Comparative Hemorheology

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Introduction

Hemorheological values vary widely among the animal species. To understand the structure-function relationships of red blood cells (RBC) together with associated physiological mechanisms, comparative studies are still a classical approach. Some animal species show extraordinary values of blood viscosity and RBC indices which would be pathologic for man. These differences can reflect an adaptation process to a specific environment or way of life. Different species also use different mechanisms to maintain blood flow, and in some cases these differences might indicate which variables limit the demand for oxygen delivery in a species or under certain circumstances. Nevertheless, when comparing values to man, it should be kept in mind that every hemorheologic “disturbance” in a healthy animal reflects a physiologic circumstance. It is necessary to know that the hemorheological profile of an animal cannot be judged by a single rheological value, but must be considered as part of the cardiovascular system in which the blood is flowing. That is, the hemorheological profile of an animal species is a conglomerate of properties which has to be considered in evaluating its cardiovascular relevance in a species-specific manner. These species-specific differences look chaotic at first sight, and no clear guiding principle has been found to combine these profiles to a universal “logic” at present. Therefore changes in parameters during disease, after interventions, or during environmental or associated changes need to be related to their species-specific reference values; describing an animal’s clinical or physiological condition using comparisons to values from other species is most likely not a valid approach.

1. General Aspects

RBC of the different classes of vertebrates have different sizes and shapes, variations which are sometimes huge due to the nucleus within non-mammalian RBC. This variation and the diversities of RBC cytoplasm and membrane constituents, together with variations in blood cell count and blood plasma composition between the species markedly influences bulk blood flow in different parts of the vasculature. A list of

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hematological parameters is given by Hawkey et al. [1] or can be found in textbooks [2, 3].

The first group of parameters which have to be considered in hemorheology are cell size and geometry. RBC size differs widely among the species – and to a lesser extent also between individuals within a species - with fish having the highest and mammals the lowest values although exceptions exist. For example, fish RBC volume can be more than 100-fold higher than those of a mammal [4]. Diversity in mammalian RBC volume alone is much smaller, ranging from a mean value of 18 fl in goat to 160 fl in giant anteater [5]. The size of the mammal has no association with its RBC volume; large species like horse may have smaller RBC than smaller species like rabbit. Mean cell volume (MCV) correlates strongly and inversely with RBC count [1, 6]. Besides hydrodynamic forces and RBC membrane mechanical properties, RBC size is most important in determining the effectiveness of tissue perfusion since it is easier for RBC to enter and transit capillaries if the cell size matches the vessel caliber and if vessels are more or less straight. Capillary geometry differs between various tissues (e.g., brain vs. kidney or skeletal muscle), with capillary density also affected by tissue metabolic demand which is measurable by capillary density per mitochondrial number. In case of skeletal muscle for instance, capillary geometry depends on the activity of the animal or on the environment the animal is adapted to [7]. Differences in rheological behavior of blood may also help to optimize O\textsubscript{2} supply to tissues in various species [8-11]. Mean capillary diameters in species reach values from 3 μm in pigeon and rat, up to 20 μm in frog with tuna, hummingbird, bat, and rabbit lying in between [12-14]. In a small number of species, capillaries have been found to be smaller than 3 μm [15], but in most animals corrosion cast measurements show capillary diameter values below 5 μm [15-17], which is indeed smaller than the diameter of most animal RBC. As part of an \textit{in vivo} study of capillary diameter and RBC geometry, frog RBC were found to travel through the capillaries with their major axis predominantly parallel to the flow direction [13]. In the presence of a greater mismatch of capillary caliber and RBC diameter as in fish, \textit{in vivo} capillary transit time might be increased as suggested by results of \textit{in vitro} filtration tests [4, 18]. However, an increased capillary transit time should enhance the time for O\textsubscript{2} delivery, which might be an advantage in animals adapted to cold temperature since it would compensate for the left shift of the O\textsubscript{2} dissociation curve. On the other hand, mammalian RBC are much smaller, which should facilitate O\textsubscript{2} diffusion across the RBC membrane by providing a greater membrane surface per unit volume of blood; the smaller size also reduces the energy required for RBC to enter and pass the capillaries. Due to the inverse correlation of RBC size and number, a greater amount of small RBC may be associated with the increased metabolic capacity in animals with constant body temperature.

A second important parameter for RBC function is cytoplasmic viscosity, reflected by mean cellular hemoglobin concentration (MCHC). MCHC is different among the species. Very high MCHC has been observed in seals, possibly to optimize O\textsubscript{2} storage during long-term dives [19]. The cost of this is an increased cytoplasmic viscosity which may affect RBC deformability, although the RBC function of carrying O\textsubscript{2}-binding proteins to guarantee adequate O\textsubscript{2} supply seems to be more important under such circumstances. MCHC is increased by about 20% in birds compared to mammals due to the presence of a nucleus which displaces part of the cytoplasm [1, 20].

Besides size and cytoplasmic hemoglobin concentration, RBC also differ in specific structural characteristics. Only mammals have RBC without a nucleus.
Additionally, in animals other than mammals, a complex tubulin structure is present which anchors to the plasma membrane and affects the specific shape of RBC [21-26]. Both nucleus and tubulin filaments increase cell stability against shear forces and lead to a specific orientation of RBC in the flow field. In contrast, mammals including man, have biconcave disc shaped RBC, and shear forces can induce greater modifications in RBC shape (e.g., folding) than in other animals. Differences in the extent of shape changes during various levels of shear stresses do exist among mammals, allowing reduction of whole blood viscosity through this mechanism. As an exception, camelids have a more intermediate status among the mammalian species. They do not have a nucleus, however their RBC are spindle shaped and some authors report the presence of a marginal band [27]. Such basic morphologic diversity between mammals and other zoological classes are among the reasons for the pronounced differences found in RBC deformability and aggregation.

These principal species differences influence both single RBC behavior and bulk blood flow. However, the prediction of blood flow phenomena in a living animal using in vitro viscometry or measurements of single cell properties is difficult because of the impact of the vascular function and geometry on the organization or stabilization of flow [14].

2. Specific Aspects

2.1. Mammals

Mammals are the most widely investigated zoological class due to their agricultural and experimental use. Many mammals have been tested. However a list of hemorheological values may never be complete, and although relevant differences might exist even among relatives, common characteristics include the following principles: mammalian RBC are non-nucleated hemoglobin-carrying biconcave disks of various sizes; hematocrit varies from 30 to 50%; RBC aggregation and deformability varies strongly among mammals.

2.1.1. Plasma and Whole Blood Viscosity

Plasma viscosity (PV) depends primarily on plasma protein concentration, indicating that PV can vary in disease [28-30]. The highest PV within a group of 9 species was found in cattle (mean: 1.72 mPa.s) and the lowest in rabbit (mean: 1.30 mPa.s) with horse, cat, dog, pig, rat, mouse, and sheep being between 1.3 and 1.7 mPa.s. These values are higher than those obtained for man by the same investigator [31]. Although PV contributes to the value of whole blood viscosity (WBV), PV and WBV values do not correlate between species. A species with low physiologic PV, compared to a “standard” of human values, can have high values of WBV. For example, horse has an increased WBV, however, its PV is less than cattle due to the high plasma fibrinogen concentration in the latter species. This high PV in cattle, however, is accompanied by a low WBV at low shear rates [31]. For further description of plasma protein concentration and PV in animal species see Chapter II.2.
Table 1. References for hemorheological values in healthy animals

<table>
<thead>
<tr>
<th>Mammalian</th>
<th>Non-Mammalian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Reference</td>
</tr>
<tr>
<td>Horse</td>
<td>[31, 32, 37-50]</td>
</tr>
<tr>
<td>Rat</td>
<td>[6, 31, 39, 47, 55-60]</td>
</tr>
<tr>
<td>Cow</td>
<td>[31, 32, 44, 45, 47-50, 59]</td>
</tr>
<tr>
<td>Sheep</td>
<td>[4, 31, 32, 34, 47, 49, 50, 55, 63, 64]</td>
</tr>
<tr>
<td>Goat</td>
<td>[4, 34, 45, 47, 64]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>[6, 31, 32, 45, 55-58, 60]</td>
</tr>
<tr>
<td>Pig</td>
<td>[31, 36, 47, 49, 50, 57, 63]</td>
</tr>
<tr>
<td>Mouse</td>
<td>[6, 31, 47, 55]</td>
</tr>
<tr>
<td>Hamster</td>
<td>[6, 47, 57]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>[6, 55, 56]</td>
</tr>
<tr>
<td>Gerbil</td>
<td>[58]</td>
</tr>
<tr>
<td>Dog</td>
<td>[4, 6, 31, 34, 47, 55, 58, 63, 64, 76]</td>
</tr>
<tr>
<td>Cat</td>
<td>[31, 57, 58]</td>
</tr>
<tr>
<td>Elephant</td>
<td>[4, 32, 34, 47, 64, 78]</td>
</tr>
<tr>
<td>Camel, Llama</td>
<td>[32, 47, 60]</td>
</tr>
<tr>
<td>Zoo Animals</td>
<td>[32, 45, 47]</td>
</tr>
<tr>
<td>Primates</td>
<td>[32, 47, 93]</td>
</tr>
</tbody>
</table>
Table 2: Whole blood viscosity (mean and median values) of various mammalian species

<table>
<thead>
<tr>
<th>Species</th>
<th>Low Shear Viscosity (mPa.s)</th>
<th>High Shear Viscosity (mPa.s)</th>
<th>Low/High Shear Rate (s^-1)</th>
<th>Hct (%)</th>
<th>T¹ (°C)</th>
<th>Device</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat (median)</td>
<td>30.2</td>
<td>4.4</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30²</td>
<td>[31]</td>
</tr>
<tr>
<td>Cattle (mean)</td>
<td>6.7</td>
<td>5.8</td>
<td>1 / 150</td>
<td>30</td>
<td>20</td>
<td>OCR-D³</td>
<td>[50]</td>
</tr>
<tr>
<td>Cattle (median)</td>
<td>5.4</td>
<td>3.6</td>
<td>0.277 / 128</td>
<td>35</td>
<td>37</td>
<td>LS30</td>
<td>[32]</td>
</tr>
<tr>
<td>Cattle (median)</td>
<td>6.6</td>
<td>4.8</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>Deer (mean)</td>
<td>45</td>
<td>4.7</td>
<td>0.277 / 128</td>
<td>42</td>
<td>37</td>
<td>LS30</td>
<td>[32]</td>
</tr>
<tr>
<td>Dog (mean)</td>
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<td>5.7</td>
<td>1 / 150</td>
<td>37</td>
<td>20</td>
<td>OCR-D</td>
<td>[50]</td>
</tr>
<tr>
<td>Dog (median)</td>
<td>62</td>
<td>5.3</td>
<td>0.277 / 128</td>
<td>50</td>
<td>37</td>
<td>LS30</td>
<td>[76]</td>
</tr>
<tr>
<td>Dog (median)</td>
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<td>5.6</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>African elephant (median)</td>
<td>28.3</td>
<td>5.4</td>
<td>0.7 / 94</td>
<td>37</td>
<td>37</td>
<td>LS30</td>
<td>[78]</td>
</tr>
<tr>
<td>Asian elephant (mean)</td>
<td>53</td>
<td>5.6</td>
<td>0.277 / 128</td>
<td>43</td>
<td>37</td>
<td>LS30</td>
<td>[32]</td>
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<tr>
<td>Horse (mean)</td>
<td>93</td>
<td>4.4</td>
<td>0.277 / 128</td>
<td>41</td>
<td>37</td>
<td>LS30</td>
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<td>12.8</td>
<td>6.5</td>
<td>1 / 150</td>
<td>44</td>
<td>20</td>
<td>OCR-D</td>
<td>[50]</td>
</tr>
<tr>
<td>Horse (median)</td>
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<td>5.2</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
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<tr>
<td>Horse (median)</td>
<td>8.3</td>
<td>4.0</td>
<td>0.75 / 230</td>
<td>40</td>
<td>37</td>
<td>Brookfield DV-II²</td>
<td>[37]</td>
</tr>
<tr>
<td>Goat (mean)</td>
<td>5.1</td>
<td>3.4</td>
<td>0.277 / 128</td>
<td>31</td>
<td>37</td>
<td>LS30</td>
<td>[32]</td>
</tr>
<tr>
<td>Mouse (median)</td>
<td>13.4</td>
<td>4.9</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>Pig (mean)</td>
<td>14.3</td>
<td>7.2</td>
<td>1 / 150</td>
<td>45</td>
<td>20</td>
<td>OCR-D</td>
<td>[50]</td>
</tr>
<tr>
<td>Pig (median)</td>
<td>24.7</td>
<td>4.9</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>Rabbit (mean)</td>
<td>12.4</td>
<td>3.8</td>
<td>0.277 / 128</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[32]</td>
</tr>
<tr>
<td>Rabbit (median)</td>
<td>8.3</td>
<td>4.0</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>Rat (median)</td>
<td>35.4</td>
<td>6.3</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>Sheep (mean)</td>
<td>4.4</td>
<td>3.9</td>
<td>1 / 150</td>
<td>31</td>
<td>20</td>
<td>OCR-D</td>
<td>[50]</td>
</tr>
<tr>
<td>Sheep (mean)</td>
<td>5.0</td>
<td>3.3</td>
<td>0.277 / 128</td>
<td>36</td>
<td>37</td>
<td>LS30</td>
<td>[32]</td>
</tr>
<tr>
<td>Sheep (median)</td>
<td>6.6</td>
<td>4.4</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
<tr>
<td>Human (median)</td>
<td>33.5</td>
<td>6.0</td>
<td>0.7 / 94</td>
<td>40</td>
<td>37</td>
<td>LS30</td>
<td>[31]</td>
</tr>
</tbody>
</table>

¹ Measurement temperature
² LS-30 Couette viscometer (Contraves GmbH, Zurich, Switzerland)
³ Oscillating Capillary Rheometer and Densitometer OCR-D (Paar GmbH, Graz, Austria)
⁴ Brookfield DV-II cone-plate viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA)
Table 3. Plasma viscosity \((mean\ and\ median\ values)\) of various mammalian species

<table>
<thead>
<tr>
<th>Species</th>
<th>Plasma Viscosity (mPa.s)</th>
<th>Device</th>
<th>°C</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat (median)</td>
<td>1.7</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Cattle (mean)</td>
<td>2.5</td>
<td>OCR-D</td>
<td>20</td>
<td>[50]</td>
</tr>
<tr>
<td>Cattle (mean)</td>
<td>1.9</td>
<td>Capillary2</td>
<td>20</td>
<td>[41]</td>
</tr>
<tr>
<td>Cattle (median)</td>
<td>1.7</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Deer (mean)</td>
<td>1.3</td>
<td>LS30</td>
<td>37</td>
<td>[32]</td>
</tr>
<tr>
<td>Dog (single value)</td>
<td>1.1</td>
<td>Coaxial2</td>
<td>37</td>
<td>[4]</td>
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<td>Dog (median)</td>
<td>1.6</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Dog (mean)</td>
<td>1.6</td>
<td>OCR-D</td>
<td>25</td>
<td>[96]</td>
</tr>
<tr>
<td>African elephant (median)</td>
<td>1.9</td>
<td>OCR-D</td>
<td>21</td>
<td>[78]</td>
</tr>
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<td>Asian elephant (mean)</td>
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<td>LS30</td>
<td>37</td>
<td>[32]</td>
</tr>
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<td>Horse (mean)</td>
<td>1.5</td>
<td>LS30</td>
<td>37</td>
<td>[32]</td>
</tr>
<tr>
<td>Horse (median)</td>
<td>1.5</td>
<td>Capillary2</td>
<td>20</td>
<td>[41]</td>
</tr>
<tr>
<td>Horse (mean)</td>
<td>1.7</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Horse (mean)</td>
<td>1.6</td>
<td>OCR-D</td>
<td>21</td>
<td>[97]</td>
</tr>
<tr>
<td>Goat (mean)</td>
<td>1.6</td>
<td>LS30</td>
<td>37</td>
<td>[32]</td>
</tr>
<tr>
<td>Mouse (median)</td>
<td>1.3</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Pig (median)</td>
<td>1.6</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Rabbit (mean)</td>
<td>1.1</td>
<td>LS30</td>
<td>37</td>
<td>[32]</td>
</tr>
<tr>
<td>Rabbit (median)</td>
<td>1.3</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Rat (mean)</td>
<td>1.0</td>
<td>Harkness3</td>
<td>37</td>
<td>[98]</td>
</tr>
<tr>
<td>Rat (median)</td>
<td>1.6</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Sheep (mean)</td>
<td>1.8</td>
<td>OCR-D</td>
<td>20</td>
<td>[50]</td>
</tr>
<tr>
<td>Sheep (median)</td>
<td>1.5</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
<tr>
<td>Sheep (mean)</td>
<td>1.6</td>
<td>OCR-D</td>
<td>25</td>
<td>[96]</td>
</tr>
<tr>
<td>Human (median)</td>
<td>1.2</td>
<td>OCR-D</td>
<td>21</td>
<td>[31]</td>
</tr>
</tbody>
</table>

1 Measurement temperature
2 Custom made device [41]
3 Harkness viscometer (Coulter Electronics Ltd., England, UK)
Viscometric data from a wide range of animals have been presented by Johnn, et al. [32], with other authors describing smaller numbers of species (see Table 1). Whole blood viscosity is strongly and directly related to hematocrit and is inversely related to temperature in species, but relations between species vary. For example, in some animals living in a cold environment (e.g., bowhead whales), WBV does not increase with decreasing temperature to the extent that would be expected for human blood [33]. WBV increases with hematocrit but the degree of viscosity increase with hematocrit is species specific, especially at low shear rates [34]. Apparent whole blood viscosity of mammalian blood shows shear thinning behavior, but the effect is low in animals with low aggregation such as goat, sheep, and cow. In vitro WBV at low shear rate (0.7 s\(^{-1}\)) is in the range of 6-7 mPa.s (40% hematocrit, 37°C) in these species. In contrast, horse, donkey, and zebra show pronounced shear thinning and increased low shear blood viscosity due to their “hyper-aggregating” blood. Their WBV at low shear rate is in the range of 38-40 mPa.s [31, 32]. Other extreme diversities in whole blood viscosity include phocid seals [35, 36] (see below). Most other mammalian species have WBV between the values for horse and goat. A ranking from data presently known can be as follows: equines> felines> chimpanzee> elephant> rat> deer> bison> mouse> rabbit> cattle> sheep> goat> camel (For references see table 1). Please note that the absolute values can sometimes not be directly compared due to the different devices used for the measurement by different laboratories. Table 2 and 3 give insight into species differences of whole blood and plasma viscosity values in mammals.

2.1.2. RBC Deformability

RBC deformability data from a wide range of animals have been presented by Smith, et al. [47]. However, due to the variability of RBC size and shape between species, values for the term “deformability” are difficult to compare because these size and shape influence the measurement outcome. Using small (\(\approx 1\) μm) micropipettes it is possible to measure membrane mechanical properties (e.g., shear elastic modulus, apparent viscosity), while larger (\(\approx 3-5\) μm) micropipettes allow aspiration of the entire cell and hence a measure of cellular deformability (e.g., entry time at a given aspiration pressure). It is of interest to note that membrane measurements made using small pipettes are insensitive to cell size, whereas cellular deformability measurements via complete cell aspiration depend strongly on the sizes of the micropipette and the cell. There are other methods to measure deformability, including cell elongation at various shear stresses (e.g., laser diffractionmetry) [47, 78, 96] or RBC capillary entry and transit time (e.g., automated filtration method) [55, 57, 64, 76]. For example, with laser diffractionmetry, RBC elongation rises with the deforming force and is characterized by a specific elongation index (EI) – shear stress relationship for each species; increased elongation at a given shear stress means increased deformability (Figure 1).

Figure 1 demonstrates that five species (rabbit, mouse, hamster, rat and pig) have more deformable RBC than the other species included in this study. It is also clear that horse and elephant RBC are the least deformable in this group, with dog RBC having an intermediate degree of deformability. Sheep RBC exhibit unique behavior with significantly lower EI values at the higher shear stress range; note that at such high shear stress levels, elongation indexes appear to be reaching a maximum for most species. Such a behavior may reflect the exceptionally small size of sheep RBC compared to other species included in this study.
Figure 1. RBC elongation index (EI) versus shear stress (SS) data obtained via ektacytometry for nine species. EI is calculated from the diffraction image as the (length-width)/(length+width) of the image.

Table 4. RBC elongation indexes (EI) measured at a shear stress of 5.38 Pa, calculated shear stress needed for one-half maximal deformation (SS_{1/2}), and mean cell volume (MCV) values for nine species. Data should be regarded as preliminary since sample size varies among species.

<table>
<thead>
<tr>
<th>Species</th>
<th>EI at 5.38 Pa</th>
<th>SS_{1/2} (Pa)</th>
<th>MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>0.448</td>
<td>2.46</td>
<td>47</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.447</td>
<td>1.91</td>
<td>46</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.447</td>
<td>1.81</td>
<td>57</td>
</tr>
<tr>
<td>Rat</td>
<td>0.446</td>
<td>2.82</td>
<td>47</td>
</tr>
<tr>
<td>Pig</td>
<td>0.419</td>
<td>3.52</td>
<td>51</td>
</tr>
<tr>
<td>Dog</td>
<td>0.381</td>
<td>5.90</td>
<td>63</td>
</tr>
<tr>
<td>Horse</td>
<td>0.279</td>
<td>12.7</td>
<td>42</td>
</tr>
<tr>
<td>Elephant</td>
<td>0.238</td>
<td>7.71</td>
<td>138</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.293</td>
<td>3.68</td>
<td>32</td>
</tr>
</tbody>
</table>

Since RBC elongation index-shear stress curves actually represent several pairs of EI-stress data, (see Chapter II.6), it is difficult to provide a single index for deformability. However, various methods have been described to derive such a parameter by calculating the shear stress for half-maximal deformation (SS_{1/2}) of the cell population [100]. SS_{1/2} values for the EI-shear stress curves shown in Figure 1 are presented in Table 4. It is also obvious from these values that, compared to the other species, elephant and horse RBC require higher shear stresses to reach half of their maximum deformation; this shear stress was 3- to 6-fold higher for elephant and horse RBC. RBC deformability of some animals (e.g., camel, llama, bank vole Myodes glareolus) cannot easily be measured using ektacytometry since RBC orientation is not
always aligned with the shear field. Rather, these cells tend to tumble and/or become perpendicular to the lines of flow (unpublished observation). The reason for these observations has not yet been investigated in detail, but most likely relates to the non-biconcave shape of these cells.

To investigate factors involved in RBC deformability, some membrane components have been tested in various species. Modifications of RBC membrane content or the function of band 4.2 and band 3 proteins have been associated with RBC shape changes [101, 102]. Percentages of band 4.2 protein have been shown in a large number of species by Guerra-Shinohara and de O’Barretto [103]. Band 4.2 protein was found to be absent in horse, guinea pig and two rodents of this study. The deficiency of band 4.2 in horse was associated with the high appearance of echinocytes in this species [39]. Band 3 has also been investigated in camelids and the rotational and lateral mobility of the membrane domain of the protein has been found to be decreased, and a tight and close connection of the cytoplasmic domain to ankyrin has been observed [104, 105].

Membrane phospholipids [63] and glycosphingolipids [106] differ among some species, but the role of dietary fatty acid intake on RBC membrane lipid content in this context is not conclusive. The quantity of unsaturated fatty acids in the RBC membrane of sheep has been observed to be twofold higher than in pig and horse [107]. This finding is surprising since sheep RBC have a lower elongation at high shear stress and a high concentration of unsaturated fatty acids of membrane phospholipids which should make the membrane more deformable. This discrepancy most likely reflects the several factors that affect RBC deformation in a shear field: membrane mechanical properties that are mainly determined by the membrane skeletal network and not by membrane lipid composition, surface area to cell volume ratio, and cytoplasmic viscosity. In another study in guinea pigs, RBC deformability remained unchanged in spite of a 50% decrease of membrane cholesterol after statin treatment [108]. Such studies indicate that differences in membrane lipid structures do not explain species specific differences in RBC deformability satisfactorily. A better insight would be provided by a systematic investigation of membrane skeletal proteins and their linkage to membrane proteins.

2.1.3. RBC Aggregation

RBC aggregation depends on cell deformability since it requires the formation of parallel membrane surfaces; poorly deformable RBC such as those containing a nucleus do not aggregate and thus almost all studies have been conducted using mammalian cells [58]. Values of aggregation range from a “hyperaggregating” group of animals (e.g., equine, rhinoceros), to a medium type (e.g., primates, pig, carnivora, many ruminants, elephants, rabbit), and to a “no” aggregation type group (e.g., cow, sheep, goat, camelids, rodents). However, as expected, exceptions will be found in future (see Table 1). Phocid seals show very unusual aggregation and will be discussed later. Interestingly, domestic bovines have very low, essentially non-measurable RBC aggregation in contrast to some wildlife relatives [32].

The diverse aggregation of cow, sheep, and goat against horse, donkey and zebra has been known for many decades [2]; aggregation in horse is so intense that even echinocytes are included into rouleaux [40]. Reasons for this diverse aggregation behaviour include variations in RBC deformability and membrane surface properties [109-111] as well as quantitative and qualitative differences in the plasma protein
A comparative study on cow, horse, and man recently showed a reduction of depletion layer thickness in cow, which could, according to the depletion model of aggregation (see II.4.b), explain the low aggregation of this species [41].

In comparing two species that reflect mammalian extremes of aggregation – horse and goat – one can postulate that in goat the distribution of RBC among a transverse section of the vessel should be more homogenous [113, 114]. Goat blood is more susceptible to turbulent flow due to the lower WBV which increases the Reynolds number [115]. This may be important for engineering studies where high Reynolds numbers are achieved, for instance during studies with mechanical devices for cardiac assist or for experimental vascular surgery. Formation of a marginal cell-free layer is decreased in non-aggregating blood and near-wall shear stress should be higher for this reason. However, one should be careful in applying results obtained in glass capillaries to in vivo conditions, since glass capillaries are over-simplified models of the circulatory system [116]. In contrast, in horse blood the parabolic velocity profile is expected to be blunted and the rate of axial migration is increased in vertical tubes. The readjustment of axial migration after junctions and branching should be quicker depending on the aggregation kinetics and tube geometries; flow partitioning at bifurcations might be increased.

There have been suggestions in the literature that athletic species are characterized by higher RBC aggregation [121]. This is true for certain species such as horse, dog, cat and antelope. However, although rodents are amongst the most active species, they have low RBC aggregation compared to larger athletic species. Again, one can speculate that this lower aggregation in rodents is consistent with shorter blood vessel lengths (i.e., body size) which prevent full development of RBC aggregates within a given vessel segment, thereby counteracting any hemodynamic advantage expected from RBC aggregation-related phenomena. In contrast, it is not possible to understand the physiological meaning of the absence of RBC aggregation in domestic bovine. The animal kingdom is full of extreme variability in RBC aggregation, even within a given species. For example, ringed seals blood exhibits no measurable aggregation while Weddell seals have very high RBC aggregation [35]. It follows from the above discussion that neither athletic capacity nor body size variations may fully explain the variations in RBC aggregation among species.

2.1.4. Unusual Mammalian Species

2.1.4.1. Camelids
Camel, dromedary, and llama have a low hematocrit resulting in a low whole blood viscosity [32], and their RBC aggregation is low (unpublished observation). Camelid RBC withstand high fluctuations of osmotic pressures during dehydration and rapid rehydration as a result of their adaptation to desert environments [122]. They also are resistant to metabolic depletion [104]. Camelid RBC are small, elliptical discs [47, 123, 124] that have an increased thickness in the central region in contrast to other mammals which show central depressions. Hemoglobin is homogenously distributed inside the cytoplasm with binding sites on band 3 protein, or, alternatively, is concentrated in crystals inside llama RBC [124]. Studies on camelid RBC membranes [125] in order to determine their principal properties have been carried out for some time. Key findings include: 1) RBC membranes of llama have increased amounts of sialic acid, and their glycoproteins differ qualitatively from human RBC membranes [104]; 2) Spectrin and
band 4.2 concentrations are equivalent to man [103], however, band 3 protein is increased threefold [104]; 3) The rotational and lateral mobility of band 3 is decreased even if parts of the membrane cytoskeleton are removed; 4) Band 3 protein has a tight and close connection to ankyrin and peripheral membrane proteins [105]; 5) Parts of a marginal band have been found [27]; 6) RBC deformability is different from other mammals due to structural reasons. However, further studies are still necessary to clarify the mechanism by which membrane rigidity is elevated in camel RBC.

2.1.4.2. Marine Mammals

Most knowledge in the field of hemorheology of this group of species has been obtained from seals. During submergence, characterized by apneic exercise with increased environmental pressure and decreased temperature, the cardiovascular adjustment and redistribution of blood flow in seals involves increased peripheral resistance and reduced cardiac output [126], termed the dive response. As an adaptation to long-term apnea, resting hematocrit, MCHC, MCV, and total blood volume are increased [88, 127]. To overcome the body’s O₂ demand during apnea, hematocrit increases further and can reach to values of 70% when the animal is diving. Although the spleen contributes to RBC storage effectively, time constants for hematocrit increase and splenic contraction favor the assistance of the hepatic sinuses for RBC delivery into the venous circulation [128]. The physiological polycythemia and the enhanced RBC hemoglobin concentration increase WBV and RBC density. The value of WBV increases with the hematocrit, however, the rise is lower than expected from terrestrial animals. O₂ transport capacity is improved for this reason [35, 88, 89]. During a dive, shear forces are reduced which affects WBV remarkably if RBC aggregation is present. Indeed, RBC aggregation reaches high values in Weddell seals, sometimes exceeding even those obtained for horse [19]. This high aggregation develops shortly after birth of pups [35]. RBC aggregation varies extremely between the species and can be ranked as follows: ringed seal < elephant seal < Weddell seal, with ringed seals showing no detectable RBC aggregation or blood sedimentation. A potential benefit of a high RBC aggregation as in Weddell seal or of “no” RBC aggregation like in the ringed seal cannot be elucidated as yet and need further investigations. The role of the more unsaturated fatty acid content in RBC membrane phospholipids in elephant and fur seals compared to man might indicate some trend toward increased RBC deformability [129].

Blood viscosity and RBC aggregation were also investigated in bowhead whales. These marine mammals also have high RBC aggregation both in autologous plasma and standard aggregation media [35]. In contrast, WBV was found to be lower than human samples when the hematocrit was adjusted to 50% for both species [33]. Additionally, bowhead whale blood exhibited significantly less temperature dependence compared to human blood, with smaller increments in WBV as the measurement temperature decreased to 5 °C [33].

2.2. Hemorheology of Other Vertebrates

Non-mammalian RBC differ structurally from mammalian cells. They contain a nucleus and most authors found a microtubular bundle connected to a membrane-associated cytoskeleton called a marginal band [3]. RBC are larger, elliptical, resistant to bending, and blood cell count is decreased. Nucleated RBC are believed to exhibit no aggregation [4, 58] although viscometric data show some degree of shear-thinning
which might be explained by some deformability and orientation of RBC under flow conditions. Studies with duck blood in glass tubes showed non-alignment between RBC and the axis of the tube [130]. This deviation was reduced at smaller tube diameters indicating better alignment with the streamlines. The authors concluded that the instability of cell orientation during blood flow may lead to an increase of viscous resistance. RBC membrane rigidity is considerably greater [18, 60, 99], although it is not clear which structures of the cell membrane are actually resisting deformation. Additionally, the nucleus is a relevant factor causing poor RBC deformability. Temperature of the environment and some forms of stress following adrenergic stimulation or hypoxia may decrease RBC deformability [3, 131].

The marginal band has been described in more detail in birds; it is found at the equator, near to the plasma membrane, in one plane only [21]. No microtubules were observed between the nucleus and the position of the marginal band in RBC of adult animals. The microtubules, whose main proteins are tubulins, curve with the profile of the cell. The number of microtubules in a RBC differ significantly among species, and has been found to vary with the size of the RBC [22]. Since polymerized actin co-localizes with the marginal band, an interaction between actin filaments and microtubules may exist [23, 24]. The response of intact marginal bands to bending, stretching and microtubule polarity has been tested in some amphibia [25, 26].

Blood viscosity in poikilotherm species is not a fixed value, but varies with the animal’s body temperature [67]. Therefore, the temperature dependence of whole blood and plasma viscosity, as well as other hemorheological values, are of interest in species adapted to low environmental temperatures. Since warming of such blood may damage red cells [18] by RBC swelling [83], comparative measurements should be made at a lower temperature. For a summary of literature containing hemorheologic data from non-mammalian vertebrates see table 1.

2.2.1. Birds

Avian RBC are spherical, lenticular, and somewhat flattened to produce an equator. They are able to deform in a specific way by folding along their major axis [130] and to orient in a shear field. The nucleus contains approximately 20% of the cytoplasmic volume; the cytoplasmic hemoglobin concentration is about 15-20% greater [1, 132] when compared with mammalian RBC and hence there is an increase of RBC density [20]. Hematocrit is lower than most mammals and hence blood viscosity is reduced; the reduction in WBV with increasing shear rate is very low in chicken blood compared to mammals. During disease [51, 133], and during development [54], blood and plasma viscosity values change as a result of variations of the animal’s hematocrit and plasma protein concentration. In broilers, a diurnal variation of whole blood viscosity was observed, influenced by the variation of environmental temperature and food and water intake [134]. Deformability of avian RBC is decreased compared to mammals [52, 130, 132], and differences in RBC rigidity is likely to exist between avian species since a difference has been found between layer and broiler chickens [53]. Despite relevant functional differences between avian and mammalian RBC, an adequate tissue O₂ supply has to be maintained in birds, since they have high metabolic rates which often exceed those of mammals.
2.2.2. Reptiles

Reptiles are poikilotherm animals, except for some metabolic intermediate species; tissue perfusion varies therefore in association with changes in ambient temperature. Blood viscosity may have a special effect on heat exchange by modulating skin blood flow in reptiles [67]. Hematocrit and mean cellular hemoglobin (MCH) are lowered, possibly associated with a reduced metabolic need of this group [1]. Whole blood viscosity is therefore lower than in mammals. Viscosity values vary with temperature [67, 70], however, a drop in temperature does not elevate viscosity as much as in mammals [62]. WBV in the painted turtle (Chrysema picta) is a function of body temperature: 3.5 mPa.s at 10°C and 1.5 mPa.s at 45°C [67]. In other turtles, plasma viscosity was found to be 1.32 mPa.s in Mauremys leprosa [62]. Others found blood viscosity values of Trachemys scripta at 20% hematocrit and 5°C of 9-12 mPa.s [68]. Blood viscosity is also decreased in Chelonia mydas hatchlings despite similar hematocrit values [69]. RBC deformability is decreased compared to mammals and values of RBC membrane shear elastic modulus are presented by Waugh [60].

Turtles are interesting species in view of their cold adaptation during hibernation. In Trachemys scripta, hematocrit is essentially maintained during cool room adaptation, leading to an increase in blood viscosity. However, hematocrit standardized values of blood viscosity were lower in cold adapted than in room adapted animals [68]. During hibernation of Sternotherus odoratus submerged in water at 5°C for five months, hematocrit increased but WBV did not as might be expected based on the hematocrit increase [135], thus indicating a possible adaptation to lower temperature.

2.2.3. Amphibians

Blood viscosity of frog and toad show hematocrit (r=0.93) and temperature (r=0.96) dependency as in other species [67, 75, 77], with the temperature dependency of blood viscosity in the bullfrog reported to resemble that in mammals [73]. Blood viscosity was reported to vary between 2.5 (toad at 27°C) [72] and 4 mPa.s (bullfrog at 5°C and 150 s⁻¹) [74]. In vivo hematocrit varies with the environmental temperature, leading to a seasonal variation in blood viscosity. Experimental data on American bullfrog showed that hematocrit and RBC count increased when the temperature of the animals’ environment was decreased to 5°C, whereas MCV decreased under these circumstances. In this circumstance hematocrit and blood viscosity did not vary significantly, however, mean viscosity values, measured at native hematocrit, increased slightly [74]. The relevance of blood viscosity for blood flow has been investigated for toads: systemic blood flow decreased as WBV increased. [77] However, general anesthesia during such studies might modify regulatory processes that maintain blood flow.

2.2.4. Fish

Fish RBC are elliptical cells which bulge in the region of their nucleus. The largest RBC volumes can be found in this class [4]. An excess of surface area is present which enables RBC to enter small capillaries of 3μm diameter. However, the shear elastic modulus was reported to be significantly higher for fish RBC and capillary entry time and transit time is increased significantly compared to man [18]. Hematocrit in fishes drops with water temperature, but it is believed that the O₂ demand is satisfied due to the increased O₂ concentration in cold water and the reduced metabolic need of the
animal in a cold environment. This hematocrit dependency on temperature should lead to diversities in blood viscosity of fishes living at different regions. Some studies focused on RBC deformability, and showed that membrane rigidity in trout was dependent on the temperature [18]. In temperature-acclimated trout, however, pipette entry time of RBC membranes has been shown to be maintained [92].

A special focus in fish hemorheology is the blood and plasma viscosity of ice fishes. These animals live at an environmental temperature down to -2 °C which should result in high viscosity values. However, some of these fish have blood with a very low cellular content which thus lowers viscosity. Antarctic ice fish show an increase of whole blood viscosity with a temperature drop from 5 to -1 °C, with the thermal sensitivity of WBV in fish being reduced compared to man. Blood of white-blooded (i.e., almost no red blood cells) fish (Chaenocephalus kathleenae) showed nearly Newtonian behavior [92], and the plasma viscosity of C. aceratus was comparable to human plasma at low temperatures. Plasma viscosity of another Antarctic ice fish with low hematocrit (7.5%) and low MCHC, Notothenia sp., was significantly lower than the value of Chaenocephalids; however, its blood viscosity was increased due to the presence of blood cells [90].

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