Whole blood viscosity, plasma viscosity and erythrocyte aggregation in nine mammalian species: reference values and comparison of data

U. Windberger *, A. Bartholovitsch, R. Plasenzotti, K. J. Korak
and G. Heinze †

Institute for Biomedical Research and † Institute for Computer Sciences, University of Vienna, Währingergürtel 18-20, A-1090 Vienna, Austria

In this study species-specific values for whole blood viscosity (WBV), plasma viscosity (PV) and erythrocyte aggregation (EA) were determined in a total of 360 animals. We used 40 individual adult animals of nine mammalian species: horse, pig, dog, cat, rat, cattle, sheep, rabbit and mouse. WBV measurements were carried out using a LS30 viscometer, PV was measured using OCR-D and EA was measured using a Myrenne aggregometer and the LS30 (aggregation index at low shear rate). At low shear rates ($0.7\ s^{-1}$ and $2.4\ s^{-1}$) haematocrit (Hct)-standardized (40 % Hct) samples showed a higher value of WBV and EA in horse, pig, dog and cat. In cattle, sheep, rabbit and mouse, EA and WBV were markedly decreased and EA was almost undetectable, although the plasma fibrinogen concentration was higher in these animals. Rats showed the highest WBV at low shear rate in native blood and WBV was not different from horse in Hct-standardized blood; however, EA was very low in the rat, a result that might be explained by mechanical or geometrical properties of the red blood cell. EA correlated with the plasma protein concentration in each species except dog and mouse. In horse, cattle and pig, EA correlated with the plasma fibrinogen concentration. At high shear rate ($94\ s^{-1}$), WBV was higher in cattle than cat and rat, and dog had higher values than horse, suggesting specific interspecies differences depending on low shear and high shear values of WBV, as a result of mechanisms that influence RBC flexibility. PV was highest in cattle and lowest in rabbit and mouse and did not correlate with WBV. Haemorheological parameters differed between the species. Each species has its own rheological fingerprint. The physiological significance of these variations among mammalian species has not yet been established. Viscosity contributes to endothelial cell shear stress. While haemorheological parameters differ across the species it may be postulated that factors influencing flow-mediated endothelial cell signal transduction are different among the species. *Experimental Physiology* (2003) **88.3**, 431–440.

Much interest has focused on comparative haemorheological studies (Amin & Sirs, 1985; Johnn *et al.* 1992; Waugh, 1992; Windberger *et al.* 1993/1994; Kumaravel & Singh, 1995; Baskurt, 1996; Baskurt *et al.* 1997, 2000; Bäumler *et al.* 2001). Despite being similar in basic structure and serving equally important physiological functions in all mammals, red blood cells differ vastly in their rheological properties in different species. Comparative studies are designed to give relevant information on the structural and functional basis of red blood cell rheological behaviour itself. Information gathered in comparative studies can thus serve as a basis for experimental models investigating flow phenomena in different animal species.

Blood viscosity contributes to endothelial shear stress (Barakat, 2001). Shear stress modulates the orientation of

endothelial cells in the direction of flow and to the 'waviness' of the luminal surface of the vessel (Davies, 1995). In addition, shear stress influences the arterial diameter (Johnson *et al.* 1981) through the release of endothelial vasoactive factors such as nitric oxide, endothelin-1 and prostaglandin I_2 (Frangos *et al.* 1985; Moncada *et al.* 1991; Malek & Izumo, 1992) and affects arterial remodelling (Langille & O'Donnel, 1986; Sumpio *et al.* 1990). However, endothelial shear stress, which is influenced by fluid viscosity and blood flow, might not be uniform between the animal species. In diseases with elevated levels of whole blood and plasma viscosity (Linke, 1996), endothelial shear stress will be even further altered.

The available data are so far only of limited use. This is partly due to the small number of animals and species

* Corresponding author: ursula.windberger@akh-wien.ac.at

evaluated in recent studies. Comparison of viscometric measurements is further complicated by differences in methodology among the individual studies as well as species-related factors.

In the following study we describe the rheological properties of blood from mammalian species that are of interest in veterinary medicine and are commonly used in experimental research. A total of 360 animals were used in this study. Blood from 40 animals of nine species was analysed employing the same protocol for all animals. It was thus possible to standardize the shear history for each sample and, at the same time, keep procedures at a minimum. Measurements were carried out by experienced investigators. Incomplete data sets were eliminated from the study. In addition to our reference values for standard haemorheological parameters, a complex comparison of whole blood viscosity, plasma viscosity, erythrocyte aggregation and related parameters is presented.

METHODS

Withdrawal of blood

Blood was withdrawn by venipuncture from healthy adult horses (Warmblut; 28 gelding, 12 mare; age, 7–13 years), pigs (28 Edelschwein × Pietrain, 12 Landschwein × Edelschwein; 8 male (castrated), 32 female; age, 5–12 months), dogs (24 beagle, 4 German shepherd dogs, 3 golden retriever, 1 boxer, 1 labrador retriever, 1 greyhound, 1 Hovawart, 1 Jack-Russell terrier, 2 collies, 2 Doberman pinschers; 17 male (6 castrated), 23 female (10 ovariectomized); age, 2–10 years), cats (37 European shorthair, 2 Siamese, 1 Karthäuser; 15 male (14 castrated), 25 female (18 ovariectomized); age, 1–11 years), cattle (34 Fleckvieh, 6 Braunvieh; 40 female; age, 2–4 years), sheep (27 Bergschaf, 13 Milchschaf; 40 female; age, 2–5 years) and rabbits (37 New Zealand White, 3 chinchilla bastard; 12 male, 28 female; age, 5–13 months). Rats (Wistar, 9 male, 31 female; age, 4–6 months) and mice (BALB/c, 7 male, 33 female; age, 4–8 months) were anaesthetized with 100 mg kg^{-1} ketamine and 1 mg kg^{-1} xylazine intraperitoneally before blood was taken by puncture of the right ventricle. No muscle relaxant drug was used. Rats and mice were killed after blood withdrawal by an I.V. bolus of 100 mg kg^{-1} thiopentone. Blood from horses, pigs, dogs, cats, cattle, sheep and rabbits flowed passively into two standard tubes containing K-EDTA. Carefully avoiding heavy suction, blood from rats and mice was withdrawn using 5 ml and 1 ml syringes, respectively. Blood samples from pigs, dogs, cats, sheep, rabbits, rats and mice were immediately placed in a centrifuge at room temperature and analysed haematologically after 1 h. However, all haemo-rheological and haematological measurements were completed within 4 h of blood sampling.

All animal experiments were carried out according to national guidelines (national approval number: GZ-Nr: 66.009/16-Pr/4/2000).

Haematological measurements

A Cell-Dyn 3500 (Abbott Laboratories, Abbott Park, IL, USA) was used for the following haematological measurements: red blood cell (RBC) count (cells × 10^6 ml^{-1}), haemoglobin concentration (g dl^{-1}), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g dl^{-1}), white blood cell (WBC) count (cells × 10^3 ml^{-1}) and platelet count (cells × 10^5

ml^{-1}). Haematocrit (Hct, %) was measured by centrifugation (Hettich, Tuttlingen, Germany).

Haemorheological measurements

Haemorheology was carried out using an LS30 viscometer (Contraves AG, Zürich, Switzerland) at three different shear rates (0.7, 2.4 and 94 s^{-1}) at 37 °C, to obtain whole blood viscosity (WBV, mPa s) and using a Myrenne aggregometer MA1 (Myrenne GmbH, Roetgen, Germany) to measure erythrocyte aggregation at room temperature (aggregation index: M0, M1). Aggregation was not checked microscopically.

Principles of measurement of aggregation:

(1) Contraves LS30. A 1 ml sample of blood is placed into the modified Couette co-axial cylinder system of the device. When the outside cylinder is rotated the viscosity of blood, which exerts mechanical strain on the freely movable inner cylinder, is measured electromagnetically. Shear rates of 0.7, 2.4 and 94 s^{-1} were used in this study. Three consecutive readings were taken for each value.

(2) Myrenne aggregometer MA1. A 30 μl sample of blood is placed between the transparent cone-plate shearing device and is spread to a cellular layer. This layer is sheared at 600 s^{-1} for 10 s to disaggregate all pre-existing aggregates, then abruptly stopped in M0 mode or rotated at 3 s^{-1} in M1 mode for another 10 s to allow aggregation. The extent of plasma gaps inside the whole blood monolayer allows more light to pass through the red cell suspension. The instrument computes an aggregation index (M0 at stasis and M1 at 3 s^{-1}) proportional to the area under the light transmission curve, which reflects the degree of aggregation attained by the end of the 10 s period (Nash *et al.* 1987). At least five readings were taken for each value of M0 and M1.

An additional aggregation index (AI) was obtained using the LS30 viscometer during the measurement of whole blood viscosity at low shear rates (0.7 and 2.4 s^{-1}) by the following procedure: after measurement of the WBV at 0.7 and 2.4 s^{-1}, the cup was further rotated at the same speed for 45 s. After 45 s the WBV was read again. AI was determined by the formula:

$$AI = (peak\ WBV - WBV\ after\ 45\ s)/peak\ WBV.$$

Calculation of AI by this formula is based upon the hypothesis that the peak value of WBV represents the characteristic viscosity of the blood sample at the given shear rate, whereas the secondary decline is thought to be affected by axial migration of red cell aggregates from the wall of the viscometer which is a measure of erythrocyte aggregation (Johnson *et al.* 1999).

Plasma viscosity (PV) was measured using OCR-D (oscillating capillary rheometer and densitometer; Paar, Graz, Austria) at 21 °C. In mouse, blood was pooled from 5–10 animals after the end of the rheological measurement and plasma viscosity was carried out from this pooled plasma.

Principles of measurement of plasma viscosity:

OCR-D. A plastic cup containing 1 ml plasma is mounted at the bottom of a glass capillary (diameter, 0.9864 mm; length, 100 mm). The plastic cup is compressed at 2 Hz exerting shear rates inside the capillary. The viscosity at the shear rate of 10 s^{-1} was determined.

Whole blood was tested first at its native haematocrit. To compare the values among the species, another portion of blood from all species except mouse was adjusted to a haematocrit of 40 % and analysed. Haematocrit standardisation was carried out by centrifugation (Rotanta RPC, Hettich, Tuttlingen, Deutschland: 2000 U min^{-1}, corresponding to RZB (relative

Table 1. Haematology, fibrinogen concentration and total protein concentration in blood from horse, pig, dog, cat, rat, cattle, sheep, rabbit and mouse

	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g dl^{-1})	Fibrinogen (mg dl^{-1})	Total protein (g dl^{-1})
Horse	35 (33/38)	45 (43/46)	17.4 (16/18)	38.8 (38/39.3)	155 (143/172)	6.1 (5.88/6.45)
Ref.	32–46	37–55	13–19	31–35	150–330	5.5–7.5
Pig	33 (29.5/40)	54 (52/61)	18.6 (18/20)	33.4 (32.6/34.3)	172 (145/260)	4.9 (4.6/5.9)
Ref.	30–42	50–68	17–23	30–40	160–390	–8.6
Dog	45 (40/52)	61 (59/62)	23.7 (23.2/24)	38.6 (38/39.4)	182 (146/235)	6.6 (6.2/6.9)
Ref.	37–55	60–77	19–23	31–34	120–290	5.4–7.5
Cat	40 (39/42)	39.3 (37.7/41)	13.9 (13.2/14.4)	35.1 (34.3/35.8)	186 (142/230)	7.2 (7/7.4)
Ref.	27–47	40–55	13–17	31–35	100–300	5.7–9.4
Rat	43 (40/44)	47 (46/48)	17.6 (17.1/17.9)	37.9 (37/38.2)	233 (215/253)	6.1 (5.4/6.5)
Ref.	35–44	50–57	18–23	36–45	—	—
Cattle	30 (27/32)	51.5 (50/54)	17.8 (17.1/18.6)	34.6 (34.3/35)	309 (252/383)	7.2 (7/7.6)
Ref.	30–40	40–60	14–19	26–34	160–560	6.0–8.0
Sheep	33 (30.8/38)	29 (27.6/30)	10.5 (10/10.8)	35.7 (35/36.3)	282 (211/360)	6 (5.6/6)
Ref.	28–39	23–48	9–13	29–38	—	5.5–7.5
Rabbit	39 (38/40)	62 (61/63.6)	21.3 (20.8/22)	33.8 (32.6/36.1)	286 (223/314)	5.6 (5.4/6)
Ref.	37–43	60–67	20–22	32–34	—	—
Mouse	40 (38.8/42)	45.5 (43.3/46.8)	16 (15/16.5)	34.8 (33.6/35.1)	283 (277/329)	4.3 (4/4.5)
Ref.	39–47	44–53	15–19	34.5–37.5	—	—

Data are expressed as median (25th/75th percentiles). Reference values from Frith *et al.* (1980); Charles River Baseline Hematology (1982); Kraft & Dürr (1997).

centrifugal acceleration) = 595, for 10 min), plasma separation and resuspension of the blood cell concentration in autologous plasma to the desired haematocrit. Blood from mouse was tested only at its native haematocrit.

Additional measurements

Fibrinogen concentration was measured according to the method of Clauss. To evaluate the health status of the animals, routine blood chemical measurements (Hitachi 904, Hitachi, Tokyo, Japan) were assessed for each sample except for mouse. In mouse, following the withdrawal of blood for haemorheological measurements, a small amount of blood was pooled from 5–10 animals to obtain fibrinogen concentration and blood chemistry values.

Protocol of measurements

After withdrawal of whole blood, the haematological measurements were made and one blood sample in a K-EDTA-containing tube was centrifuged for haematocrit adjustment. Meanwhile, whole blood viscosity at the native haematocrit and erythrocyte aggregation were measured. Then the same haemorheological parameters were evaluated using standardized blood. Blood from individual animals was pooled and centrifuged, and plasma viscosity was measured on the next day (plasma sample stored at 4 °C). By using this protocol, a maximum of 10 samples could be measured within 4 h of withdrawal of blood.

Statistics

Descriptive analysis of native and haematocrit-standardized data was performed according to the method of Harrell & Davis (1995). Data are presented as median and 25th and 75th percentile. Values within this percentile range were used as 'reference values'. Differences between the animal species were

evaluated from data obtained from the standardized blood samples only and were calculated first using the Kruskal-Wallis test. In a second step, pairwise comparisons of all species were performed using the Wilcoxon rank sum test. The resulting *P* values were corrected by the Bonferroni-Holm procedure (Holm, 1979). Correlations between haemorheological data and total protein concentration, fibrinogen concentration, MCV and MCHC were assessed by the Spearman correlation coefficient, *r*. *P* values were corrected using the Bonferroni-Holm procedure. Values of $P < 0.05$ were considered statistically significant. The software SAS System V 8.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

We are well aware that the term 'reference value' is difficult to apply to the haemorheological values determined in this study due to the inherent variations in methodology among different laboratories. The established numerical values are therefore used as 'laboratory-specific' reference values. However, the consistency of the applied procedures in combination with the experience of the investigators justifies the interspecies comparison of the gathered data.

RESULTS

Haematological measurements

All values were within physiological limits. MCV was greatest in rabbit followed by pig, cattle, rat, mouse, horse and sheep. MCH was greatest in rabbit followed by dog, pig, cattle, rat, horse, mouse, cat and sheep. MCHC was greatest in horse followed by dog, rat, sheep, cat, mouse, cattle, pig and rabbit. Table 1 shows the haematological

Table 2. Whole blood viscosity (standardized blood samples, 40 % Hct) and plasma viscosity of horse, pig, dog, cat, rat, cattle, sheep, rabbit and mouse

	WBV ($0.7\ s^{-1}$) (mPa s)	WBV ($2.4\ s^{-1}$) (mPa s)	WBV ($94\ s^{-1}$) (mPa s)	PV (mPa s)
Horse	38.173 (31.657/42.547)	20.179 (17.227/24.384)	5.175 (4.795/5.512)	1.66 (1.57/1.84)
Pig	24.692 (17.262/31.033)	14.861 (11.786/19.205)	4.938 (4.337/5.500)	1.58 (1.40/1.69)
Dog	22.885 (18.819/27.752)	14.584 (12.358/17.476)	5.591 (4.939/6.689)	1.61 (1.57/1.69)
Cat	30.194 (26.899/33.971)	15.349 (13.871/17.251)	4.442 (4.183/4.671)	1.71 (1.62/1.78)
Rat	35.400 (26.335/40.902)	19.748 (16.038/22.111)	6.288 (5.883/6.920)	1.59 (1.55/1.65)
Cattle	6.556 (6.015/7.349)	6.290 (5.487/6.949)	4.799 (4.483/5.142)	1.72 (1.63/1.94)
Sheep	6.587 (5.850/7.192)	6.092 (5.731/6.793)	4.373 (4.095/4.682)	1.49 (1.44/1.57)
Rabbit	8.313 (6.163/10.243)	7.442 (6.351/8.713)	4.043 (3.750/4.375)	1.30 (1.24/1.41)
Mouse	13.367 (10.691/16.568)	10.563 (8.929/12.289)	4.879 (4.506/5.345)	1.31 (1.29/1.34)
Man	*33.487 (26.264/39.584)*	*18.529 (15.729/21.493)*	*5.996 (5.140/6.938)*	*1.24 (1.20/1.31)*

Data are expressed as median (25th/75th percentiles). Data from man were included from our own unpublished investigations.

data from the tested animal species, with reference values from healthy animals.

Haemorheological measurements

'Reference' values are presented in Table 2 (viscometric data of standardized blood samples) and Table 3 (erythrocyte aggregation data of standardized blood samples). Due to the number of animals (40 per species) the 25th and 75th percentile distance was taken as the reference range.

At a standardized haematocrit value, whole blood viscosity at the three different shear rates, the aggregation indices and plasma viscosity were different among the animal species, as identified by the Kruskal-Wallis test. Within the

Figure 1

Plasma viscosity (PV; mPa s) in blood from horse (ho), pig (pi), dog (do), cat (ca), rat (rt), cattle (ct), sheep (sh), rabbit (rb) and mouse (m). The graph shows the median value and the 25th and 75th percentiles. The error bars show the highest and the lowest value inside the 1½ interquartile range. The circles show data that are outside of this range. PV is highest in cattle and lowest in rabbit and mouse.

data some parameters from the various animal species were not significantly different following Bonferroni-Holm correction. All data are presented as median with the 25th and 75th percentiles in parentheses.

Figures 1 to 5 show the values of PV, WBV at $0.7\ s^{-1}$, WBV at $94\ s^{-1}$, AI at $2.4\ s^{-1}$ and M0 of the standardized samples, respectively.

Differences in plasma viscosity

The values of PV were in the range 1.30 (1.24/1.41) to 1.72 (1.63/1.94) in all the species tested. The highest value was measured in blood from cattle, followed by cat, horse, dog, rat, pig, sheep, mouse and rabbit. There were two groups of animals. In the first group, the plasma viscosity was higher (cattle, cat, horse, dog, rat and pig) than in the other group (sheep, mouse and rabbit) (see Fig. 1).

Differences in whole blood viscosity at low shear rate

Shear rate $0.7\ s^{-1}$. The values of WBV were in the range 6.556 (6.015/7.349) to 38.173 (31.657/42.547) in all the species tested. The highest value was measured in blood taken from horse followed by rat, cat, pig, dog, mouse, rabbit, sheep and cattle (see Fig. 2).

Shear rate $2.4\ s^{-1}$. The values of WBV were in the range 6.092 (5.731/6.793) to 20.179 (17.227/24.384) in all the species tested, and the species distribution followed the same pattern as above.
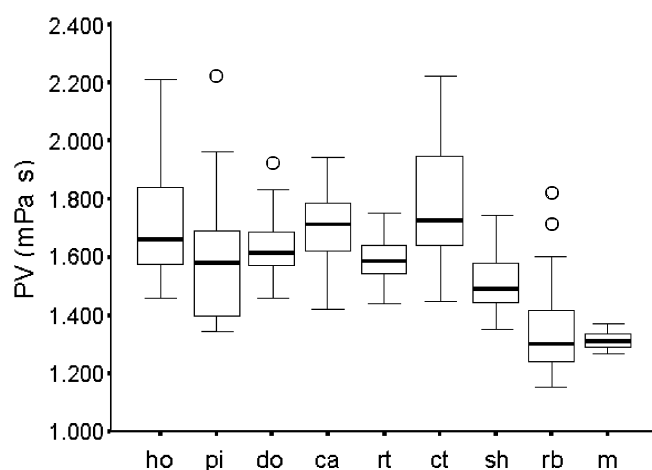
Differences in whole blood viscosity at high shear rate ($94\ s^{-1}$)

The values of WBV were in the range 4.043 (3.750/4.375) to 6.288 (5.883/6.920) in all the species tested. The highest value was measured in blood from rat followed by dog, horse, pig, mouse, cattle, cat, sheep and rabbit (see Fig. 3).

Differences in erythrocyte aggregation

Aggregation index (AI) measured using the LS30 viscometer:

AI at shear rate $0.7\ s^{-1}$. The values of AI were in the range 0.0 (0.0/0.0) to 0.30 (0.27/0.36) in all the species tested.

Table 3. Erythrocyte aggregation (standardized blood samples, 40 % Hct) in blood from horse, pig, dog, cat, rat, cattle, sheep, rabbit and mouse

	AI ($0.7\ s^{-1}$)	AI ($2.4\ s^{-1}$)	M0	M1
Horse	0.30 (0.27/0.36)	0.32 (0.27/0.37)	13.2 (11.7/14.5)	57.6 (44.0/67.7)
Pig	0.05 (0.02/0.08)	0.04 (0.02/0.6)	3.7 (2.2/4.6)	26.2 (20.9/31.4)
Dog	0.07 (0.05/0.13)	0.11 (0.07/0.18)	3.0 (2.7/4)	23.5 (21.1/27.9)
Cat	0.17 (0.13/0.21)	0.10 (0.07/0.14)	5.2 (4.7/6.3)	29.6 (22.8/40.1)
Rat	0.01 (0/0.04)	0.01 (0/0.04)	0.7 (0.3/1.2)	2.4 (1.5/4.2)
Cattle	0 (0/0)	0 (0/0)	1.7 (1.1/2.2)	11.6 (8.3/14.4)
Sheep	0.01 (0/0.02)	0 (0/0.01)	0 (0/0)	6.6 (3.5/8.8)
Rabbit	0.02 (0.01/0.03)	0 (0/0)	0 (0/0.1)	13.3 (8.2/17.2)
Mouse	0 (0/0)	0 (0/0)	0.2 (0.1/0.3)	0.6 (0.5/0.9)
Man	*0.09 (0.08/0.11)*	*0.10 (0.06/0.14)*	—	—

Data are expressed as median (25th/75th percentile). Data from man were included from our own unpublished investigations.

The highest value was measured in blood from horse followed by cat, dog and pig. The values in blood from rabbit, mouse, rat, cattle and sheep showed nearly no measurable aggregation index.

AI at shear rate $2.4\ s^{-1}$. The values of AI were in the range 0.0 (0.0/0.0) to 0.32 (0.27/0.37) in all the species tested. The highest value was measured in blood from horse followed by dog, cat and pig. Almost no aggregation index was measured in blood from rabbit, mouse, rat, cattle and sheep (see Fig. 4).

Aggregation index measured using the Myrenne aggregometer:

M0. The values of AI were in the range 0 (0/0) to 13.2 (11.7/14.5) in all the species tested. The highest value was measured in blood from horse followed by cat, pig, dog, cattle, rat, mouse, rabbit and sheep (see Fig. 5).

M1. The values of AI were in the range 0.6 (0.5/0.9) to 57.6 (44.0/67.7) in all the species tested. The highest value was measured in blood from horse followed by cat, pig, dog, rabbit, cattle, sheep, rat and mouse.
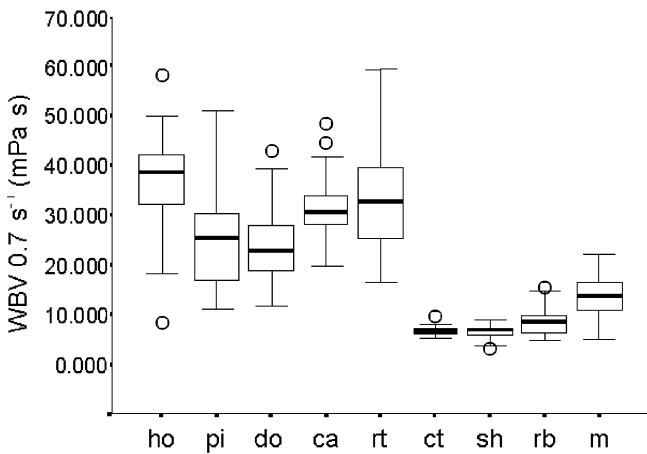
Figure 2

Whole blood viscosity at low shear rate (WBV at $0.7\ s^{-1}$; mPa s) in standardized blood samples (Hct = 40 %) from 9 mammalian species. Abbreviations as in Fig. 1. The graph shows the median value and the 25th and 75th percentiles. The error bars show the highest and the lowest value inside the 1½ interquartile range. The circles show data that are outside of this range. Horse, pig, dog, cat and rat show elevated WBV, whereas cattle, sheep, rabbit and mouse have very low WBV at low shear rate.
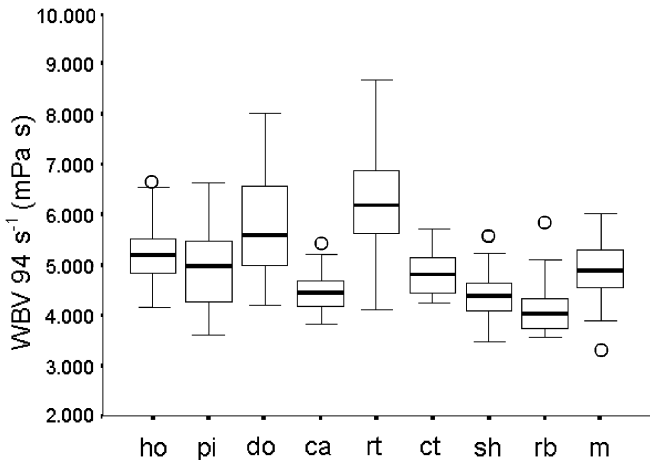
Figure 3

Whole blood viscosity at high shear rate (WBV at $94\ s^{-1}$; mPa s) in standardized blood samples (Hct = 40 %) from 9 mammalian species. Abbreviations as in Fig. 1. The graph shows the median value and the 25 % and 75 % percentile. The error bars show the highest and the lowest value inside the 1½ interquartile range. The circles show data that are outside of this range. Differences in WBV at high shear rate are not as prominent as differences at low shear rate. Rat shows the highest WBV at this shear rate.

Table 4. Results of the correlation tests

	Horse	Pig	Dog	Cat	Rat	Cattle	Sheep	Rabbit	Mouse
TP *vs.* PV	—	$r = 0.56$ $P < 0.01$	—	—	—	—	$r = 0.37$ $P < 0.05$	—	$r = 0.76$ $P = 0.06$
Fibrinogen *vs.* PV	—	$r = 0.66$ $P < 0.01$	$r = 0.54$ $P < 0.05$	—	—	—	—	—	—
TP *vs.* WBV (0.7 or 2.4 s^{-1})	$r = 0.48$ $P < 0.05$	$r = 0.54$ $P < 0.01$	—	$r = 0.47$ $P < 0.05$	—	$r = 0.55$ $P < 0.01$	$r = 0.38$ $P < 0.05$	$r = 0.45$ $P < 0.05$	$r = 0.41$ $P = 0.08$
TP *vs.* AI	—	—	—	—	$r = 0.48$ $P < 0.05$	—	—	—	—
TP *vs.* WBV (94 s^{-1})	—	$r = 0.46$ $P < 0.05$	—	$r = 0.46$ $P < 0.05$	—	$r = 0.62$ $P < 0.001$	$r = 0.38$ $P = 0.05$	—	—
Fibrinogen *vs.* WBV (0.7 or 2.4 s^{-1})	$r = 0.43$ $P < 0.05$	$r = 0.54$ $P < 0.01$	—	—	—	$r = 0.42$ $P < 0.05$	—	—	—

r values are given where there is significant correlation. Note that a strong correlation coefficient in mouse was associated with a low *P* value due to the small sample size. TP, total protein; PV, plasma viscosity; WBV, whole blood viscosity; AI, aggregation index.

Relationships between the parameters within the animal species

In all species, except dog, a positive correlation was found between the plasma total protein concentration and parameters related to erythrocyte aggregation, such as WBV at low shear rate and aggregation indices, both native and standardized. In mouse, a trend was observed suggesting a relationship between total protein concentration and WBV at low shear rate in native blood. In dog and pig, a positive correlation was found between the plasma fibrinogen concentration and the plasma viscosity. Positive correlation of fibrinogen concentration with parameters which indicate RBC aggregation were found in horse, cattle and pig. These results are summarized in Table 4.
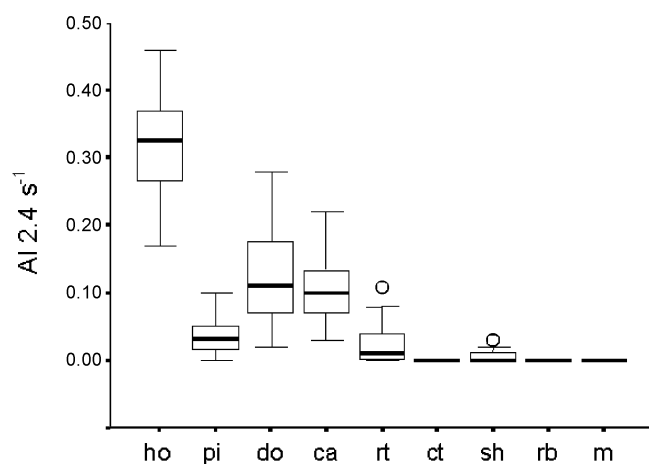

Figure 4

Aggregation index measured using the LS30 viscometer (AI at 2.4 s^{-1}) in standardized blood samples (Hct = 40 %) from 9 mammalian species. Abbreviations as in Fig. 1. The graph shows the median value and the 25th and 75th percentiles. The error bars show the highest and the lowest value inside the 1½ interquartile range. The circles show data that are outside of this range. Horse shows intensive erythrocyte aggregation. Aggregation was low or almost unmeasurable in rat, cattle, sheep, rabbit and mouse.
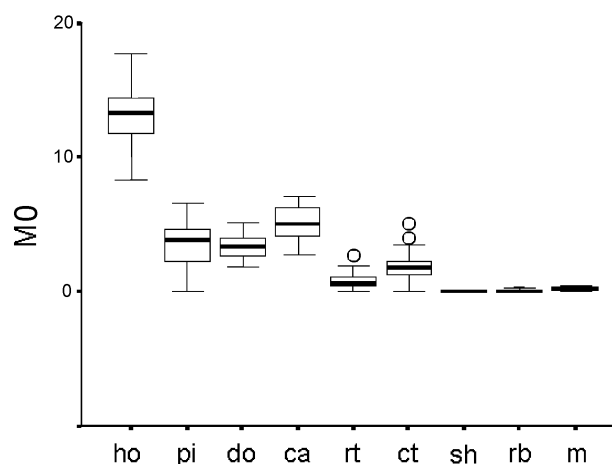

Figure 5

Aggregation index measured by Myrenne aggregometer (M0) in standardized blood samples (Hct = 40 %) from 9 mammalian species. Abbreviations as in Fig. 1. The graph shows the median value and the 25th and 75th percentiles. The error bars show the highest and the lowest value inside the 1½ interquartile range. The circles show data that are outside of this range. There is intensive erythrocyte aggregation in horse, but low erythrocyte aggregation in rat, cattle, sheep, rabbit and mouse.

Haemorheological values correlated with erythrocyte indices in only a few of the animal species. In dog, MCHC correlated significantly with the aggregation index AI ($r = 0.476$, $P < 0.01$) and in rabbit, MCHC correlated with the aggregation index M0 ($r = 0.467$, $P < 0.05$). No correlation was found between the MCV and haemorheological parameters.

Additional measurements

Plasma total protein and fibrinogen concentration, as well as haematocrit were different among the animals tested (see Table 1). Cattle demonstrated the highest total protein. Cattle also demonstrated the highest fibrinogen concentration, followed by rabbit, mouse, sheep, rat, cat, dog, pig and horse. Species with low erythrocyte aggregation had higher plasma fibrinogen concentrations. Dog showed the highest haematocrit followed by rat, mouse, cat, rabbit, horse, pig, sheep and cattle.

Blood chemistry data were within normal ranges (Kraft & Dürr, 1997) in all the animals. Values of the plasma fibrinogen and total protein concentration of animals are included in Table 1.

DISCUSSION

The values of whole blood viscosity, erythrocyte aggregation and plasma viscosity differed among the mammalian species investigated. Resembling a fingerprint, each species had its own individual haemorheological characteristics. In the following discussion, these individual characteristics as well as the respective variations among the species will be considered.

Differences in WBV at low shear

Based on the WBV under low shear, the animals could be divided into two groups: one group with an elevated WBV including horse, rat, pig, dog and cat, and a second group with a low WBV including cattle, sheep, rabbit and mouse.

It is generally accepted that pronounced red cell aggregation is a major factor contributing to increased whole blood viscosity at low shear. In eight out of nine species this assumption could be verified. However, in rats our finding of high WBV values in combination with a low erythrocyte aggregation contrasted with this general assumption despite the fact that erythrocyte aggregation was measured by two different methods. A slightly elevated WBV despite a decreased erythrocyte aggregation in rats was also observed by Baskurt *et al.* (1997). It seems that the extent of erythrocyte aggregation does not influence the high WBV at low shear in the rat. Another possible parameter affecting WBV is the haematocrit. In this study the possible influence of the haematocrit on WBV was eliminated by standardizing the blood samples. When these standardized blood samples were compared there was still no difference between the WBV of rat and horse, the latter having pronounced WBV and high erythrocyte aggregation. Plasma viscosity, total protein concentration and fibrinogen concentration further influence WBV. However, these parameters were not increased in the rat. In order to

exclude possible bias, a second series of 40 rats was tested by different investigators. The results of this second series supported the findings of our first series. Increasing the shear rate led to a decline in WBV, but WBV at 94 s^{-1} was still higher in the rat than in the other animals. It seems that mechanisms other than erythrocyte aggregation, haemoconcentration or suspending phase viscosity are responsible for the high WBV in the rat. These mechanisms might include mechanical or geometrical properties of the red blood cell (Meiselman, 1978).

Differences in erythrocyte aggregation

Erythrocyte aggregation was increased in horse, pig, dog and cat, whereas erythrocyte aggregation in rat, cattle, sheep, rabbit and mouse was low or undetectable. Interestingly, animals with low erythrocyte aggregation tended to have a higher plasma fibrinogen concentration than animals with high red cell aggregation. As expected, erythrocyte aggregation within a species correlated with the plasma concentration of total protein. This correlation was not found in the dogs investigated in this study, probably due to the differences in breed, age and sex amongst the animals in this group. These factors have been correlated with notable differences in haemorheological variables among individuals of the same species in the past (Bodey & Rampling, 1998).

In horse, cattle and pig, a positive correlation was found between the plasma fibrinogen concentration and parameters that indicate erythrocyte aggregation. In the other species, erythrocyte aggregation and fibrinogen did not correlate. Nevertheless, the lack of methodologically dependent numerical correlation does not indicate an absence of a relationship between these parameters. The measure of fibrinogen concentration depends on various factors such as methods of blood sampling or blood storage. Furthermore, the observed aggregation index was sometimes small to start with and a potential relation between these parameters was therefore difficult to establish.

The origin of red blood cell aggregation is complex and can be explained by two theories: the bridging theory (Chien & Sung, 1987; Brooks, 1988) and the more recently proposed depletion theory (Bäumler *et al.* 1999; Armstrong *et al.* 1999). Aggregation takes place at low or zero shear conditions. Plasma factors such as the concentration of the macromolecules fibrinogen or dextran and cellular factors including the affinity of the red cell for macromolecules, geometric factors, red cell surface charge and the membrane and cytoskeletal composition all influence red blood cell aggregation.

The physiological function of erythrocyte aggregation and its haemorheological effects on blood flow are still controversial. For example, in a feline gastrocnemius muscle model (Cabel *et al.* 1997), an increase in erythrocyte aggregation was associated with an increase in venous flow resistance. These findings were supported by a study which showed that red blood cell aggregation led to increased venous flow resistance as arterial pressure and flow rate

were reduced (Bishop *et al.* 2001). In contrast, when human blood was used in a guinea pig model, an increase in red cell aggregation was related to a decrease in venous flow resistance (Baskurt *et al.* 1999).

Studies with hyperaggregating blood indicate that axial migration is larger and directly related to the aggregation tendency of the blood (Reinke *et al.* 1986; Cokelet & Goldsmith, 1991; Qin *et al.* 1998). Erythrocyte aggregation in turn augments the axial migration of red blood cells during blood flow. As an advantage, the hydrodynamic resistance is thus reduced, while at the same time the margination of leucocytes and platelets is facilitated (Stoltz *et al.* 1999).

Under pathological conditions, such as sepsis or diabetes, red blood cell aggregation is often increased as well. In contrast to the effect of EA during physiological conditions, oxygen distribution and peripheral perfusion are impaired under these circumstances.

Red blood cell aggregation is different between animal species. However, its physiological and evolutionary impact has so far not been elucidated. Besides all mentioned effects, the vasculature of the various animals should have adapted to the degree of red cell aggregation. Even in horses, the species with 'hyperaggregating' blood, no impairment of oxygen distribution is observed when physical performance is associated with increased haematocrit and viscosity, at least in trained animals (B. Stoiber, personal communication). Further factors leading to species-dependent differences in red blood cell aggregation must be taken into account. The viscosity of the cellular contents of the erythrocyte may vary; for example, dense erythrocytes are more rigid (Linderkamp *et al.* 1993). Estimation of internal viscosity based on the MCHC (Pfafferott *et al.* 1982) differed among the animals. However, an interspecies relationship to red blood cell aggregation could not be ruled out. Red blood cell shape might also play a role, since the MCV is different in all animals, but no relationship was detected. However, species differences might exist in the molecular weights of fibrinogen and other macromolecules which are important for red blood cell aggregation (Andrews *et al.* 1992), but this was not tested in the present study.

Further, there is evidence that sedentary animals have lower blood viscosity than athletic animals (Popel *et al.* 1994). Horses are athletic animals that can perform extensive muscle work in the presence of increased red cell aggregation. Hyperaggregating blood leads to increased plasma skimming. Based on this, one might speculate that capillary perfusion is augmented under these circumstances. Here, increased plasma skimming allows the activity-dependent adaptation of the capillary haematocrit and, at the same time, eases the flow of blood with a high haematocrit through arteriovenous anastomoses. This would enable the intensively retracted erythrocyte column to act as an O_2-carrier reservoir.

Finally, vessel length plays a role in axial migration. Because erythrocyte aggregation is a time-consuming process, a minimum tube length is required for sufficient plasma skimming. In the venous network, constant infusions of blood from the daughter vessels impair axial migration (Bishop *et al.* 2001). For the interpretation of erythrocyte aggregation in different animals, the geometric variability of the vascular network has to be considered. However, comparative studies of the vasculature of small or large animals and of animals with high or with low erythrocyte aggregation, are lacking. Speculatively, we assume that the necessary dimensions for erythrocyte aggregation might not exist in small animals such as the mouse, rat and rabbit.

Differences in WBV at high shear stress

During high shear stress, differences in WBV among the species decrease. Interestingly, animals with elevated whole blood viscosity at low shear rate do not necessarily have an increased viscosity at high shear rate. For instance, whole blood viscosity at 94 s^{-1} was higher in cattle than in cat. Also rat and dog had a higher whole blood viscosity than horse at this shear rate. During high shear stress, the red cell aggregates are destroyed. Erythrocytes become oriented in the direction of flow while their cytoskeleton reorganizes. The main cellular factor influencing whole blood viscosity is erythrocyte deformability. Erythrocyte deformability is affected by the mean shear rate, tube diameter, external fluid viscosity, cell shape and geometry, membrane and cytoskeletal viscoelasticity, internal fluid viscosity and physicochemical properties of haemoglobin. Although erythrocyte deformability was not measured in this study, there may be some differences among the species. These differences might not be uniform in animals which have erythrocyte aggregation or in those animals where it is undetectable.

Differences in plasma viscosity

Plasma viscosity plays an important role in the perfusion of the microvasculature. Since the haematocrit is dramatically reduced according to the Fahraeus-Lindqvist effect, viscosity in the arterioles and capillaries is reduced almost to the value of viscosity in the plasma. Therefore plasma viscosity, in addition to intraluminal pressure and flow pattern, is a major determinant of endothelial shear stress in this particular region. Plasma viscosity is used as a marker for different diseases in humans such as coronary artery disease (Koenig *et al.* 2000; Lowe *et al.* 2000), lupus erythematosus (Reid & DeCeulaer, 1999; Rosenson *et al.* 2001) and rheumatoid arthritis (Grassi *et al.* 1998). However, an increase in plasma viscosity with the progression of disease (Hoffmeister *et al.* 2001) or with therapy (Gibbs *et al.* 2001) was not observed.

Plasma viscosity was highest in cattle, presumably because of the increased plasma protein content in this species and was lowest in rabbit and mouse. Despite these differences in plasma viscosity, these animals have uniform WBV at low shear because of minimal red cell aggregation.

Therefore, animals with a low WBV do not necessarily have low plasma viscosity.

The findings of this study do not show a uniform relationship between WBV, plasma viscosity and erythrocyte aggregation. Plasma viscosity is increased by raised levels of macromolecules such as fibrinogen. Fibrinogen also increases erythrocyte aggregation. Cattle, rabbit, sheep and mouse tend to have higher fibrinogen levels than horse, pig, dog, cat and rat. This is interesting, since rabbit and mouse, both have a low aggregation tendency and a low plasma viscosity while at the same time their fibrinogen level is high. Cattle have a very low red blood cell aggregation in the presence of high fibrinogen levels. However, in contrast to rabbit and mouse, cattle have a high plasma viscosity. The reason for this discrepancy is unclear. Generally, species with low plasma viscosity such as rabbit and mouse might elicit lower endothelial shear stress than animals with high plasma viscosity such as cattle. The clinical significance of an increased plasma viscosity is unclear, since the results of a recent study showed that plasma viscosity was rather the result of coronary artery disease than its cause (Pfafferott *et al.* 1999).

Conclusion

Haemorheological parameters differed between the species studied. Each species has its own rheological fingerprint. The physiological significance of these variations in different mammalian species is not entirely clear at present.

Viscosity is a contributor to endothelial cell shear stress. The findings of this study show that viscosity, as well as other haemorheological parameters, differs across the species tested. We therefore postulate that factors influencing flow-mediated endothelial cell signal transduction are also different among the species.

Amin TM & Sirs JA (1985). The blood rheology of man and various animal species. *Q J Exp Physiol* **70**, 37–49.

Andrews FM, Korenek NL, Sanders WL & Hamlin RL (1992). Viscosity and rheologic properties of blood from clinically normal horses. *Am J Vet Res* **53**, 966–970.

Armstrong JK, Meiselman HJ & Fisher TC (1999). Evidence against macromolecular "bridging" as the mechanism of red blood cell aggregation induced by nonionic polymers. *Biorheology* **36**, 433–437.

Barakat AI (2001). A model for shear stress-induced deformation of a flow sensor on the surface of vascular endothelial cells. *J Theor Biol* **210**, 221–236.

Baskurt OK (1996). Deformability of red blood cells from different species studied by resistive pulse shape analysis technique. *Biorheology* **33**, 169–179.

Baskurt OK, Bor-Kucukatay M & Yalcin O (1999). The effect of red blood cell aggregation on blood flow resistance. *Biorheology* **36**, 447–452.

Baskurt OK, Bor-Kucukay M, Yalcin O & Meiselman HJ (2000). Aggregation behavior and electrophoretic mobility of red blood cells in various mammalian species. *Biorheology* **37**, 417–428.

Baskurt OK, Farley RA & Meiselman HJ (1997). Erythrocyte aggregation tendency and cellular properties in horse, human and rat: a comparative study. *Am J Physiol* **273**, H2604–2612.

Bäumler H, Neu B, Donath E & Kiesewetter H (1999). Basic phenomena of red blood cell rouleaux formation. *Biorheology* **36**, 439–442.

Bäumler H, Neu B, Mitlohner R, Georgieva R, Meiselman HJ & Kiesewetter H (2001). Electrophoretic and aggregation behavior of bovine, horse and human red blood cells in plasma and in polymer solutions. *Biorheology* **38**, 39–51.

Bishop JJ, Nance PR, Popel AS, Intaglietta M & Johnson PC (2001). Erythrocyte margination and sedimentation in skeletal muscle venules. *Am J Physiol Heart Circ Physiol* **281**, H951–958.

Bodey AR & Rampling MW (1998). A comparative study of the haemorheology of various breeds of dog. *Clin Haemorheol Microcirc* **18**, 291–298.

Brooks DE (1988). Mechanism of red cell aggregation. In *Blood Cells, Rheology and Aging*. ed. Platt D, pp. 158–162. Springer, Berlin.

Cabel M, Meiselman HJ, Popel AS & Johnson PC (1997). Contribution of red cell aggregation to venous vascular resistance in skeletal muscle. *Am J Physiol* **272**, H1020–1032.

Charles River Baseline Hematology (1982). Clinical chemistry values for Charles River Wistar rats. Charles River, Kislegg, Germany.

Chien S & Sung LA (1987). Physicochemical basis and clinical implications of red cell agggregation. *Clin Haemorheol* **7**, 71–91.

Cokelet GR & Goldsmith HL (1991). Decreased hydrodynamic resistance in the two-phase flow of blood through small vertical tubes at low flow rates. *Circ Res* **68**, 1–17.

Davies PF (1995). Flow-mediated endothelial mechanotransduction. *Physiol Rev* **75**, 519–560.

Frangos JA, Eskin SG, McIntire LV & Ives CL (1985). Flow effects on prostacyclin production by cultured human endothelial cells. *Science* **227**, 1477–1479.

Frith CH, Suber RL & Umholtz R (1980). Haematological and clinical chemistry findings in control BALB/c and C57BL/6 mice. *Lab Anim Sci* **30**, 835–840.

Gibbs CR, Blann AD, Watson RDS & Lip GYN (2001). Abnormalities of haemorheological, endothelial and platelet function in patients with chronic heart failure in sinus rhythm. *Circulation* **103**, 1746–1751.

Grassi W, Deangelis R, Lamanna G & Cervini C (1998). The clinical feature of rheumatoid arthritis. *Eur J Radiol* **27**, S18–S24.

Harrel R & Davies M (1995). In *Statistical Bases of Reference Values in Laboratory Medicine*, ed. Harris A & Boyd M. Dekker, New York.

Hoffmeister A, Rothenbacher D, Bäzner U, Fröhlich M, Brenner H, Hombach V & Koenig W (2001). Role of novel markers of inflammation in patients with coronary heart disease. *Am J Cardiol* **87**, 262–266.

Holm ST (1979). A simple sequentially rejective multiple test procedure. *Scand J Statistics* **6**, 65–70.

Johnn H, Phipps C, Gascoyne S, Hawkey C & Rampling MW (1992). A comparison of the viscometric properties of the blood from a wide range of mammals. *Clin Haemorheol* **12**, 639–647.

Johnson PC (1981). The myogenic response. In *Handbook of Physiology*, section 2, *The Cardiovascular System,* vol. II, *Vascular Smooth Muscle*, chap. 15, pp. 409–442. American Physiological Society, Bethesda, MD, USA.

Johnson PC, Bishop JJ, Popel S & Intaglietta M (1999). Effects of red cell aggregation on the venous microcirculation. *Biorheology* **36**, 457–460.

Koenig M, Sund M, Löwel H, Döring G & Ernst E (2000). Association between plasma viscosity and all-cause mortality: results from the MONICA-Augsburg Cohort Study 1984–92. *Br J Haematol* **109**, 453–458.

Kraft W & Dürr UM (1997). *Klinische Labordiagnostik in der Tiermedizin*. 4th edn, Schattauer, Stuttgart, New York.

Kumaravel M & Singh M (1995). Sequential analysis of aggregation process of erythrocytes of human, buffalo, cow, horse, goat and rabbit. *Clin Haemorheol* **15**, 291–304.

Langille BL & O'Donnel F (1986). Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* **231**, 405–407.

Linderkamp O, Friederichs E & Meiselman HJ (1993). Mechanical and geometrical properties of density separated neonatal and adult erythrocytes. *Pediatr Res* **34**, 688–693.

Linke J (1996). Untersuchungen zur Plasmaviskosität beim Hund. *Tierärztl Prax* **24**, 524–528.

Lowe G, Rumley A, Norrie J, Ford I, Shepherd J, Cobbe S, MacFarlane P & Packard CH (2000). Blood rheology, cardiovascular risk factors and cardiovascular disease: the West of Scotland Coronary Prevention Study. *Thromb Haemost* **84**, 553–558.

Malek K & Izumo S (1992). Physiological fluid shear stress causes down-regulation of endothelin-1 mRNA in bovine aortic endothelium. *Am J Physiol* **263**, C389–396.

Meiselman HJ (1978). Rheology of shape-transformed human red cells. *Biorheology* **15**, 225–237.

Moncada S, Palmer RM & Higgs EA (1991). Nitric oxide: physiology, pathophysiology and pharmacology of nitric oxide. *Pharmacol Rev* **43**, 109–142.

Nash GB, Wenby RB, Sowemimo-Coker SO & Meiselman HJ (1987). Influence of cellular properties on red cell aggregation. *Clin Haemorheol* **7**, 93–108.

Pfafferott C, Moessmer G, Ehrly AM & Bauersachs RM (1999). Involvement of erythrocyte aggregation and erythrocyte resistance to flow in acute coronary syndromes. *Clin Haemorheol Microcirc* **21**, 35–43.

Pfafferott C, Wenby RB & Meiselman HJ (1982). Morphologic and internal viscosity aspects of RBC rheologic behavior. *Blood Cells* **8**, 65–69.

Popel AS, Johnson PC, Kameneva MV & Wild MA (1994). Capacity for red blood cell aggregation is higher in athletic mammalian species than in sedentary species. *J Appl Physiol* **77**, 1790–1794.

Qin Z, Durand LG & Cloutier G (1998). Kinetics of the "black hole" phenomenon in ultrasound backscattering measurements with red blood cell aggregation. *Ultrasound Med Biol* **24**, 245–256.

Reid HL & DeCeulaer K (1999). Abnormal plasma and serum viscosity in systemic lupus erythematosus (SLE): a Jamaican study. *Clin Haemorheol Microcirc* **20**, 175–180.

Reinke W, Johnson PC & Gaethgens P (1986). Effect of shear rate variation on apparent viscosity of human blood in tubes of 29 to 94 μm diameter. *Circ Res* **59**, 124–132.

Rosenson RS, Shott S & Katz R (2001). Elevated blood viscosity in systemic lupus erythematosus. *Semin Arthritis Rheum* **31**, 52–57.

Stoltz JF, Singh M & Riha P (1999). Microrheological parameters. In *Haemorheology in Practice*, chap. 4, pp. 27–52. IOS Press, Amsterdam, Berlin, Oxford, Tokyo, Washington DC.

Sumpio BE, Banes AJ, Link GW & Iba T (1990). Modulation of endothelial phenotype by cyclic stretch: inhibition of collagen production. *J Surg Res* **48**, 415–420.

Waugh RE (1992). Red cell deformability in different vertebrate animals. *Clin Haemorheol* **12**, 649–656.

Windberger U, Ribitsch V, Resch KL & Losert U (1993/94). The viscoelasticity of blood and plasma in pig, horse, dog, ox and sheep. *J Exp Anim Sci* **36**, 89–95.