047(\text{HCT})^2 \quad (230.4\ \text{sec}^{-1}) \qquad \text{(Eq. 6.22);}$$

$$\text{VISCOSITY} = 6.84 - 0.053\ (\text{SHEAR}) + 0.000152(\text{SHEAR})^2 \qquad \text{(Eq. 6.23).}$$

The relationships for seals are:

$$\text{VISCOSITY} = 2.96 + 0.163(\text{HCT}) + 0.00034(\text{HCT})^2 \quad (11.5\ \text{sec}^{-1}) \qquad \text{(Eq. 6.24);}$$

$$\text{VISCOSITY} = 1.16 + 0.040(\text{HCT}) + 0.00234(\text{HCT})^2 \quad (23.0\ \text{sec}^{-1}) \qquad \text{(Eq. 6.25);}$$

$$\text{VISCOSITY} = 3.04 - 0.0115(\text{HCT}) + 0.00189(\text{HCT})^2 \quad (46.1\ \text{sec}^{-1}) \qquad \text{(Eq. 6.26);}$$

$$\text{VISCOSITY} = 1.42 + 0.0931(\text{HCT}) + 0.000125(\text{HCT})^2 \quad (115.2\ \text{sec}^{-1}) \qquad \text{(Eq. 6.27);}$$

$$\text{VISCOSITY} = 1.51 + 0.0545(\text{HCT}) + 0.00058(\text{HCT})^2 \quad (230.4\ \text{sec}^{-1}) \qquad \text{(Eq. 6.28);}$$

$$\text{SHEAR} = 47.6 - 1.84\ (\text{VISCOSITY}) + (\text{VISCOSITY})^2 \qquad \text{(Eq. 6.29).}$$

Oxygen consumption does not become dependent upon viscosity at shear rates comparable to those during the experiments calculated using <u>in vivo</u> flow probe data (see Equations 6.7, 6.9, 6.14, 6.23 and 6.29).

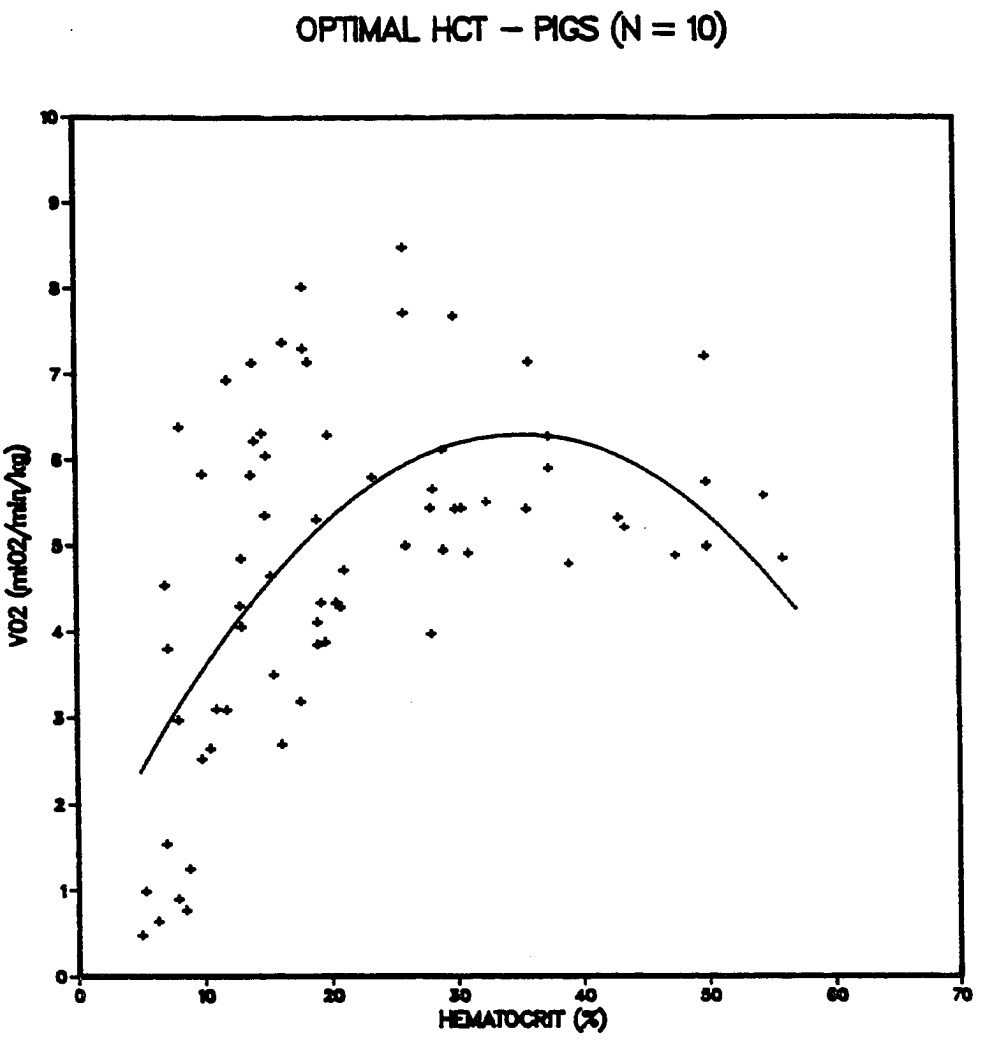


Figure 6.2. Plot of oxygen consumption ($\dot{V}O_2$) for whole body versus HCT for ten open-chest domestic pigs. Data for blood gases are from Instruments Laboratories IL-813 and IL-282 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microsphere data.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

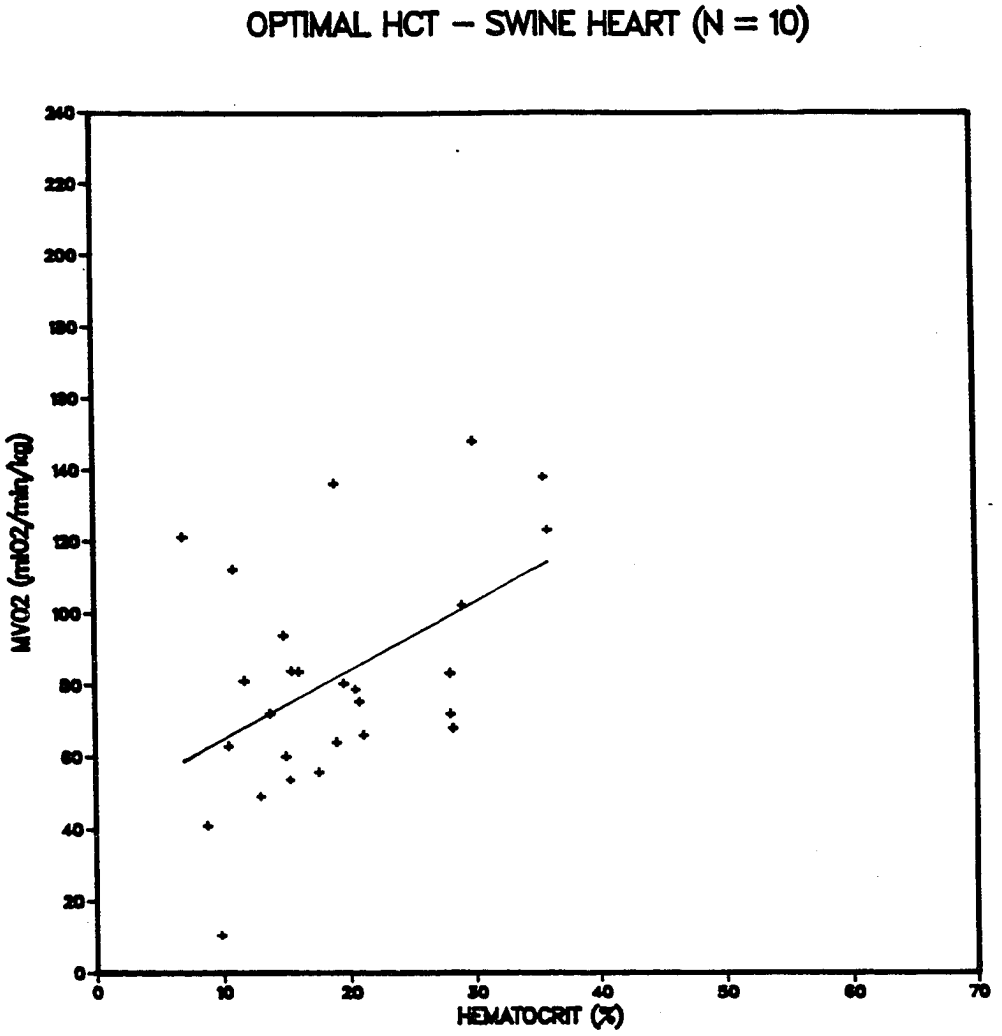


Figure 6.3. Plot of myocardial oxygen consumption ($M\dot{V}O2$) versus HCT for ten open-chest domestic pigs. Data for blood gases are from Instruments Laboratories IL-813 and IL-282 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microsphere data.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

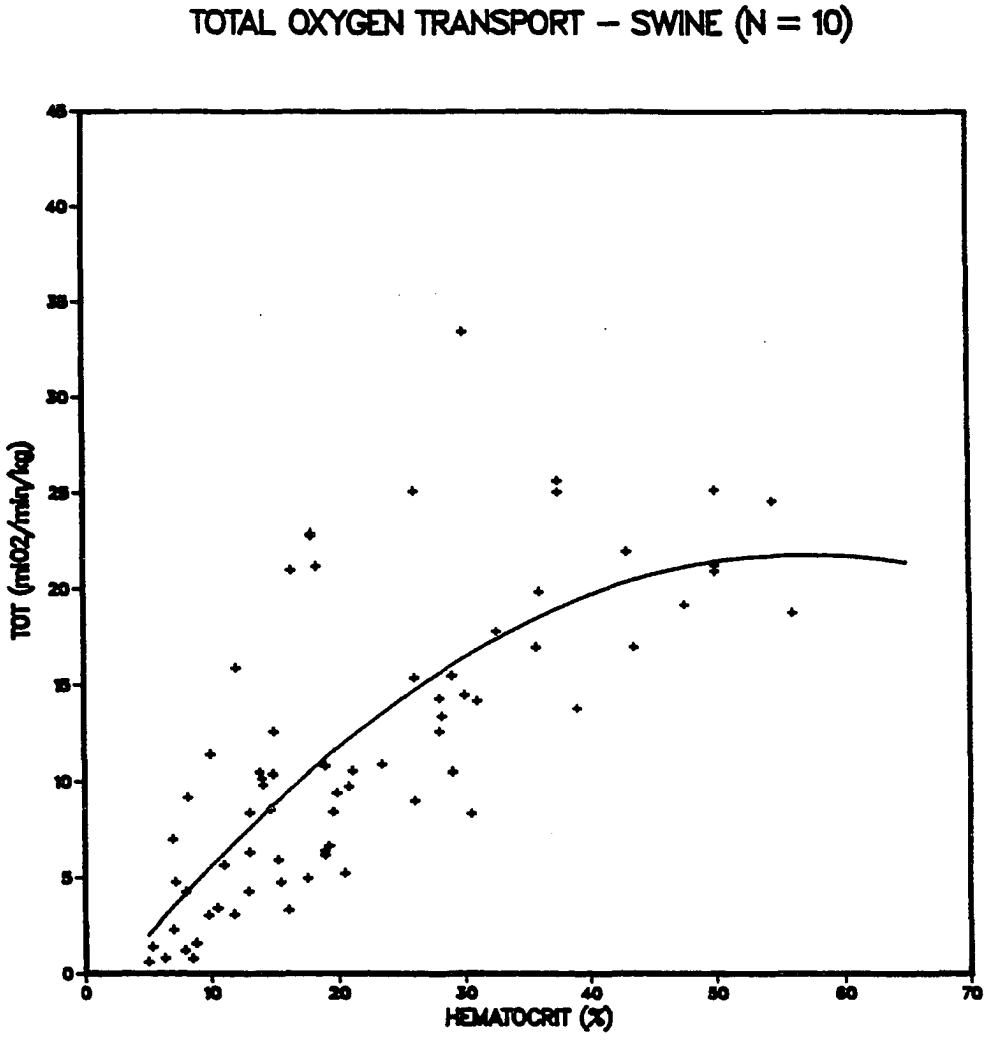


Figure 6.4. Plot of total oxygen transport (TOT) versus HCT for ten open-chest domestic pigs. Data for blood gases are from Instruments Laboratories IL-813 and IL-282 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microsphere data.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

$$TOT = CaO_2 \times Q_T$$

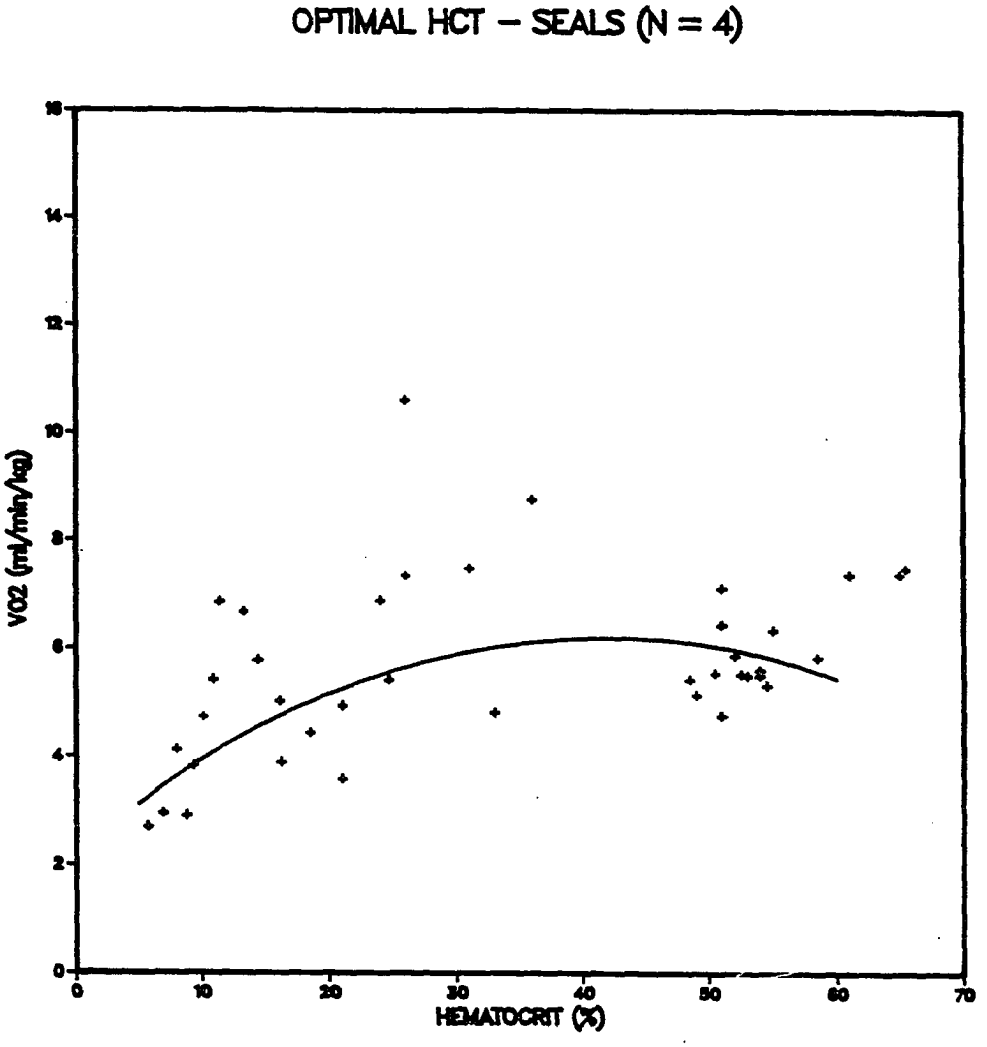


Figure 6.5. Total body oxygen consumption ($\dot{V}O_2$) versus HCT for four open-chest seals. Blood gases were determined using an Instruments Laboratories (IL-813 and IL-282) blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

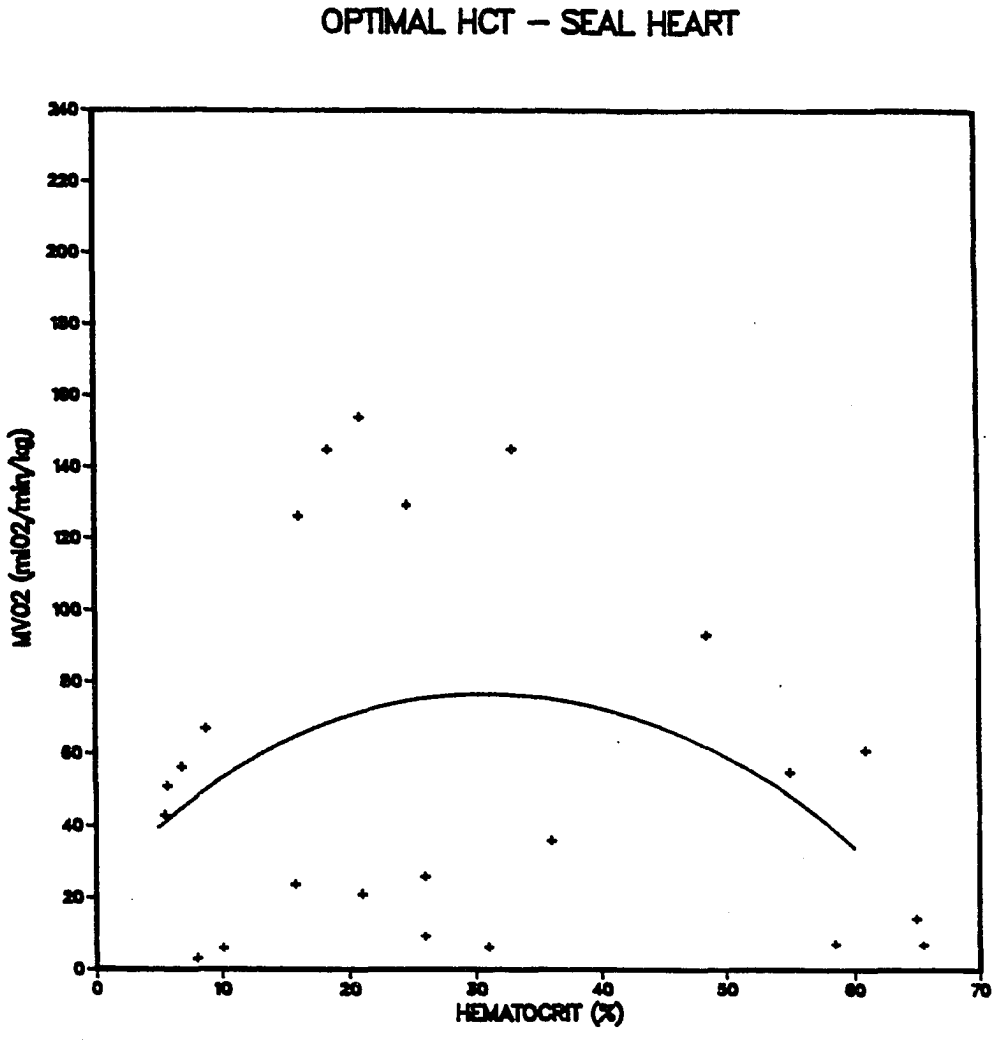


Figure 6.6. Myocardial oxygen consumption ($\dot{M}VO_2$) versus HCT for four open-chest seals. Blood gases were determined using an Instruments Laboratories (IL-813 and IL-282) blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

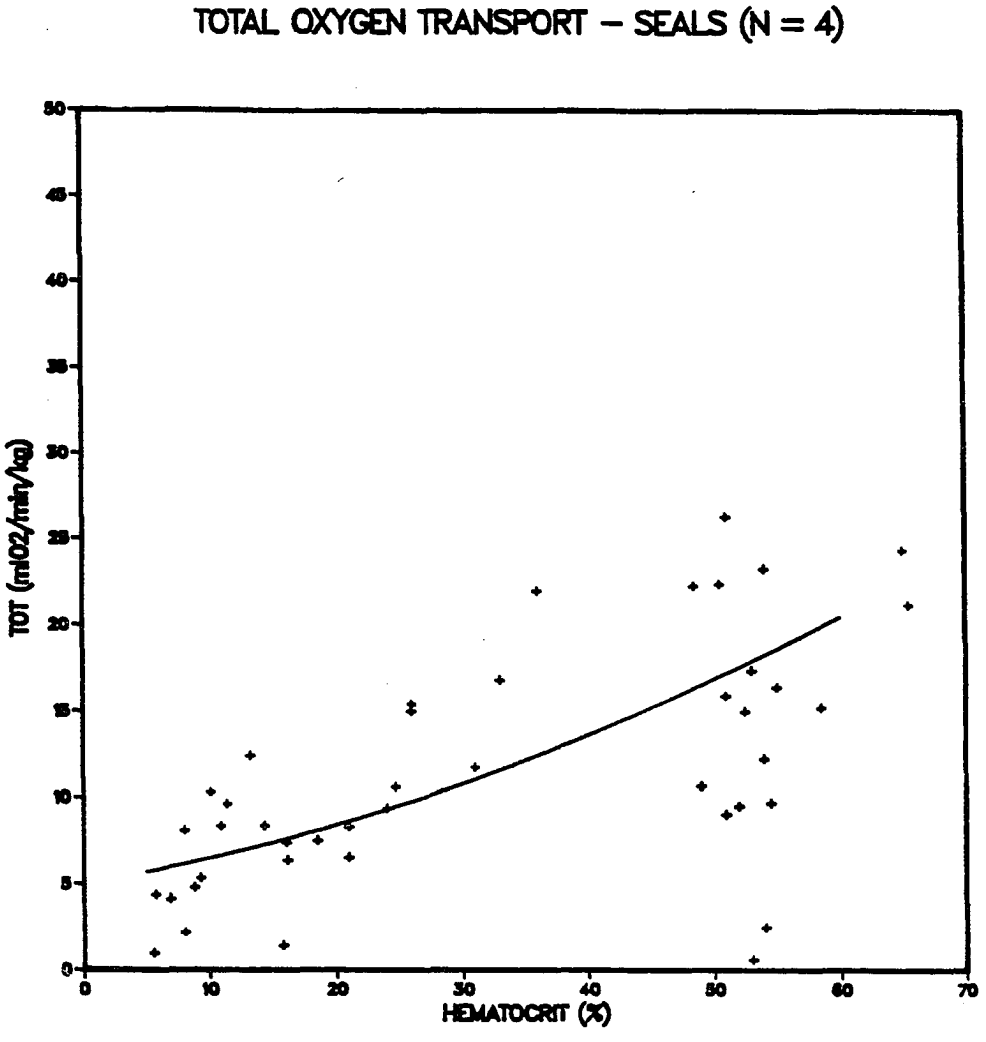


Figure 6.7. Total oxygen transport (TOT) versus HCT for four open-chest seals. Blood gases were determined using an Instruments Laboratories (IL-813 and IL-282) blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

$TOT = CaO_2 \times Q_T$

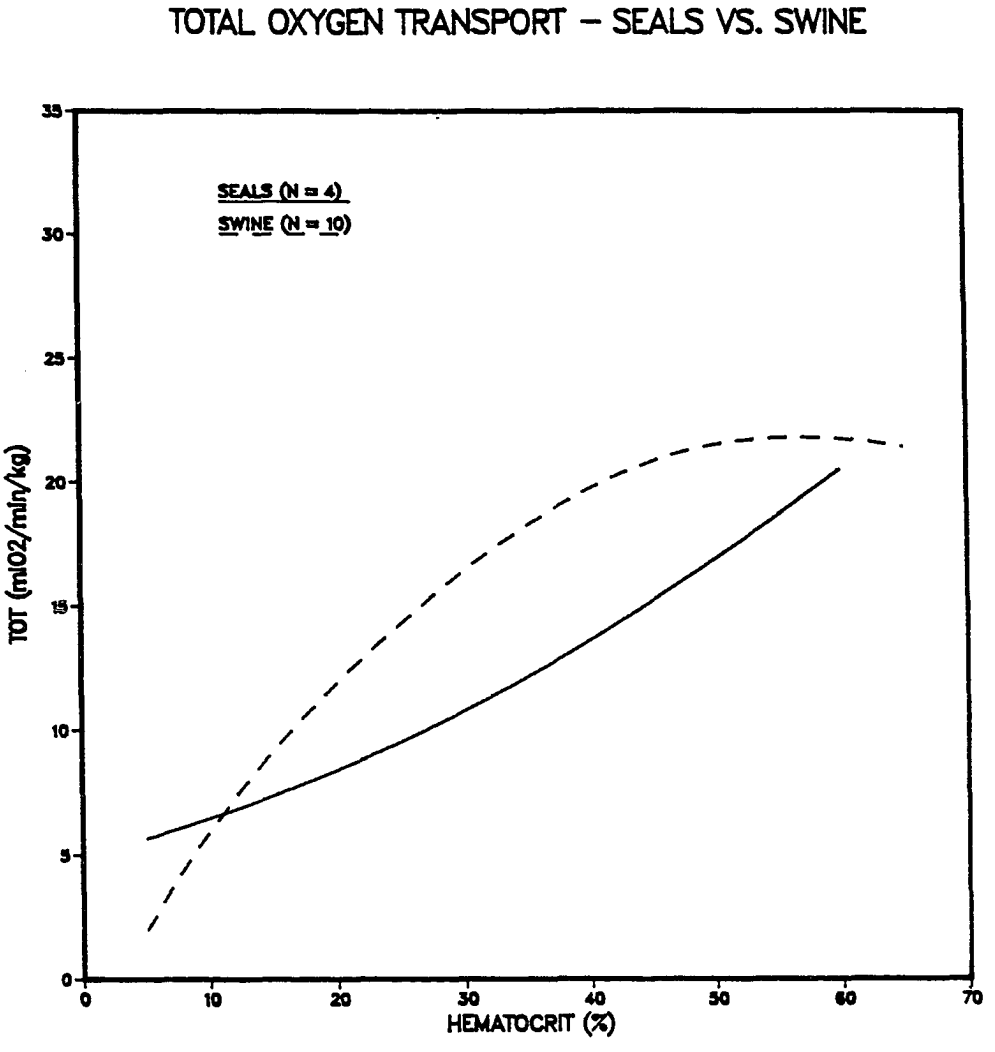


Figure 6.8. Comparisons of seals and pigs in terms of total oxygen transport (TOT) in response to alteration of HCT using a Haemonetics 30-S blood processor. Blood gases were measured using an Instruments Laboratories IL-813 and IL-283 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

$TOT = CaO_2V \times Q_T$

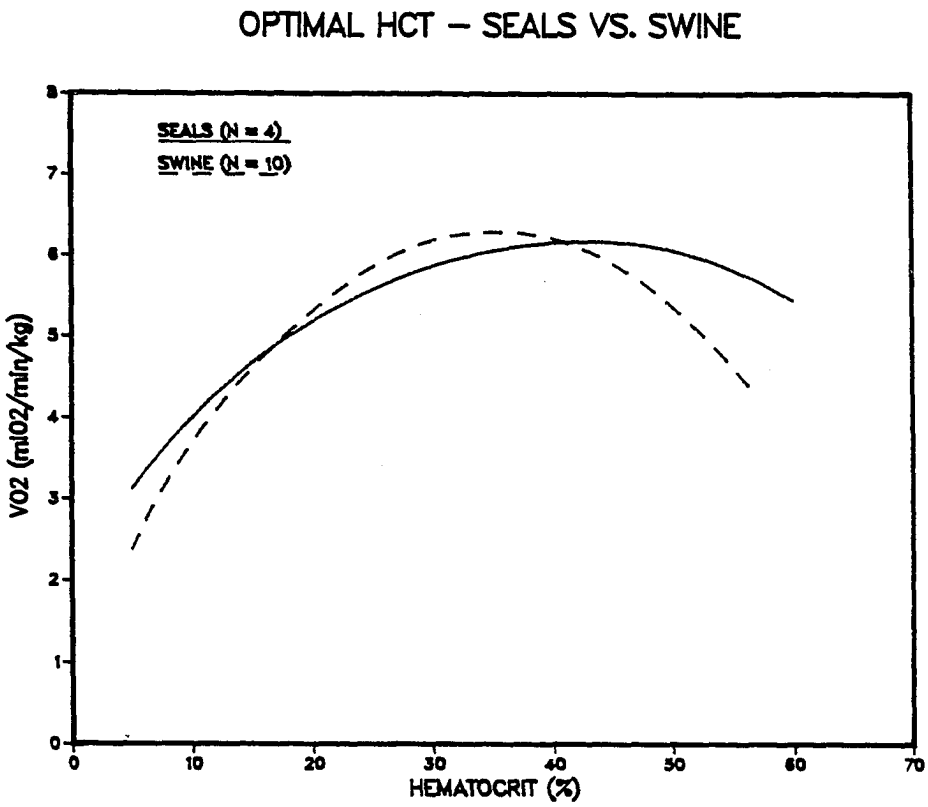


Figure 6.9. Comparisons of seals and pigs in terms of whole body oxygen consumption ($\dot{V}O_2$) in response to alteration of HCT using a Haemonetics 30-S blood processor. Blood gases were measured using an Instruments Laboratories IL-813 and IL-283 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

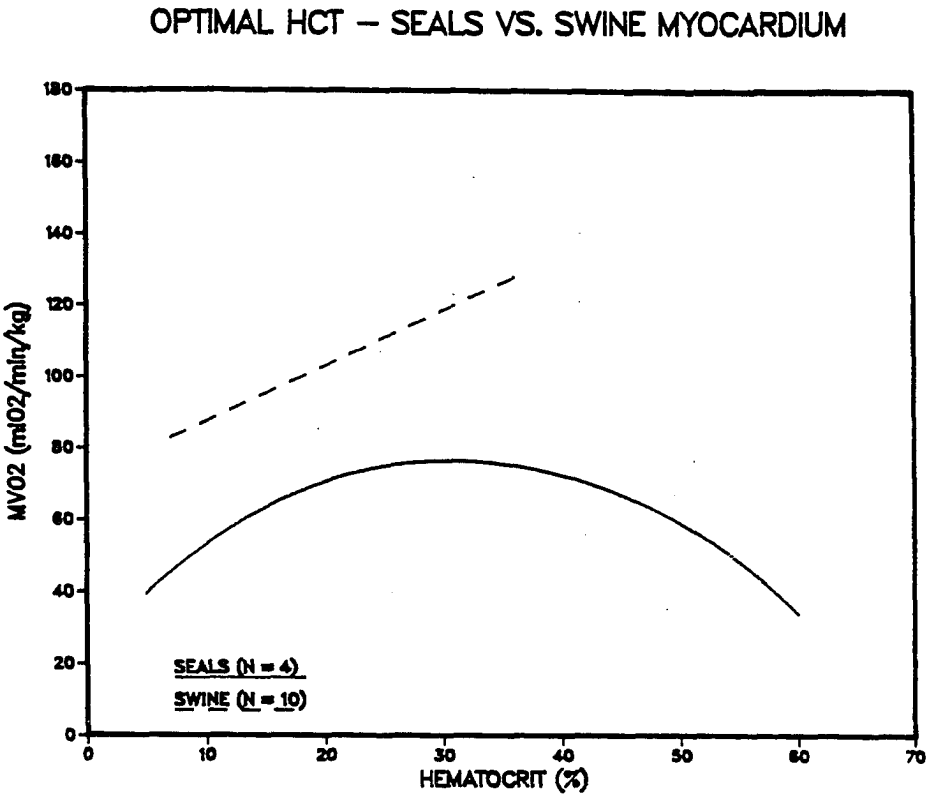


Figure 6.10. Comparisons of seals and pigs in terms of myocardial oxygen consumption ($M\dot{V}O_2$) versus HCT using a Haemonetics 30-S blood processor. Blood gases were measured using an Instruments Laboratories IL-813 and IL-283 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

$$\dot{V}O_2 = Q_T \times (CaO_2 - CvO_2)$$

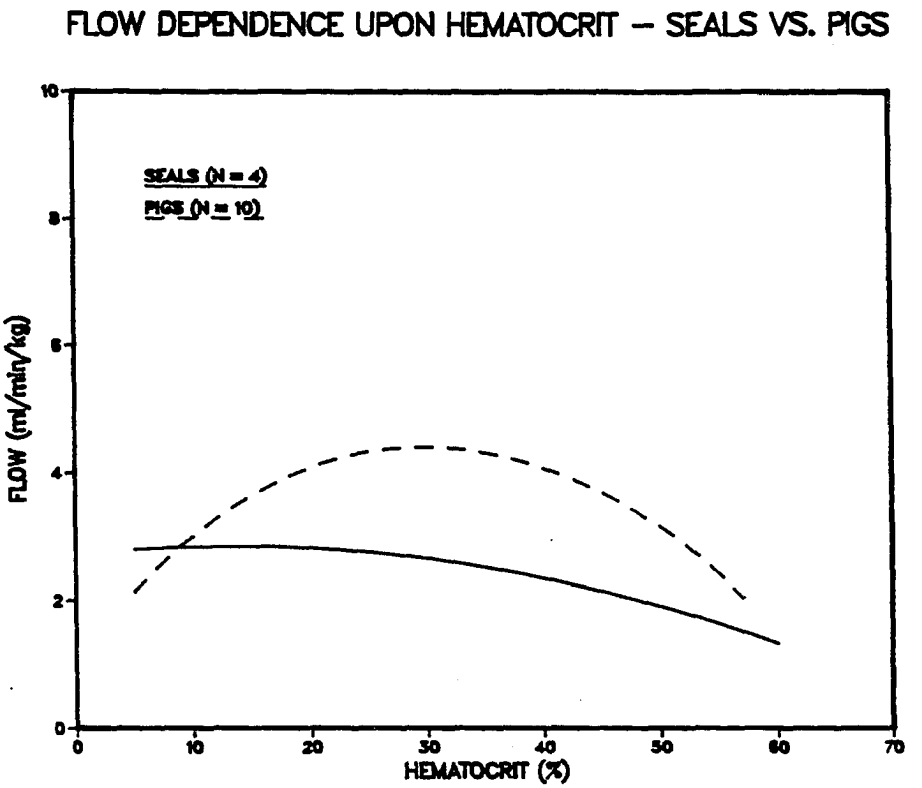


Figure 6.11. Comparisons of seals and pigs in terms of flow rate versus hematocrit in response to alteration of HCT using a Haemonetics 30-S blood processor. Blood gases were measured using an Instruments Laboratories IL-813 and IL-283 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

SHEAR RATE = $\dot{\gamma}$ = 4 X flow rate / vessel radius

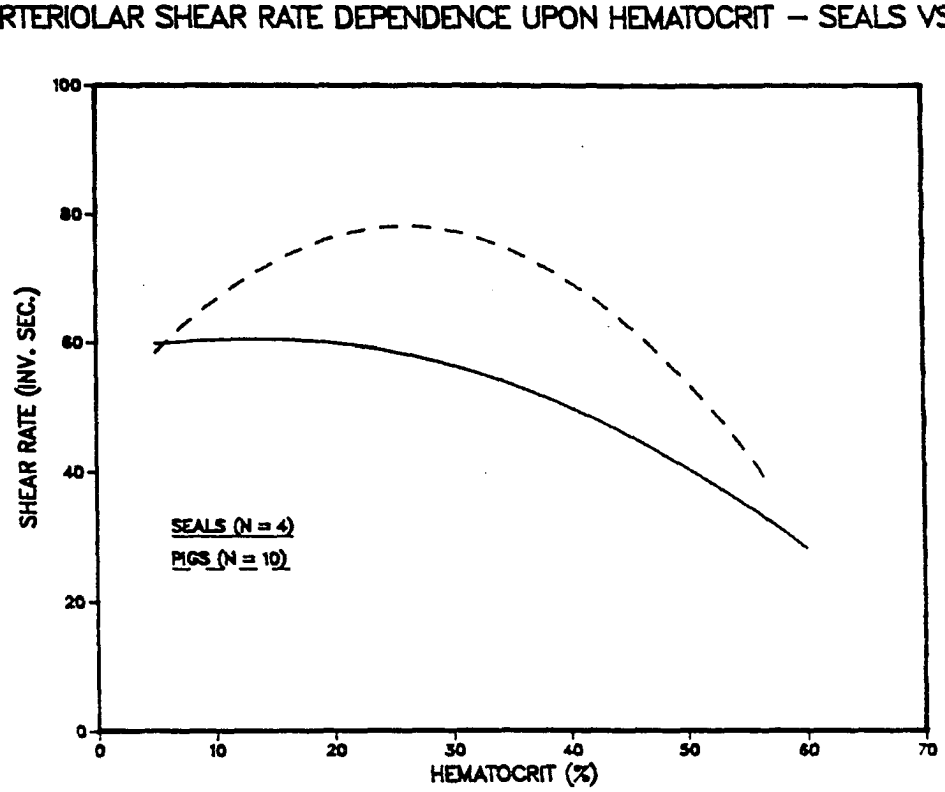


Figure 6.12. Comparisons of seals and pigs in terms of arteriolar shear rate versus hematocrit in response to alteration of HCT using a Haemonetics 30-S blood processor. Blood gases were measured using an Instruments Laboratories IL-813 and IL-283 blood gas analyzer and co-oximeter. Flow data are from implanted electromagnetic flow transducers and radiolabeled microspheres.

ARTERIOLAR SHEAR RATE =
$\dot{\gamma}$ = 4 X flow rate / vessel radius (r = 100 μm)

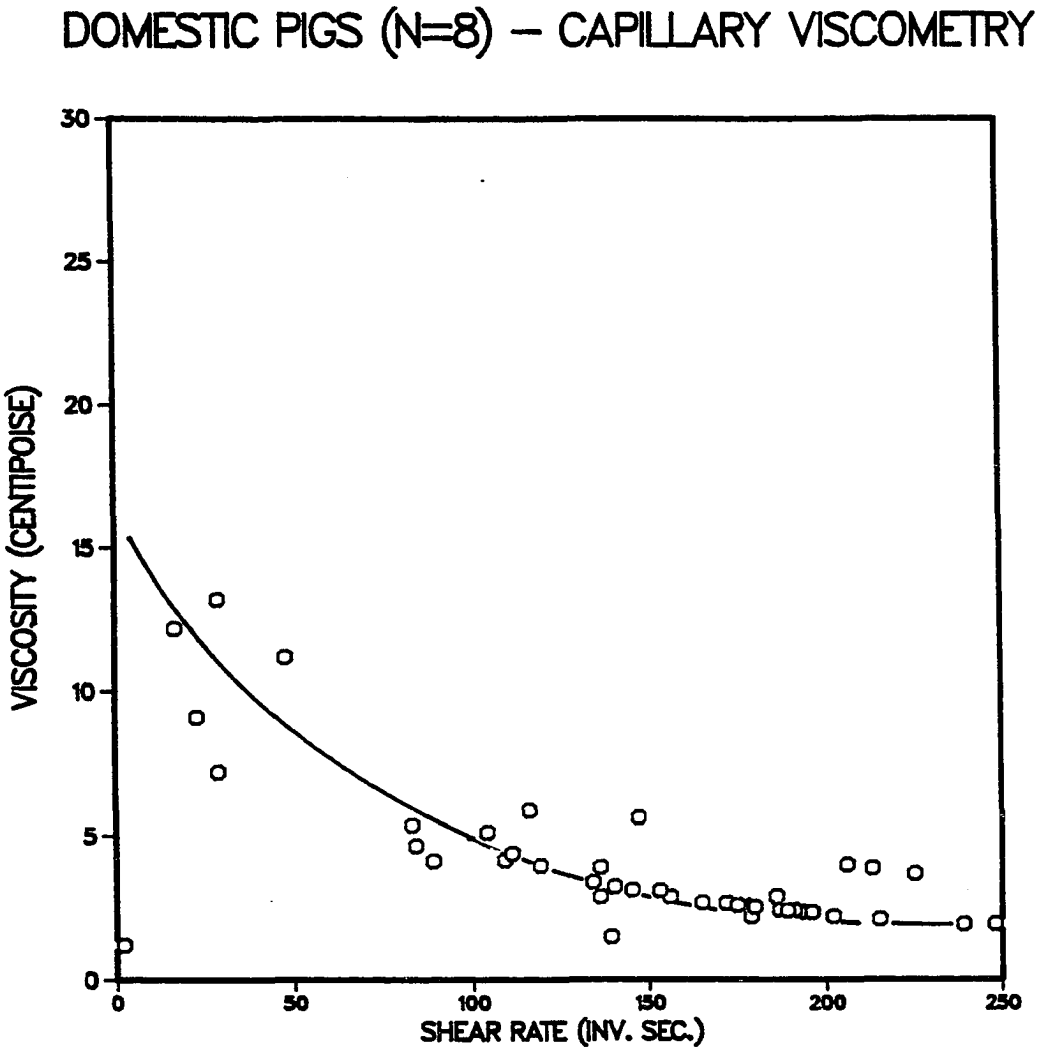


Figure 6.13. Apparent viscosity (cP) versus shear rate (sec^{-1}) for blood from eight open-chest domestic pigs during hemodilution and hemoconcentration. Viscosity was calculated using Poiseulle's Equation. All measurements were made at 37°C in a capillary viscometer (radius = 500μm).

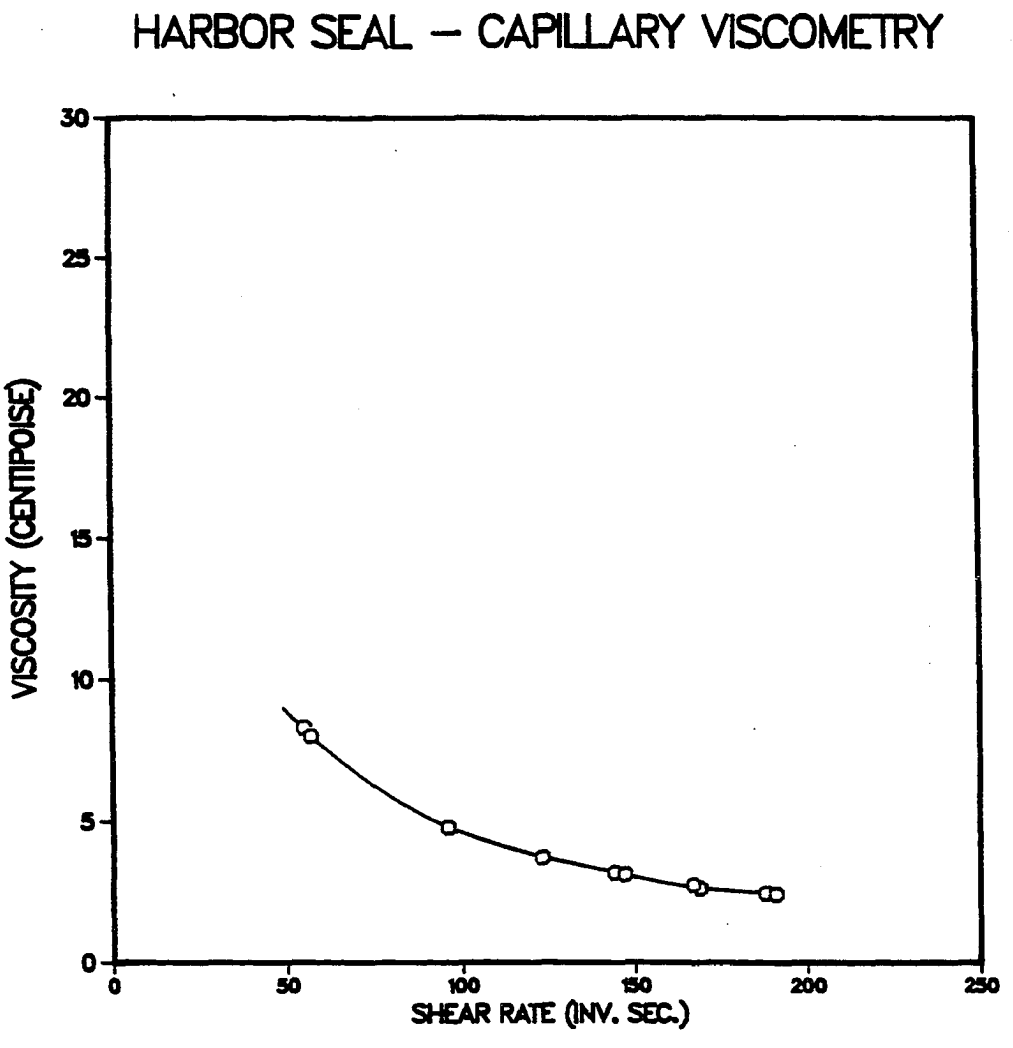


Figure 6.14. Apparent viscosity (cP) versus shear rate (sec^{-1}) for whole blood from one harbor seal during open-chest hemodilution and hemoconcentration. Viscosity was calculated using Poiseuille's Equation. Measurements were made in a capillary viscometer (radius = 500μm) operated at 37°C.

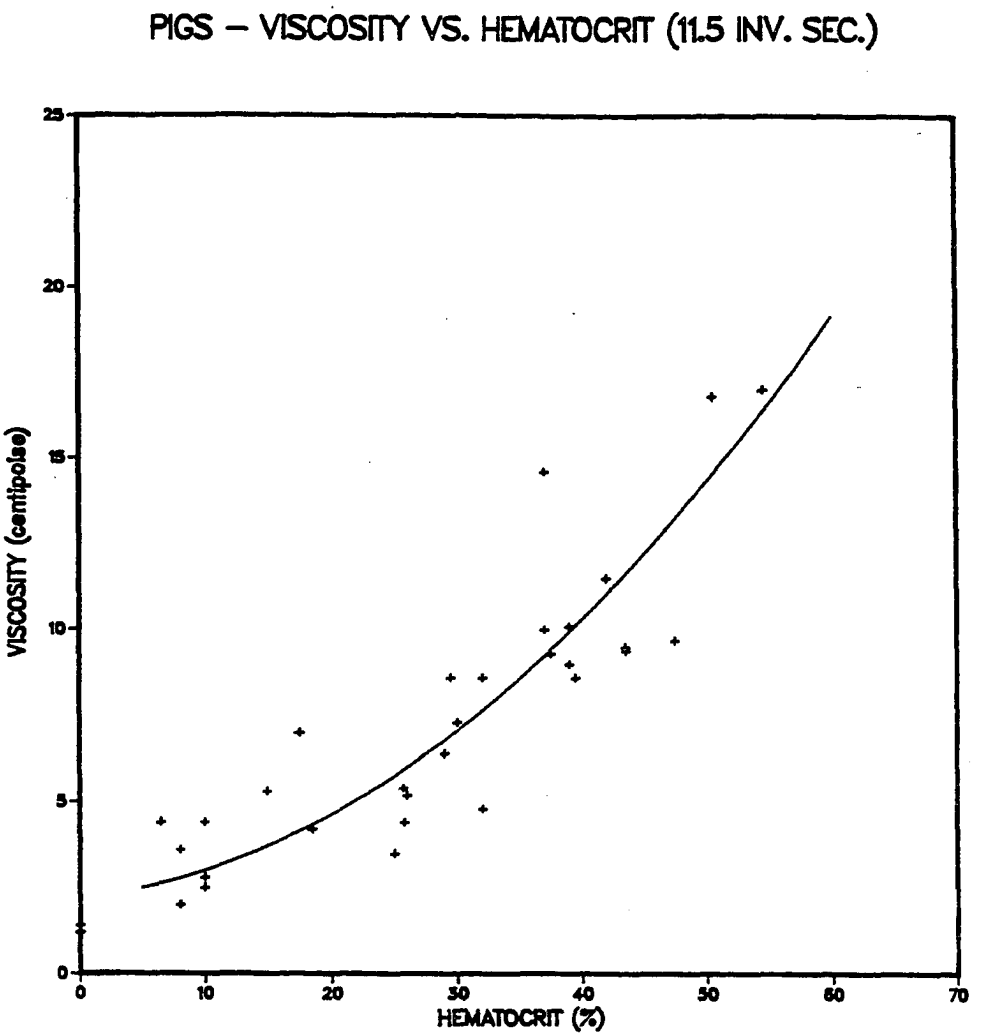


Figure 6.15. Relationship between absolute blood viscosity (cP) and HCT (%) at 11.5 sec^{-1} during hemoconcentration and hemodilution experiments on 10 open-chest pigs. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

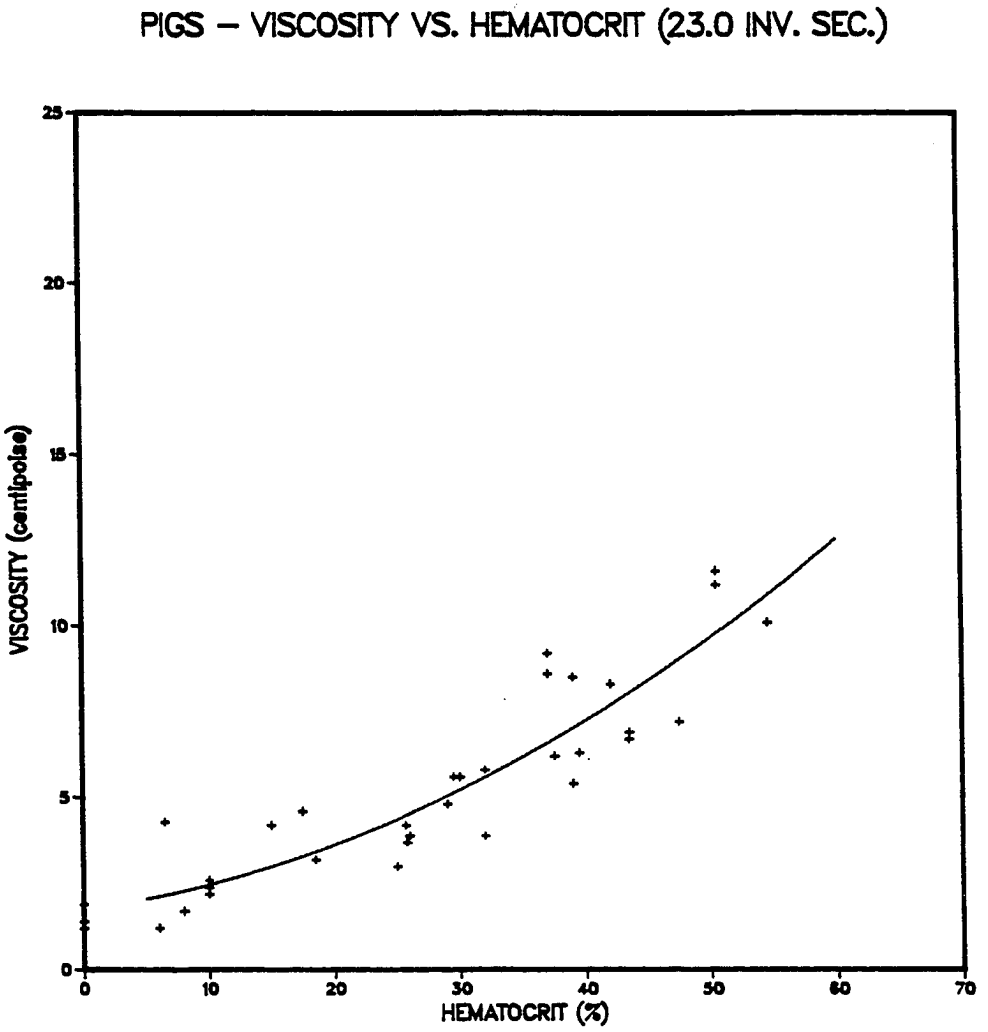


Figure 6.16. Relationship between absolute blood viscosity (cP) and HCT (%) at 23.0 sec^{-1} during hemoconcentration and hemodilution experiments on 10 open-chest pigs. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

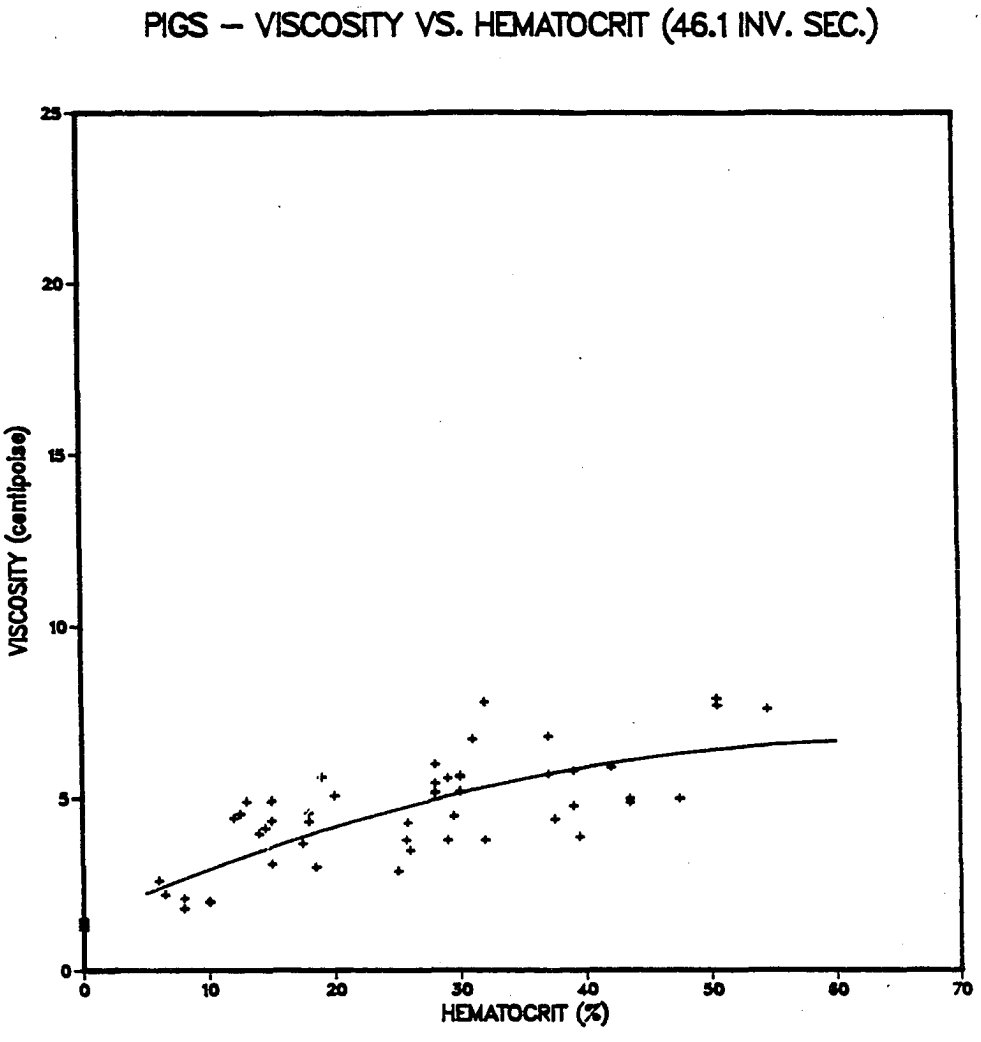


Figure 6.17. Relationship between absolute blood viscosity (cP) and HCT (%) at 46.1 sec^{-1} during hemoconcentration and hemodilution experiments on 10 open-chest pigs. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

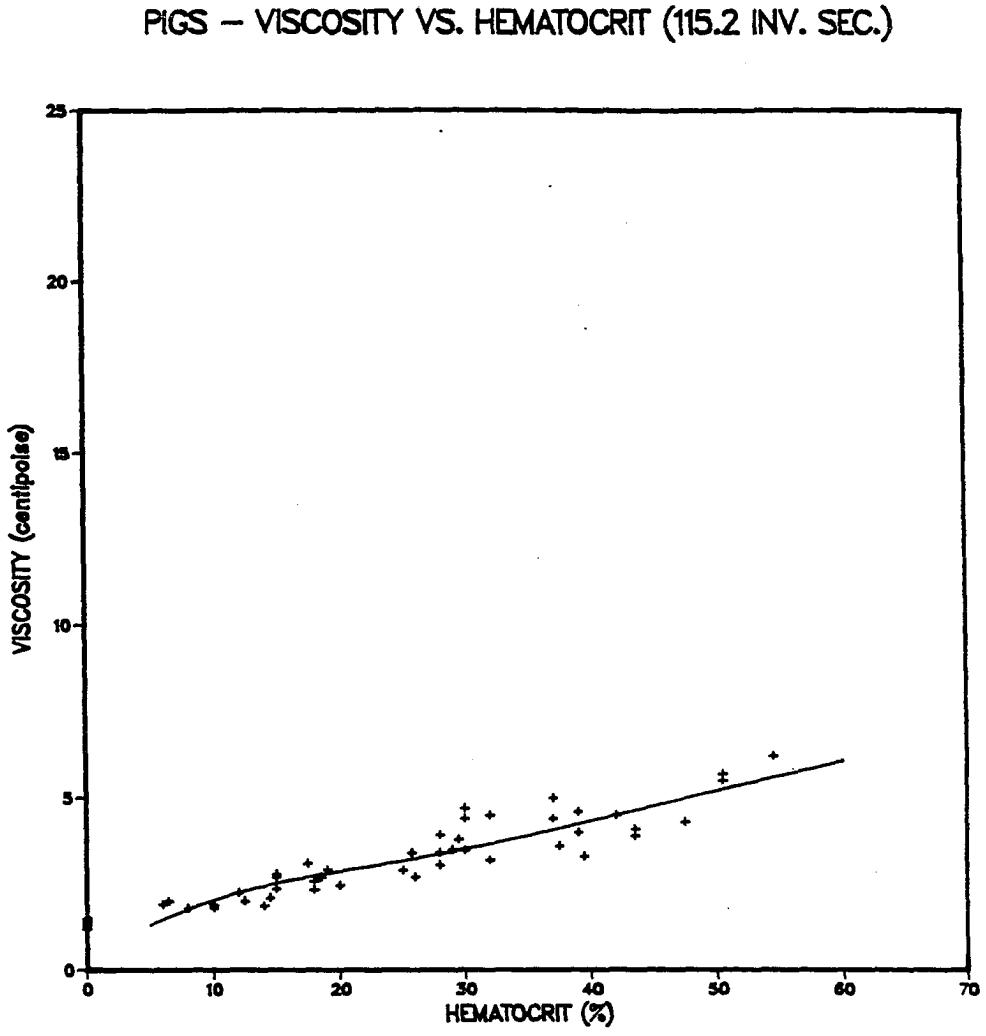


Figure 6.18. Relationship between absolute blood viscosity (cP) and HCT (%) at 115.2 sec^{-1} during hemoconcentration and hemodilution experiments on 10 open-chest pigs. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

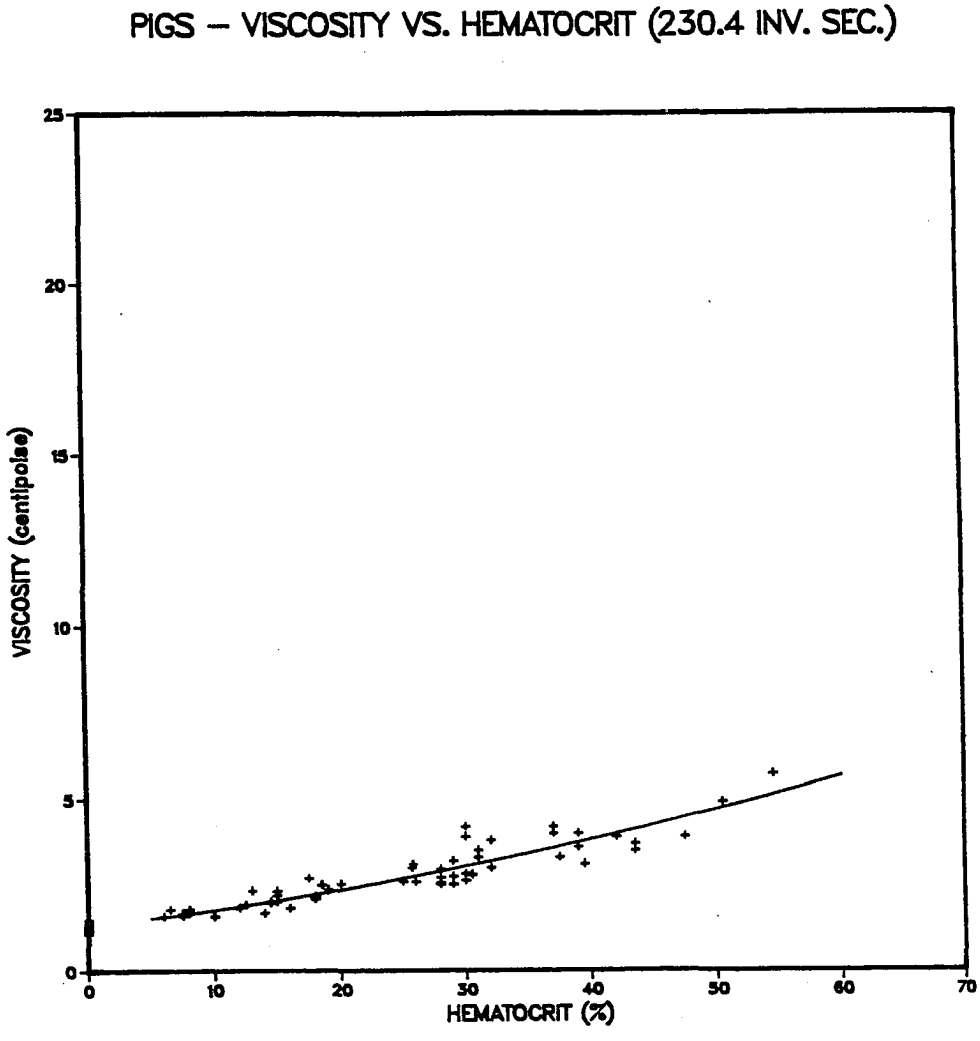


Figure 6.19. Relationship between absolute blood viscosity (cP) and HCT (%) at 230.4 sec^{-1} during hemoconcentration and hemodilution experiments on 10 open-chest pigs. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

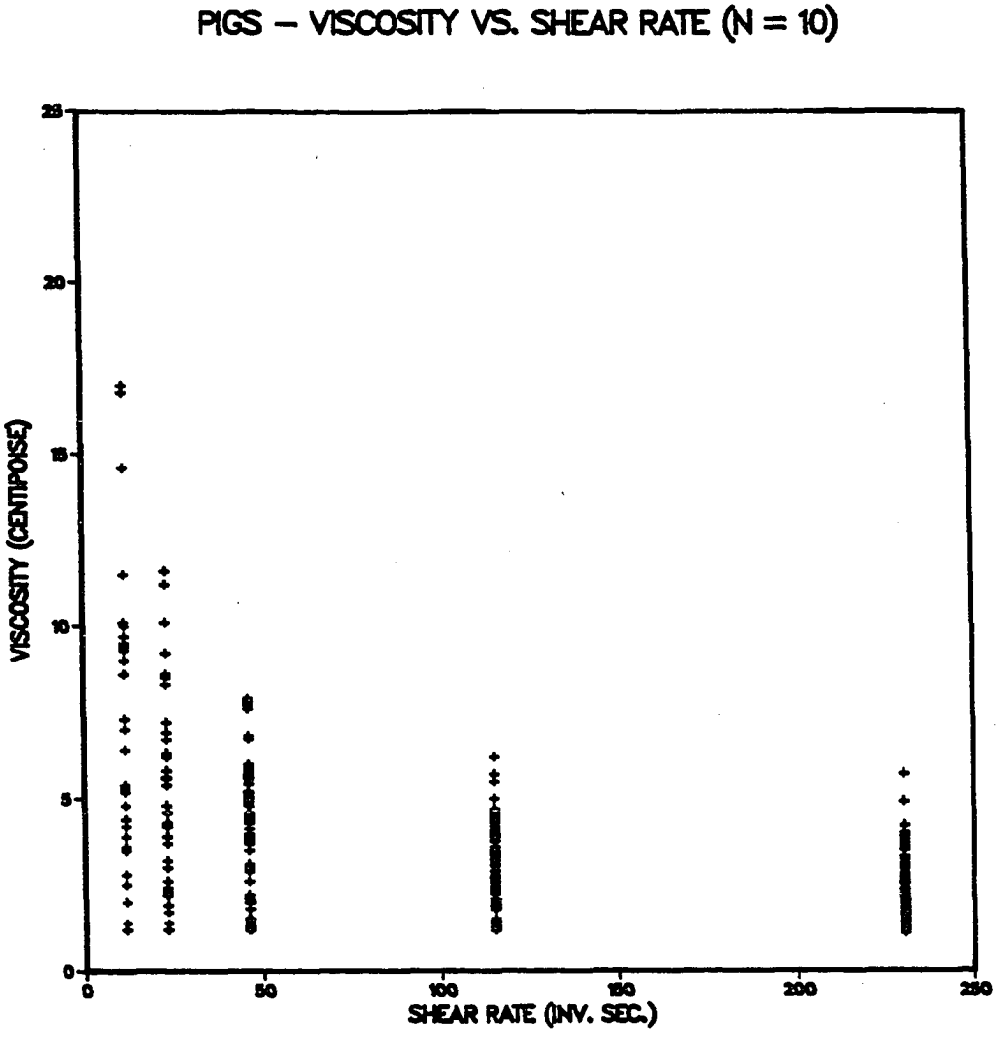


Figure 6.20. Relationship between absolute viscosity (cP) and shear rate (sec^{-1}) during experimental polycythemia and anemia experiments (n=10) on domestic pigs. Viscosity was determined from measurements made using a Wells-Brookfield (LVT) cone-plate viscometer at 37°C.

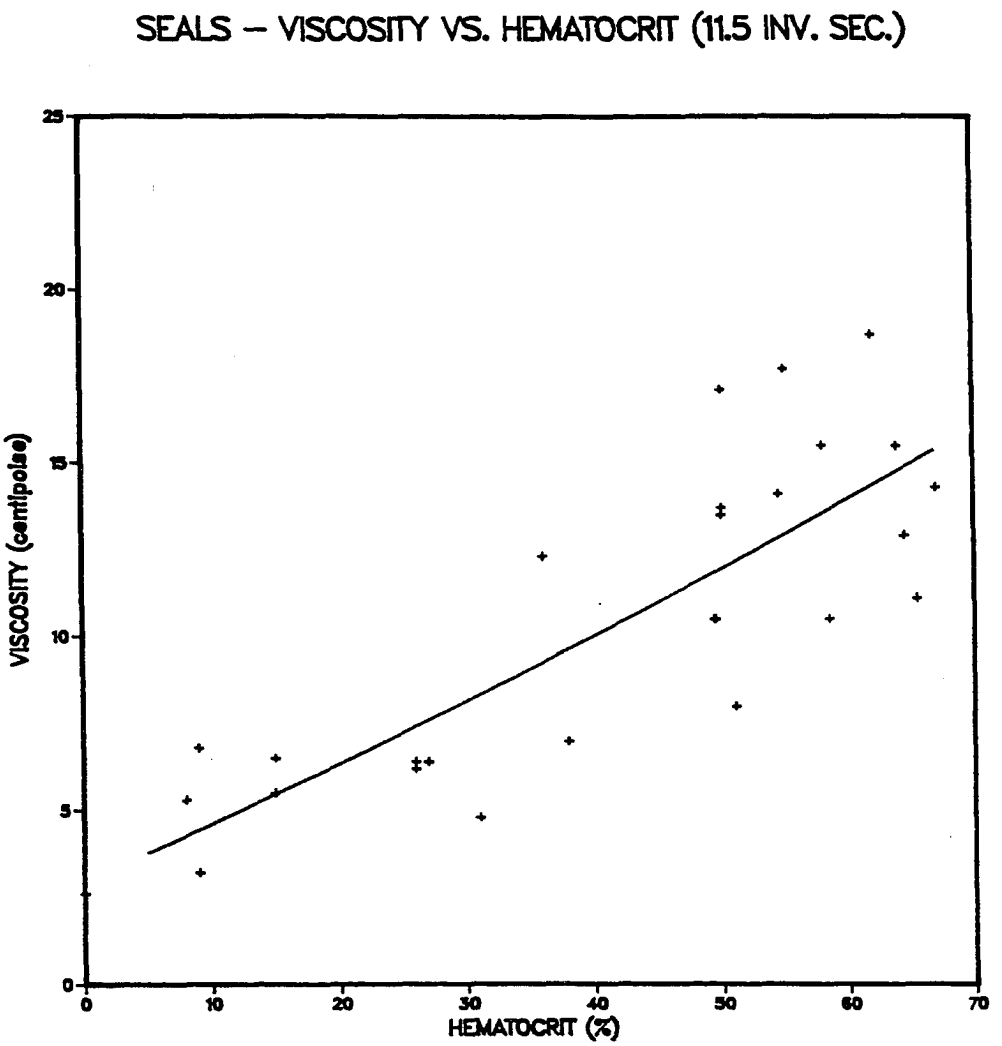


Figure 6.21. Relationship between absolute blood viscosity (cP) and HCT (%) at 11.5 sec^{-1} during hemoconcentration and hemodilution experiments on 4 open-chest seals. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

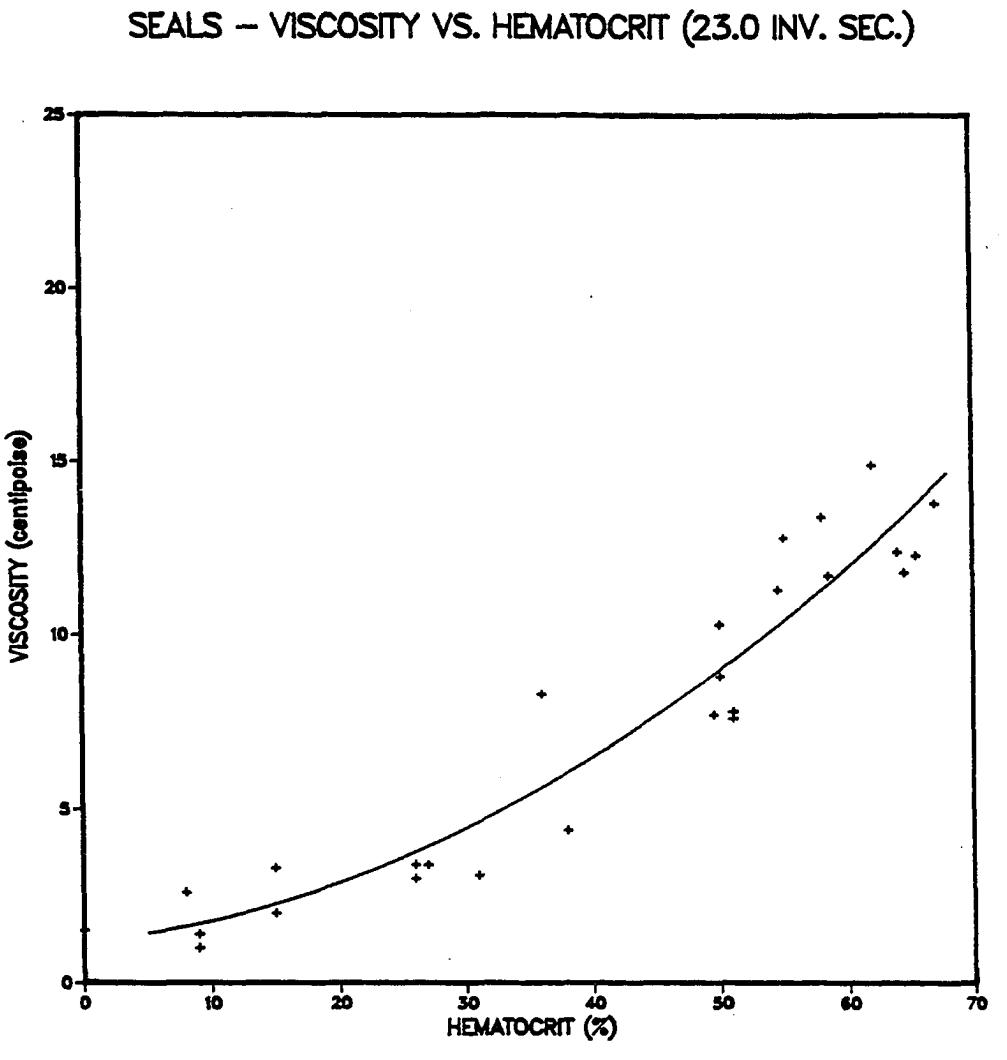


Figure 6.22. Relationship between absolute blood viscosity (cP) and HCT (%) at 23.0 sec^{-1} during hemoconcentration and hemodilution experiments on 4 open-chest seals. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

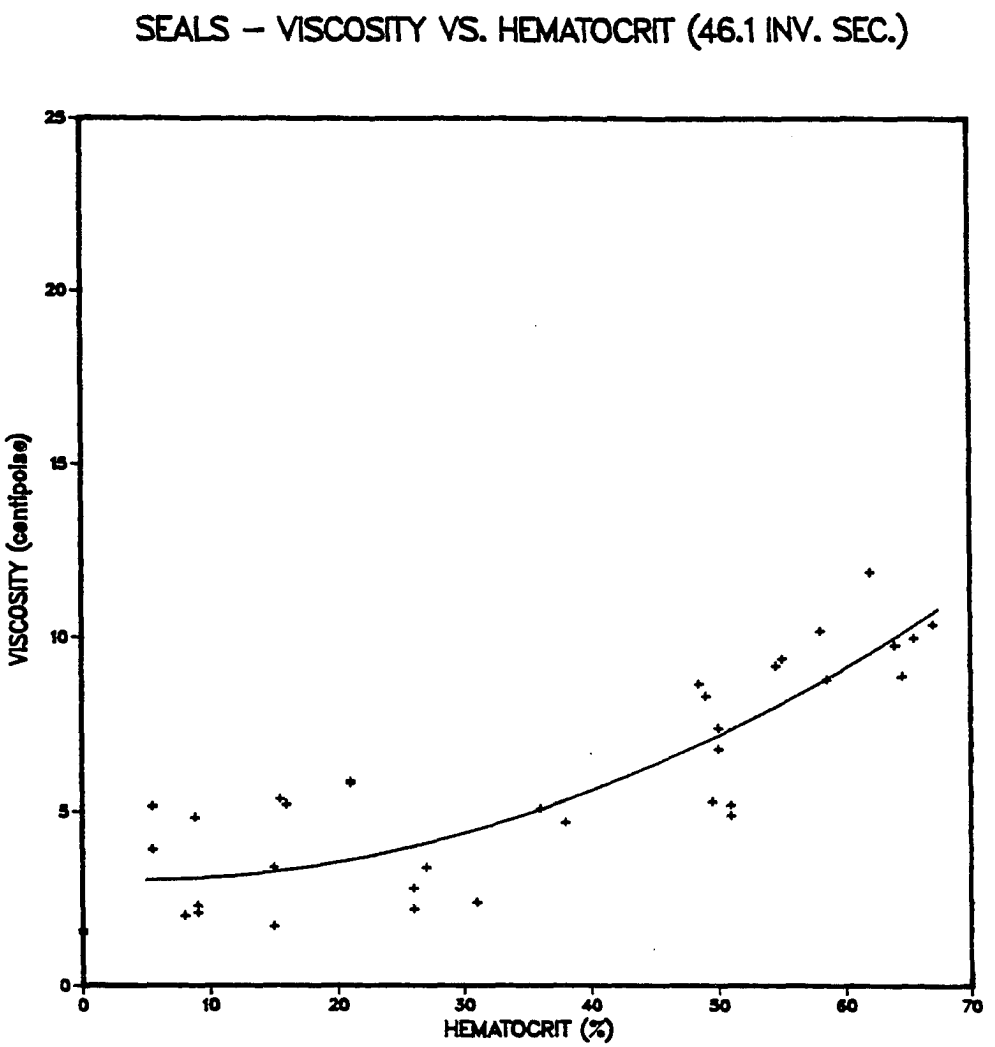


Figure 6.23. Relationship between absolute blood viscosity (cP) and HCT (%) at 46.1 sec^{-1} during hemoconcentration and hemodilution experiments on 4 open-chest seals. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

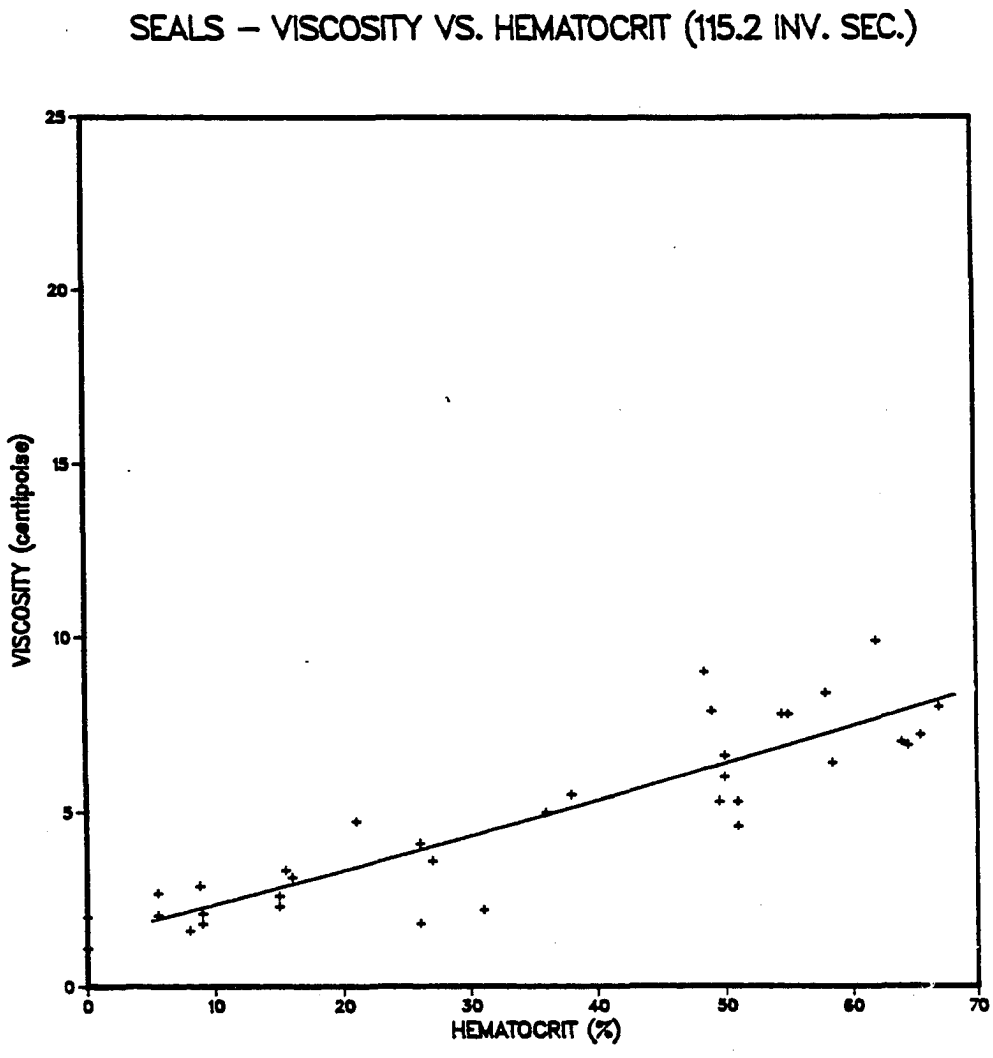


Figure 6.24. Relationship between absolute blood viscosity (cP) and HCT (%) at 115.2 sec^{-1} during hemoconcentration and hemodilution experiments on 4 open-chest seals. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

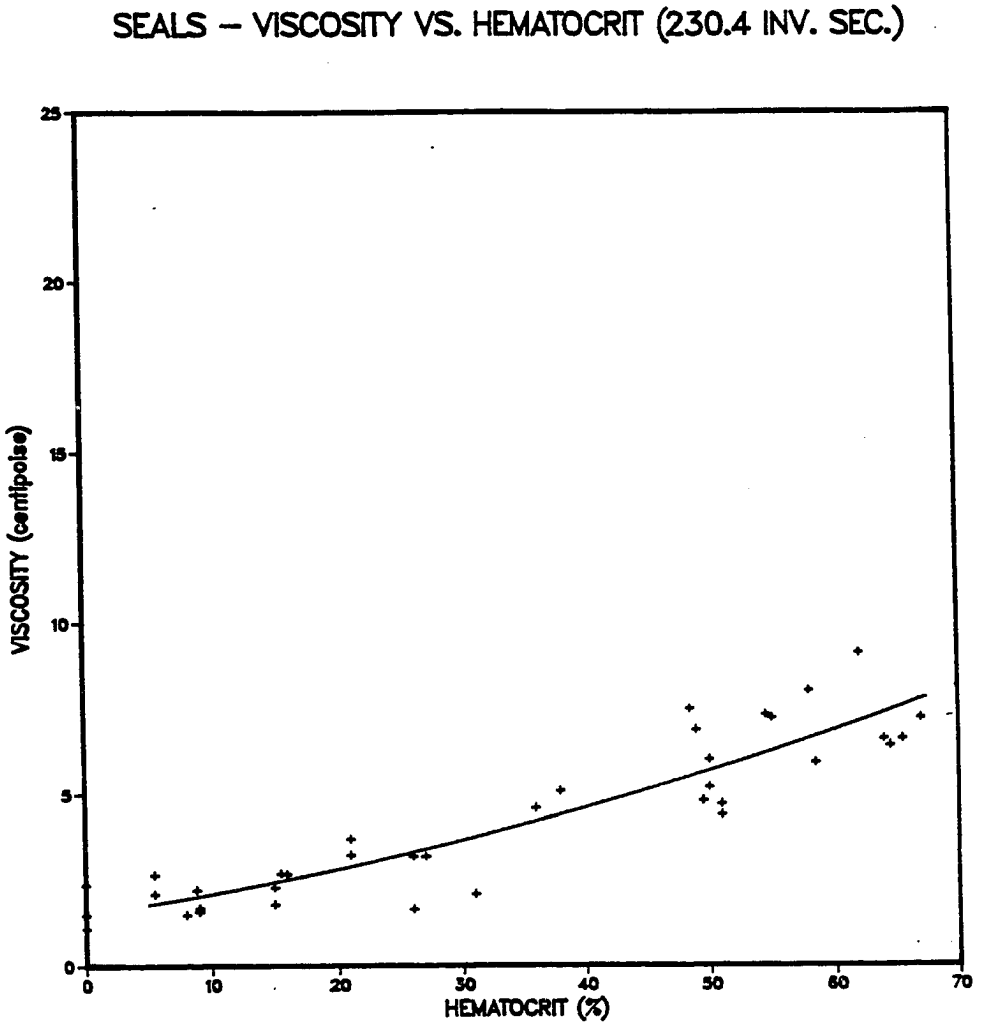


Figure 6.25. Relationship between absolute blood viscosity (cP) and HCT (%) at 230.4 sec^{-1} during hemoconcentration and hemodilution experiments on 4 open-chest seals. All viscosity measurements were made at 37°C on a Wells-Brookfield (LVT) cone-plate viscometer.

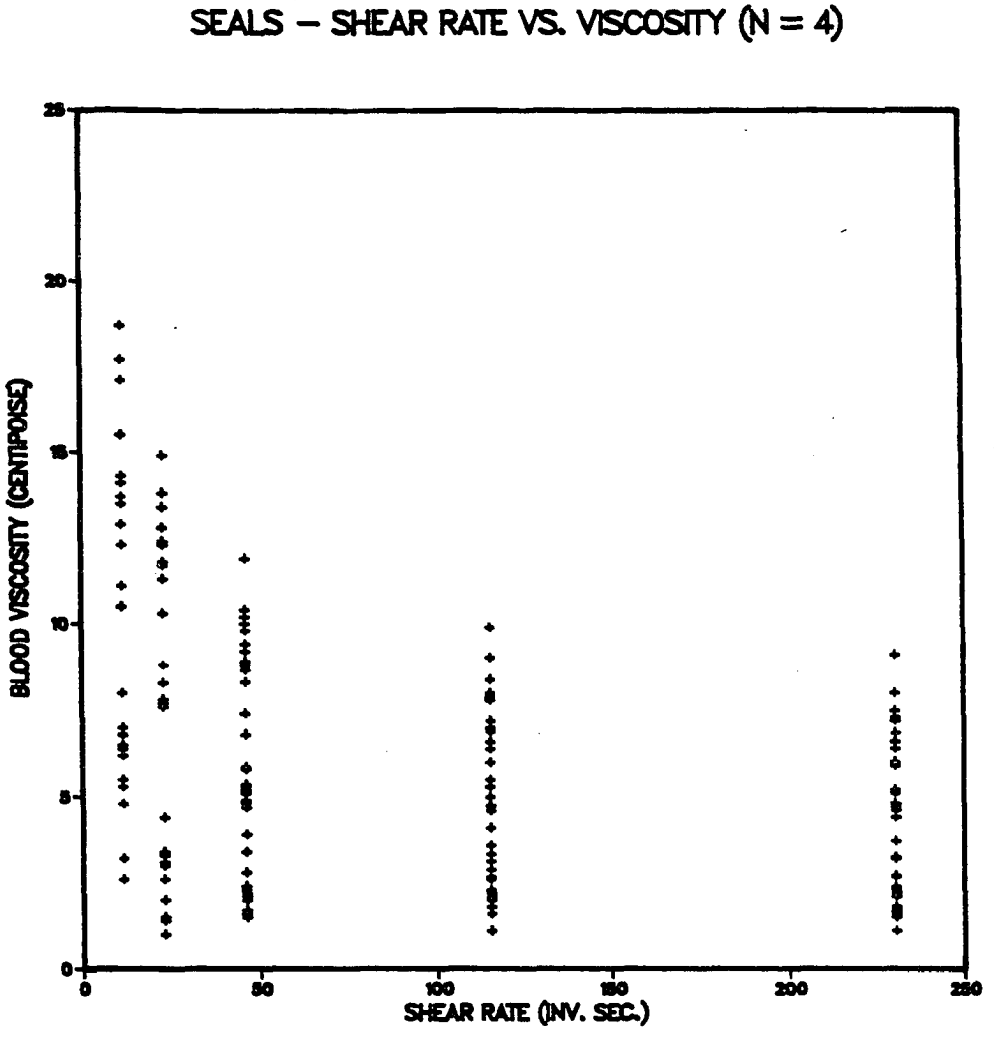


Figure 6.26. Relationship between absolute viscosity (cP) and shear rate (sec^{-1}) during experimental polycythemia and anemia experiments (n=4) on harbor seals. Viscosity was determined from measurements made using a Wells-Brookfield (LVT) cone-plate viscometer at $37^{o}C$.

DISCUSSION

The circulatory system is responsible for the transport of oxygen to the tissues. The rate of oxygen transport or oxygen supply to the tissues (TOT) is determined by the product of arterial oxygen content (CaO_2) and the blood flow rate (Q_T). Arterial oxygen content is a function of the arterial oxygen saturation and the hematocrit. Blood flow rate (Q_T) is determined by arterial pressure and the resistance to flow ($F = P/R$), while the latter is proportional to blood viscosity and vessel geometry (vascular hindrance). Under ideal conditions where circulatory and respiratory function are constant, the relationship between oxygen transport and HCT is defined by blood viscosity (Jan and Chien, 1977). The mathematical relationships between blood viscosity and hematocrit (Equations 6.18-6.22 and 6.24-6.28) indicate that the relationship between oxygen transport and HCT is a non-linear curve with the maximal value for TOT at what is termed the optimal HCT (Figures 6.4 and 6.7). The results of many investigations support this contention (Crowell and Smith, 1967; Johansson, et al., 1967; Restorff, et al., 1975; Fan, et al., 1980; Baer, et al., 1987). However, to extrapolate from these findings to the cardiovascular dynamics within the body is difficult at best.

Tissue oxygen consumption, whether it be for the whole body ($\dot{V}O_2$) or for a specific organ such as the heart ($M_{\dot{V}O2}$), depends upon oxygen extraction as well as oxygen transport (Eq. 6.1). Therefore, any variable which affects tissue metabolic rate or the dissociation of oxygen may also affect the relationship between oxygen transport and oxygen consumption (Suga, et al., 1987). It is then obvious that tissues with differing levels of activity and thus metabolic rate will show differing relationships between TOT and $\dot{V}O_2$. The regulation of vascular geometry (hindrance) may also differ and thereby affect local shear rate and rheology of the microcirculation (Jennett, et al., 1976). Additionally, the wall surface condition in which the blood is flowing affects blood viscosity (Scott-Blair, 1958). Blood viscosity is reduced in tube flow and varies inversely with the radius (Poiseulle 1841 and 1842; Fahraeus and Lindquist, 1931). Further reduction of blood viscosity in vivo has been established by the work of Whittaker and Winton (1933), Kramer and Winton (1939) and Pappenheimer (1941a and 1941b) and further quantified by Djojosugito, et al., (1970) and Benis, et al., (1973). This phenomenon is presumed to be due to endothelial surface proteins and vessel compliance. The integration of all these variables are responsible for the optimal HCT for tissues

and explains why each organ may have its "own" optimal HCT. In the present study, the effects of HCT on oxygen transport and utilization were determined for the total body and for the heart in pigs and seals.

Optimal HCT in terms of whole body oxygen consumption ranged between 25% and 45% for domestic pigs while the curve for harbor seals lies further to the right, with a maximum range between 25% and 55% (Figure 6.9). Thus, it appears that, similar to other mammals studied, both domestic pigs and harbor seals are living at their optimal HCT.

Total oxygen transport data plotted against HCT resemble curves constructed for humans, dogs and cats in other studies. However, there is no decreasing trend in TOT at high HCTs for pigs or seals (Figures 6.4 and 6.7).

Optimal HCT for myocardium of pigs could not be determined from the data as $M_{\dot{V}O2}$ increased up to 40% HCT and no data were available at higher HCTs (Figure 6.3). A range of HCT from 20% to 50% produced the highest values of $M_{\dot{V}O2}$ for seal hearts (Figure 6.6).

Comparisons of curves for pigs and seals for $\dot{V}_{O2}$, $M_{\dot{V}O2}$, flow resistance and arteriolar shear resistance all illustrate the broad range of hematocrits over which values for seals appear to plateau while corresponding curves for the pigs more closely resemble inverted

parabolas (Figure 6.11 and 6.12). It is possible that these plateaus represent greater autoregulation in seals. Perhaps these diving mammals have adapted themselves to their high hematocrit by having increased vessel compliance in order to withstand changes in viscosity. The large myoglobin stores of seal heart may also confer the advantage of enhanced facilitation of diffusion of oxygen to seal myocardium for aerobic metabolism, as well as increased buffering capacity for anaerobic metabolism (Dreidzic, et al., 1982; Castellini and Somero, 1981; Kjeckshus, et al., 1982). Seal heart has fewer mitochondria per unit volume than does pig myocardium (Sordahl, et al., 1983) which suggests a lower capacity for aerobic metabolism as is apparent from this study (Figure 6.10).

Recent studies by Elsner, et al. (1987) using similar experimental procedures on pigs and seals, but using ventilation with low partial pressures of oxygen to bring about hypoxia rather than anemia as in this study, may provide further insight. They found that myocardial energetic resources were higher in seals when compared to pigs. Oxidative and glycolytic energy reserves were 2.5 and 3.0 times that of pigs, respectively. However, the proportion of total energy derived from glycolysis was similar in seals and pigs (0.33 and 0.28, $P<0.05$). The

points of critical oxygen transport when myocardial lactate production begins were at arterial oxygen saturations of 35% (PaO_2 = 24 Torr) for seals and 57% (PaO_2 = 36 Torr) for pigs ($P<0.05$). In other experiments, White, et al. (1988) made seals and pigs acutely hypoxic until cessation of cardiac output (seals: 17.5 min.; pigs: 7.4 min., $P<0.05$) and then reoxygenated them. Seals recovered promptly to control levels of cardiac mechanical function while none of the pigs recovered. Six different pigs were beta blocked (beta-adrenergic nerves which control myocardial contractility) with propanolol and subjected to acute hypoxia which they tolerated 64% better than control pigs. The conclusions were that seals resist hypoxia better than do pigs as a result of decreased cardiac demand by modulation of beta adrenergic activity and increased oxygen and glycolytic storage.

It is important to realize that the independent variable in the experiments by White and co-workers and in the present study is cardiac mechanical function. The limitations of the seal and pig models used in this study are the assumptions of constant demand on the heart which are, in actuality, variable. Therefore, the values for whole body and myocardial oxygen consumption below the critical hematocrit at which lactate production

begins (the onset of glycolysis) become invalid. For seals and pigs the critical hematocrits are approximately 15% (F.C. White, pers. comm.). However, seals and pigs regulate oxygen requirements differently and this should be taken into consideration when evaluating the results of this study.

Knowledge of the limitations of the models used in this study allow postulation of experimental designs which may provide additional information. The use of an autoperfused heart preparation would allow measurement of the mechanical resistance and basal metabolism of the myocardium in a stable, steady-state system without the added variablity of reflex-associated vessel dilation. Whole animal experiments during which the animals (pigs and seals) were splenectomized, injected with red blood cells to increase their hematocrit (both acutely and chronically) and exercised while measuring oxygen consumption via collection of respiratory gases would, perhaps, better determine the significance of increased hematocrits upon oxygen transport and oxygen consumption.

In conclusion, the whole body and myocardial oxygen consumptions of domestic pigs and harbor seals do not appear to become dependent upon viscosity at arteriolar shear rates or at flow conditions measured during these experiments. The optimal hematocrits for whole body

oxygen consumption for pigs and seals are approximately 35% and 55%, respectively. Optimal HCTs for myocardium could not be determined for pigs but, those for seals covered a broad range from 20% to 45%. Therefore, seals do not seem to have made a trade-off by having high oxygen carrying capacity at the expense of reduced oxygen transport since whole body oxygen consumption for seals does not decline until a HCT of 60% is reached. Perhaps not surprisingly, pigs and seals appear to have optimal HCTs very close to their respective "natural HCTs" of 35% and 53%.

CHAPTER VII.

SUMMARY AND CONCLUSIONS OF THE THESIS

SUMMARY

Comparisons among marine mammals and terrestrial mammals in terms of hemorheology revealed many insights into the relationship of blood viscosity variations to the physiology and ecology of the animals.

The first studies of comparisons among different marine mammal species in terms of hemorheology and variables which affect it were broad in scope and provided baseline data upon which further hypotheses were based. Many of the hematologic characteristics had already been studied previously, but it was necessary to try to link this data with the flow behavior in order to present a comprehensive view of the mechanics of the flow properties which had not been previously accomplished. Treatment of marine mammals as a group in the present study resulted in negation of the earlier hypothesis which stated that the ecology and diving behavior were reflected in blood rheology. However, upon regrouping the animals into classes, the trends in hematology and rheology became apparent. Indeed, those animals with

higher hemoglobin, hematocrit and blood viscosity values were also the longer divers (i.e. northern elephant seals, ringed seals and harbor seals in the Pinnipedia and false killer whales in the Cetacea). A study involving species which are closely related phylogenetically but with greater differences in lifestyle would perhaps illustrate these trends more clearly. Hemorheological characteristics of the marine mammals showed no statistically significant differences with respect to age or sex and blood viscosity was independent of erythrocyte size as measured by mean corpuscular volume. However, all marine mammal blood studied exhibited the typical mammalian dependence of viscosity upon hematocrit and shear rate.

Contrary to the popular belief in acclimatization responses, comparisons of free-ranging and captive northern elephant seals and sea otters which were newly-captured and those in captivity for three weeks showed no statistical differences in hemorheologic variables except for increased white blood cell (WBC) counts. The reasons for the increased WBC counts is unknown but changes in diet, water, ambient noise, as well as interaction with humans may be responsible. Increased plasma protein concentrations and hence, higher plasma viscosity, as well as reduced mean corpuscular size in

free-ranging northern elephant seals were believed to result from dehydration during the spring fast. All blood variables studied were well within previously reported ranges for captive and free-ranging animals.

Comparative marine and terrestrial mammalian hemorheology revealed significant differences among phocid seals and domestic pigs (the terrestrial mammalian model). In addition to the increased oxygen carrying capacity afforded the seals by way of increased blood volume, hematocrit and mean corpuscular hemoglobin concentration, seals had larger erythrocytes and increased leukocyte counts and plasma protein concentrations.

The reconstitution of pig blood in order to compare the bulk flow properties at the same volume concentration revealed reductions in seal blood viscosity relative to that of pigs up to 22% at low shear rates. The differences were shear rate dependent and became insignificant at high flow rates (> 50 sec^{-1}). Increases in plasma proteins and leukocytes which normally result in increased viscosity and the lack of correlation with erythrocyte geometry suggested that the viscosity reduction in seals was due to RBC interactions. This led to the hypothesis that reduced aggregation was responsible for the low viscosity of seal blood at low

shear rates. The reduced viscosity is thought to represent an adaptation to diving recovery to reduce the force necessary to reinitiate flow in stagnant venous capacitance vessels.

The only other reported studies of phocid seal blood viscosity stated that there were no rheological differences between seals and various terrestrial mammals (Guard and Murrish, 1975; Hedrick, et al., 1986). However, Hedrick and co-workers (1986) did not make any rheologic measurements at low shear rates and inspection of their high shear data supports the contentions of this dissertation. Guard and Murrish (1975) imply that the blood viscosity for Antarctic seals is actually higher than that for humans at the same temperature. However, if one looks at their table of viscosity values, it is apparent that the values listed for comparisons are derived from the Casson Equation used to define yield stress values. The viscosity values reported for seals were measured at much lower shear rates than those for humans and tropical species which will obviously make the seal values appear higher in comparison. Calculation of shear rates for their viscosity values using their reported shear stress values and corresponding viscosity values (shear rate = shear stress / viscosity) provide results which are comparable to those for elephant seals

and harbor seals at 37 °C reported in this dissertation.

Studies of RBC aggregation and the dynamic viscoelasticity of blood from seals, humans and pigs provide further support for the contention that seal blood viscosity shows a pronounced reduction near stasis and that reduced aggregation is partly responsible for this behavior. However, the studies also revealed significant differences among the seals. The reasons for this are unclear, but reduced fibrinogen levels and increased electrophoretic mobility of seal RBCs seems to indicate that the erythrocytes of some species may actually repel one another and thereby reduce aggregation, sedimentation rate and low shear viscosity.

The effects of all these various rheological phenomena upon the cardiovascular dynamics was investigated during experimental normovolemic hemodilution and hemoconcentration in harbor seals and domestic pigs. The objective was to determine the optimal hematocrits for whole body and myocardial oxygen consumption in seals and swine and to answer the primary question of the dissertation: Have seals made a trade-off by having such high oxygen carrying capacity that the ability to transport oxygen is reduced and results in lower tissue oxygen consumption? An attempt by Hedrick, et al. (1986) to answer this question theoretically using in vitro

viscosity measurements and plotting theoretical oxygen transport (Hb/viscosity) against hematocrit resulted in the typical inverted parabola and the conclusion that the seals have made such a trade-off. However, based on the method of curve construction, an inverted parabola will always result from such calculations and does not answer the question. The measurement of *in vivo* oxygen consumption is necessary to determine whether or not the cardiovascular system is able to compensate for viscosity variations.

Perhaps not surprisingly, the results indicate that both seals and pigs are living within the range of what is defined as their optimal or "ideal" hematocrit for both whole body and myocardial oxygen consumption. Seal total body and myocardial oxygen consumption did not begin to decrease until after their natural hematocrit of 53% was reached. Total oxygen transport for both seals and pigs never showed a decline even at hematocrits up to 68%. Plots of total body oxygen consumption for pigs closely resembled the theoretical curve (inverted parabola) found for other terrestrial mammals while curves for seals were more broad and flat. Actual values for oxygen consumption were also lower for seals.

These results indicate that seals have lower overall oxygen demands for whole body and myocardial metabolism

and an increased ability to compensate for changes in hematocrit and hence, variations in viscosity. In view of the striking changes in hematocrit within muscle during exercise (Klitzman and Duling, 1979) and those reported for seals during different physiologic states (Castellini, et al., 1986; Qvist, et al., 1986), perhaps this makes sense. Reduced levels of mitochondria (Sordahl, et al., 1983) and increased myoglobin in seal heart may further support this contention. Increased myoglobin would facilitate oxygen transport and increase the buffering capacity of the myocardium (Castellini and Somero, 1981; Dreidzic, et al., 1982) thus reducing aerobic requirements and providing neutralization of end products of anaerobic metabolism during dives (Kjekshus, et al., 1982).

CONCLUSIONS

The general conclusions of this thesis are:

1. Trends in hematology and hemorheology were reflected in the ecology and diving behavior of marine mammals when grouped into classes. Deeper and longer divers had increases in oxygen-carrying capacity and increased blood viscosity. However, there were exceptions among some species and the trends were not statistically significant.

2. There appears to be no acclimatization response to captivity in northern elephant seals and sea otters in terms of hematology and hemorheology.

3. Some significant differences exist among terrestrial and marine mammals and they are generally quantitative in nature rather than qualitative. Seals have increased oxygen-carrying capacity due to increased hematocrits and mean corpuscular hemoglobin concentrations, larger erythrocytes and thus, fewer erythrocytes at the same volume concentration, as well as increased white blood cell counts and plasma protein concentrations. However, seal blood exhibits less of a viscosity-dependent reduction in transport at low shear rates. These quantitative differences may be, in part, responsible for the remarkable diving abilities of these marine mammals.

4. Further comparisons of phocid seals and terrestrial mammals revealed more pronounced reductions in blood viscosity and elasticity near stasis due to reduced red cell aggregation. Reduced fibrinogen levels and increased cell surface charge seem to contribute to this behavior.

5. Seals seem to be living at their optimal hematocrit and therefore have made no evolutionary trade-off by having oxygen-carrying capacity such that their oxygen consumption declines.

Many unanswered questions remain regarding mechanisms of interactions among erythrocytes and the rheology of individual cells, their membranes and their interactions with the suspending medium. The mechanisms by which seals compensate for hematocrit variations also remain a mystery. Do seals have an enhanced ability to autoregulate? Is this compensatory ability based upon vessel geometry or upon humoral interactions based on fluid balance, blood volume and peripheral resistance (pressure) or distribution of hematocrit within the body and where does rheology fit into this scheme in terms of the adaptation of the animal to its environment?

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APPENDIX

T-TEST FOR SIGNIFICANCE AMONG HEMATOLOGIC AND RHEOLOGIC VARIABLES FOR CAPTIVE AND FREE-RANGING ELEPHANT SEALS AND SEA OTTERS

SPECIES	VARIABLE	P-VALUE AT 95% CONFIDENCE LEVEL
Mirounga angustirostris		
	age	0.320
	Hb	0.096
	HCT	0.790
	RBC	0.250
	MCV	0.001
	MCHC	0.780
	PP	0.017
	WBC	0.020
	viscosity @	
	11.5 inv. sec.	0.920
	23.0 inv. sec.	0.820
	46.1 inv. sec.	0.230
	115.2 inv. sec.	0.430
	230.4 inv. sec.	0.480
Enhydra lutris		
	age	1.000
	Hb	0.095
	HCT	0.910
	RBC	0.280
	MCV	0.440
	MCHC	0.030
	PP	0.380
	viscosity @	
	11.5 inv. sec.	0.910
	23.0 inv. sec.	0.380
	46.1 inv. sec.	0.240
	115.2 inv. sec.	0.740
	230.4 inv. sec.	0.150

GLOSSARY

absolute viscosity - viscosity measured in a non-tubular viscometer.

acclimatization - the climatic adaptation of an organism that has been moved to a new environment.

acute - having a short and severe course.

aerobic scope - the number of times an organism can raise its oxygen consumption above the basal or resting rate.

anaerobic metabolism - that proportion of an organism's metabolism supported by the glycolytic pathway without use of oxygen.

apnea - a state of cessation of respiration; breath-holding

biorheology - the study of the deformation and flow of materials within living organisms.

capacitance - in reference to the vessels of the venous system as to their ability to dissipate the volume and pressure changes in response to the arterial side of the circulatory system.

Cetacea - that class of marine mammals which includes whales, dolphins and porpoises.

claudication - see Intermittent.

critical HCT - the hematocrit at which anaerobic metabolism begins as evidenced by lactate production.

deformation - an alteration in shape due to pressure or stress.

Delphinidae - a family of marine mammals which includes the dolpins.

diapedesis - the passage of blood or blood constituents through narrow channels such as pores in blood vessel walls.

differential (WBC) - the analysis of the proportions of different white blood cell types in a white blood cell count, represented as percentages.

eta - shear stress.

flow - deformation proceeding irreversibly with time.

gamma - shear rate.

Hb - hemoglobin concentration (g/dl).

HCT - hematocrit, the percentage of red blood cells in whole blood; packed cell volume of blood (%).

hematocrit - (see HCT).

hematology - the study of blood and it's constituents.

hemoconcentration - the concentration of the blood by addition of erythrocytes. The process is termed normovolemic if volume is kept constant throughout the procedure.

hemodilution - the opposite of hemoconcentration; dilution of the blood with plasma or a plasma expander such as low molecular weight dextran and saline.

hydrostatic pressure - that pressure exerted by a column of water or fluid (rho.g.h) (dynes, cm H2O, mmHg).

intermittent claudication - a complex of symptoms characterized by absence of pain or discomfort in a limb when at rest, the commencement of pain, tension and weakness after walking is begun, intensification of the condition until walking becomes impossible, and the disappearance of the symptoms after a period of rest. The condition is often caused by occlusive arterial disease due to venous stasis and ensuing blood sludging.

isovolemic - see normovolemic.

MCV - mean corpuscular volume (cubic micrometers or cubic microns).

MCHC - mean corpuscular hemoglobin concentration (%); pertaining to erythrocytes.

myocardium - the tissue of the heart.

Newtonian - a fluid which exhibits flow behavior in which the shear stress is directly proportional to shear rate; the plot of viscosity versus shear rate is a straight line.

normovolemic - a process in which volume is kept constant (e.g. normovolemic hemodilution).

Ostwald viscometer - a viscometer in which the sample fluid hydrostatic pressure causes it flow through a U-shaped tube and fill a bulb to a measured mark; passage time and geometry are used calculate viscosity. This type of viscometer usually requires several hundred milliliters of sample fluid.

parturition - the act of pupping; giving birth.

PCV - packed cell volume of erythrocytes in whole blood (%) see HCT.

phocid - pertaining to those seals of the family Phocidae; true seals, earless seals.

poise - a unit of measurement of viscosity; equal to mPa.s.

polycythemia - an increase in the total red cell mass of the body; increase in total body hematocrit.

RBC - red blood cell, erythrocyte; also RBC count (millions per cubic millimeter of blood).

rheology - the science of the study of deformation and flow of materials.

rheopexy - a characteristic of time-dependent flow behavior in which viscosity increases directly with time.

rouleau - a geometric conformation of red blood cells in which individual cells stack up into rolls which look like a stack of coins (pl. rouleaux).

shear - an applied force or system of forces that tend to produce a shearing strain (shear stress).

shear stress - (tau) see shear.

shear rate - (gamma) a condition in or deformation of an

elastic body or fluid caused by forces that tend(s) to produce an opposite but parallel sliding motion of the body's planes; relative rate of fluid velocity in adjacent fluid laminae; proportional to flow rate.

splenectomy - the surgical removal of the spleen.

tau - see shear stress.

thoracotomy - surgical incision of the wall of the chest.

torque - (T) the moment of a force, a measure of its tendency to produce torsion and rotation about an axis, equal to the vector product of the radius from the axis of rotation to the point of application of the force by the force applied; a twisting or turning force.

viscometer - an instrument that measures viscosity.

viscosity - the ratio of shear stress to shear rate for a material. The internal friction within a deforming material. The consistency of a fluid or material.

WBC - white blood cell, leukocyte, or a count of the number of white cells (thousands) in a cubic millimeter of blood.