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Hemorheology and oxygen transport in vertebrates. A role in thermoregulation?

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We studied the effect of temperature on blood rheology in three vertebrate species with different thermoregulation and erythrocyte characteristics. Higher fibrinogen proportion to total plasma protein was found in turtles (20%) than in pigeons (5.6%) and rats (4.2%). Higher plasma viscosity at room temperature than at homeotherm body temperature was observed in rats (1.69 mPa·s at 20 °C *vs.* 1.33 mPa·s at 37 °C), pigeons (3.40 mPa·s at 20 °C *vs.* 1.75 mPa·s at 40 °C), and turtles (1.74 mPa·s at 20 °C *vs.* 1.32 mPa·s at 37°C). This fact allow us to hypothesize that thermal changes in protein structure may account for an adjustement of the plasma viscosity. Blood viscosity was dependent on shear rate, temperature and hematocrit in the three species. A different behaviour in apparent and relative viscosities between rat and pigeon at environmental temperature was found. Moreover, the blood oxygen transport capacity seems more affected by a reduction of temperature in rats than in pigeons. Both findings indicate a greater influence of temperature on mammalian erythrocyte than on nucleated red cells, possibly as a consequence of differences in thermal sensitivity and mechanical stability between them. A comparison between the three species revealed that apparent blood viscosity measured at homeotherm physiological temperature was linearly related to the hematocrit level of each species. However, when measured at environmental temperature, rat blood showed a higher apparent viscosity than those found in species with non-nucleated red cells, thus indicating a higher impact of temperature decrease on blood viscosity in mammals. This suggest that regional hypothermia caused by cold exposure may affect mammalian blood rheological behaviour in a higher extent than in other vertebrate species having nucleated red cells and, consequently, influencing circulatory function and oxygen transport.

Key words: Blood viscosity, Comparative hemorheology, Eryhtrocytes, Vertebrates, Thermoregulation.

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The thermal sensitivity of blood viscosity and erythrocyte microrheology has been thoroughly studied in past years but some aspects are still under current discussion. Some pioneering reports demonstrated an increased viscosity of human blood during hypothermic conditions (19). This finding, together with the relationship between body temperature, blood viscosity, oxygen carrying capacity and metabolic requirements, lead to the idea that there is a thermal shift of the "optimal hematocrit" (22, 27). Although some authors have also described an optimal adjustment of erythrocyte rheological properties at physiological temperatures in different vertebrate species (9, 11, 15, 4, 20), the mechanisms underlying these responses are still not clear. Moreover, there is no agreement in the interpretation and physiological meaning of the blood viscosity differences observed at low temperature between temperate and Antarctic species (6, 3, 21). Changes in blood viscosity induced by environmental temperature have been postulated as a dominant factor affecting heat exchange, by modifying peripheral blood flow, in heterothermic species (9). However, the extent of this mechanism on the peripheral microcirculation of endotherms has not been evaluated.

The aim of this work was to study the effect of temperature on the hemorheological behaviour in three representative species of vertebrates with different thermoregulative strategies. A fresh-water turtle *(Mauremys leprosa)* was selected as representative of ectothermic species, whilst the laboratory rat *(Rattus norvegicus)* and the urban pigeon *(Columba livia)* were chosen for being typical endotherms. In addition, choosing a mammal and a bird, allowed to study the presumable differences in hemorheology caused by the internal structural characteristics of red blood cells (non-nucleated versus nucleated) in endotherms. We have not considered any other morphological characteristics of erythrocytes affecting the hemorheological behaviour, such as size and shape, for two reasons. First, because the role of these factors has already been deeply described in previous reports (1, 23, 24); and second, due to the impossibility of using vertebrate species having at the same time different thermoregulative patterns and erythrocytes with similar morphometrical characteristics.

Materials and Methods

Animals.– Laboratory rats (*Rattus norvegicus* from the Sprague-Dawley strain) were obtained from a commercial dealer and fed with the usual equilibrated diet pellets. Feral pigeons *(Columba livia)* were caught from free-living urban population in Barcelona and fed with a mixture of wheat (35%), minced corn (35%) and vetch (30%), a sulfamyde based coccidiostatic (Coccitaber) was added to drink water (0.5%) during an initial quarantine period of three weeks. Rats and pigeons had constant access to food, drunk water "ad libitum" and were maintained at room temperature (20 °C). Spanish terrapins (*Mauremys leprosa)* were collected at Laguna de Adra (Almería, Spain) and were maintained in the animal facility at the University of Barcelona into cages with free access to water ponds, which were renewed daily. Turtles were fed with minced meat including bone meal and occasionally fish. They drunk water "ad libitum" in an environmental temperature maintained at 20 °C.

Blood withdrawal was achieved after at least one month of acclimation to confinement conditions. All the animals were

apparently healthy, with no clinical signs of disease. Hematological and body weight control showed no significant change during the period of captivity conditions.

Blood sampling procedure.– Blood samples were collected by cardiac puncture in rats (ether anaesthesia) and turtles (ice bath anaesthesia) and from the radial vein in pigeons. Sodium heparin was used as anticoagulant. Blood was immediately stored in an ice bath until all analyses were performed. A fraction of each blood sample was separated for immediate hematological analysis, which were always completed within two hours of blood withdrawal. A second portion of the sample was simultaneously processed for blood rheology determinations. The cellular portion of a third part of the blood sample was removed by centrifugation, and plasma obtained was separated without delay. An aliquot was used for viscosity determination or red blood cell (RBC) suspension preparation, and the remaining was collected into capillary tubes, which were immediately frozen for biochemical analysis. Hematological and hemorheological values were determined immediately after collection and plasma biochemical analyses were carried out during the days following blood withdrawal.

Hematology.– Hematocrit values were determined using microhematocrit centrifugation technique. Hemoglobin concentration was measured by Drabkin technique. The RBC count was determined with an electronic cell counter (Coulter Counter ZF). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Total plasma protein concentration was measured using a folin reagent conventional technique, plasma fibrinogen was determined by the sulphite precipitation method (18). Plasma proteins were separated by cellulose acetate electrophoresis (Cellogel, Atom), fractions were identified and quantitatively estimated by densitometric curves (Cellomatic Digiscan Atom). The ratio albumine/globuline was calculated as the quotient of the protein fraction of high mobility (prealbumines+albumines) and the remaining protein fractions (globulines).

Blood rheology.– The apparent viscosity (η_a) of blood and plasma was measured using a cone-plate microviscosimeter (Wells-Brookfield LTVII) connected to a thermostatic bath. All the determinations on whole blood and RBC suspensions in autologous plasma were obtained immediately after gently shaking the sample in a roller mixer during at least 5 minutes. A sample volume of 0.5 ml was tested at different shear rates ($\dot{\gamma}$) ranging from 2.25 to 450 s^{-1}. Different samples were used for measurements at room temperature (20 °C) for the three species and also at 37°C for rat and turtle and 40 °C for pigeon. Due to its low viscosity values, and in order to obtain the highest accuracy, plasma viscosity was measured only at 450 s^{-1}, since it is well established that plasma presents Newtonian behaviour.

In order to avoid the effect of RBC fraction volume on rheological behaviour, freshly heparinized blood was used to prepare 40% hematocrit samples. Blood was centrifuged, "buffy coat" and surrounding plasma was removed, and the hematocrit was adjusted to 40% by autologous plasma addition or substraction. No evidence of hemolysis or change in RBC volume occurred during this procedure. The small volume and the low

hematocrit of the blood samples obtained in turtles did not allow to include it in this part of the study.

The effect of plasma viscosity on whole blood apparent viscosity was considered by studying the relative viscosity (η_r) of the whole blood, which is expressed as the quotient of the apparent viscosity of whole blood and the viscosity of the plasma. In the same way, the relative viscosity of RBC suspensions (adjusted at a hematocrit value of 40%) was calculated by dividing the apparent viscosity of RBC suspensions by the viscosity of the plasma.

According to CHIEN *et al.* (2) erythrocyte aggregability (RBC_a) and deformability (RBC_d) are the main factors affecting blood viscosity at low and high shear rates, respectively. As a consequence, the contribution of these microrheological characteristics to blood flow properties could be estimated from the variation of apparent blood viscosity values into low (when cell-plasma protein interactions are strong) and high (with high probabilities of cell-cell interaction) ranges of shear rate respectively. Thus, we applied the following formulae, also defined as the degree of shear dependence (23).

$$RBC_a = (\eta_{2.25}-\eta_{4.5})/\eta_{4.5}$$
$$RBC_d = (\eta_{225}-\eta_{450})/\eta_{450}$$

where η_x indicates the apparent blood viscosity at a given shear rate level ($\dot{\gamma}$) = x.

Finally, some calculations were performed in order to evaluate the impact of blood viscosity changes induced by temperature on oxygen transport function. We calculated two different coefficients previously defined in the literature. First, the oxygen delivery index (Hc/η) (10), which shows the relationship between the hematocrit (Hc) and blood viscosity (η). Second, the blood oxygen potential transport capacity (β[Hb]/η) (7), which relates the hemoglobin oxygen capacity (β) and hemoglobin concentration [Hb] with blood viscosity (η); β values were assumed as 1.22 ml $O_2 \cdot g^{-1}$ at 20°C and 1.34 ml $O_2 \cdot g^{-}1$ for 37-40 °C for the three species.

Statistics.– All data are presented throughout the text and tables as sample means ± 95% confidence intervals. Three-way ANOVA test was used to compare viscosity data between the three different variables involved in the study: temperature, shear rate and hematocrit. Comparison between different experimental conditions for each individual and between different species at the same conditions were carried out by means of the Tukey's honest significant difference test. Descriptive statistics and analyses of normality of data were made with SigmaStat (Jandel Scientific), whereas 3-way ANOVA and Tukey's test were performed by the application of suitable subroutines from the package SPSS/PC+ (SPSS Inc.). Differences were considered statistically significant for $p<0.05$.

Results

Hematological and plasmatic parameters.– Hematological parameters for the three species are given in Table I. The application of one-way ANOVA and Tukey's tests showed significant differences between the three species in all the showed haematological parameters. Hematocrit and hemoglobin values were highest in the pigeon and lowest in the turtle. The greatest MCVs and MCHs were found in the turtle whilst the lowest in the rat. As exception, the difference in MCHC between turtles and rats was not significant, but both species had signifi-

Table I. *Hematological parameters, plasma viscosity, fibrinogen and total protein concentrations and proteinogram for the three species studied.*

Mean values ± confidence intervals at 95%. MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: Red blood cells. Statistical significance codes: [a]significant differences between the three species, [b]significant differences between turtle and homoeotherm species, [c]significant differences between pigeon and the other two species,

	Turtle (n = 6)	Rat (n = 20)	Pigeon (n = 25)
Hematocrit (%)	[a]21.7 ± 1.7	37.6 ± 0.9	49.9 ± 2.8
Hemoglobin (g/dl)	[a]8.18 ± 0.3	12.33 ± 0.7	14.2 ± 1.6
RBC count (10^6/µl)	[b]0.513 ± 0.03	5.334 ± 0.31	4.894 ± 0.26
MCV (µm^3)	[a]423.2 ± 27.0	71.2 ± 3.7	101.9 ± 2.3
MCH (pg)	[b]160.1 ± 9.4	23.4 ± 1.8	29.0 ± 2.2
MCHC (%)	[a]38.0 ± 2.4	32.9 ± 1.9	28.5 ± 2.0
Plasma viscosity at 37-40 °C (mPa·s)	[c]1.32 ± 0.07	1.33 ± 0.23	1.75 ± 0.29
Plasma viscosity at 20 °C (mPa·s)	[c]1.74 ± 0.08	1.69 ± 0.27	3.40 ± 0.39
Total protein (g/l)	[b]17.29 ± 5.82	42.89 ± 2.54	38.35 ± 4.61
Fibrinogen (mg/ml)	[a]3.46 ± 0.19	1.79 ± 0.28	2.15 ± 0.17
Pre-albumin (%)	-	-	16.5 ± 1.35
Albumin (%)	28.45 ± 3.84	45.88 ± 2.48	30.7 ± 5.00
α_1-globulin (%)	[b]18.55 ± 1.56	11.11 ± 2.22	12.0 ± 1.507
α_2-globulin (%)	-	9.98 ± 1.54	-
β-globulin (%)	30.85 ± 5.45	20.88 ± 0.52	23.7 ± 3.36

cantly higher MCHC values than those found in pigeons.

Significant differences between the three species were found in plasma fibrinogen levels, with a higher concentration in the turtle (Table I). When analysing total plasma protein concentration, no significant differences between pigeons and rats were found, although both values were significantly higher than those values found in turtles. Each species showed a characteristic electrophoretic pattern. The pigeon presented the highest proportion of proteins with high motility and the highest albumin/globulin ratio. In the turtle the albumin fraction showed lower values than in pigeons and rats, which is the responsible for the lower albumin/globulin ratio.

Blood rheology.– All blood samples showed a non-Newtonian shear-thinning behaviour manifested as a clear reduction of viscosity as applied shear rate increased (Figs. 1 and 2). When the apparent viscosity of the native whole blood was compared by means of a three-way ANOVA test, a significant effect of the factors species, shear rate and temperature was found on blood viscosity (Fig. 1). However, this effect was not present on the apparent blood viscosity at the uniform hematocrit value of 40% (Fig. 2). The ANOVA analysis shows that the effect of temperature is different depending on the species, whereas the effect of shear rate is quite uniform and not dependent on the species. Tukey's test detected significant differences in apparent blood viscosity at the uniform hematocrit value of 40% when species and shear rate were considered, but not significant effect of the temperature factor was observed. In contrast, when the relative blood viscosity is con-

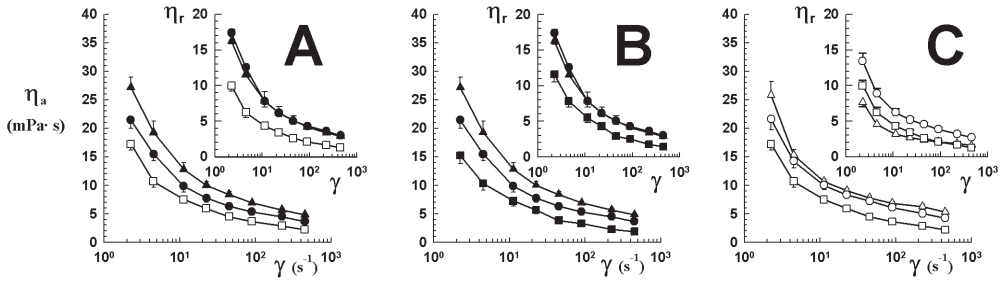

Fig. 1. *Rheograms of apparent blood viscosity* (η_a) *and relative viscosity* (η_r) *for native blood at the physiological hematocrit of the three species studied: turtles (squares), rats (circles) and pigeons (triangles).* Bars represent 95% confidence intervals. Panel A: Values at physiological temperatures for each species (rat: 37 °C, pigeon: 40 °C, and turtle: 20 °C). Panel B: Values at 37 °C (rat and turtle) and 40 °C (pigeon). Panel C: All species at 20 °C. Filled symbols: 37 °C-40 °C; hollow symbols: 20 °C.

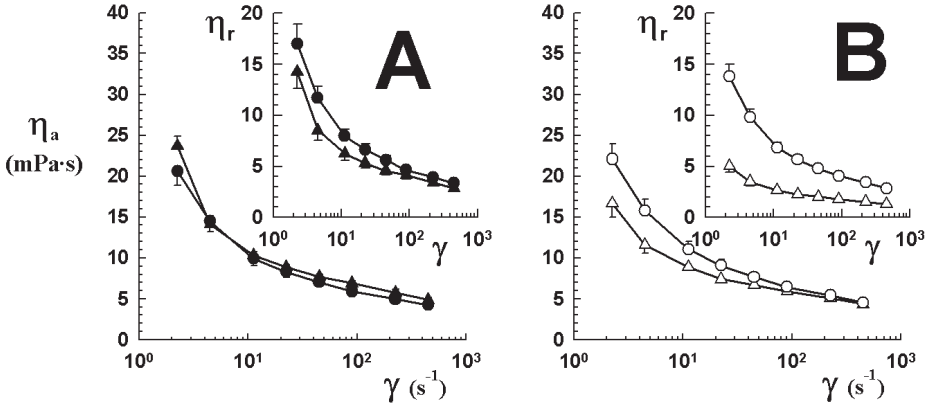

Fig. 2. *Rheograms of apparent viscosity* (η_a) *and relative viscosity* (η_r) *of red blood cell suspensions in autologous plasma of rats* (circles) *and pigeons* (triangles) *with a fixed hematocrit of 40%.*
Bars represent 95% confidence intervals. Panel A: Values at 37 °C (rat) and 40 °C (pigeon). Panel B: Values at 20 °C.

sidered, a significant effect of the three factors: species, temperature and shear rate, is clear at the uniform hematocrit value of 40%.

The relationship between blood apparent and relative viscosities and the hematocrit at the two ranges of temperature studied is plotted in Fig. 3 showing a conspicuous linearity at 37-40 °C between blood viscosity and hematocrit. However, this trend is not observed at environmental temperature, due to the deviation found in the relationship between hematocrit and viscosity caused by the increased values of the rat blood.

Whereas RBC aggregability indexes were quite uniform, ranging from 0.53 to 0.63, the RBC deformability indexes widely differ between the three species: around 0.38 in pigeon; 0.43 in rat and 0.61

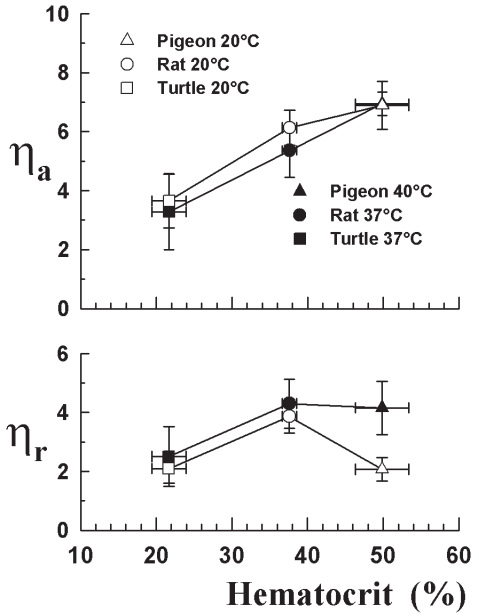

Fig. 3. *Temperature effect on the apparent blood viscosity* (upper panel) *and relative viscosity* (lower panel) *relationships with the hematocrit level of the three species.*
Values at 37 °C–40 °C, (filled symbols) and 20 °C, (hollow symbols). Bars represent 95% confidence intervals.

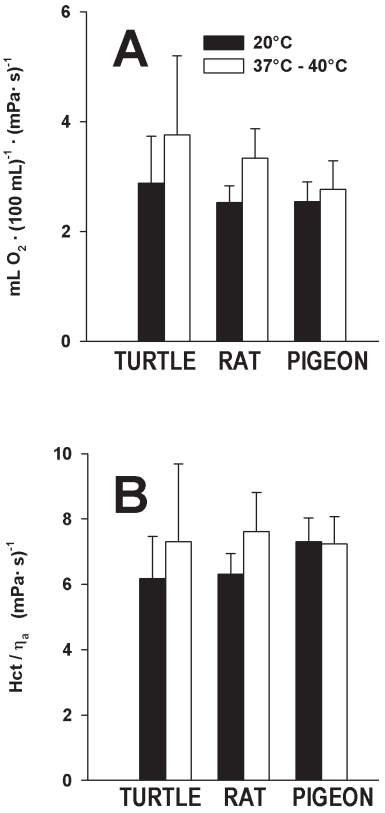

Fig. 4. *Blood oxygen potential transport capacity* (Panel A) *and oxygen delivery index* (Panel B) *for the three species at 37 °C for rats and turtles and 40 °C for pigeons* (filled bars) *and 20 °C (hollow bars).*
Lines represent 95% confidence intervals.

in turtle. The effect of temperature not resulted statistically significant for the three species. However, these results must be considered with caution because that indexes indicate the rheological behaviour and red cell interactions in cone-plate conditions, which are distinct to those *in vivo* into microcirculatory vessels.

Blood oxygen potential transport capacity and on the oxygen delivery indexes are plotted in Figure 4. It has to be noted that, at the same temperature, both indexes showed close values, being much more homogeneous at room temperature. The effect of temperature decrease from homeotherm to environmental levels on blood oxygen potential transport capacity (Fig. 4; upper graph) and on oxygen delivery index (Fig. 4; lower graph) was not statistically significant, although a more apparent temperature dependence seems to be in rat and turtle, thus indicating that oxygen supply to the tissues can be reduced under local hypothermia conditions, obviously this phenomenon can be more critical, due to the wide different metabolic requirements, in rat than in turtle.

Discussion

The hematological parameters and oxygen transport indices found for the three species were within the range of the results previously found in our laboratory (17, 25, 26). Higher hematocrit and hemoglobin concentration values in the pigeon are a consequence of the high metabolic rate demanded by flapping flight, whilst the high MCV and MCHC found in turtles compensate the low hematocrit and hemoglobin concentration of the turtle blood.

In relation to plasmatic parameters, no direct relationship between plasma protein concentration and plasma viscosity can be observed when comparing data of the three species studied. This could be caused by the different levels of plasma fibrinogen, since the proportion of fibrinogen in relation to total plasma protein is significantly higher in the turtle (about 20%) than in the pigeon (5.6%) and the rat (4.2%). Two conclusions may be inferred from this finding: first, that a low plasma viscosity is found at the physiological temperature of the rat than in the nucleated species; and second, that changes in protein structure may be responsible for the alteration of the plasma viscosity as a result of exposure to different temperatures.

The linearity in the relationship between blood viscosity and hematocrit observed at homeotherm temperature when considering the three species is noteworthy, specially if it is taken into account that the relationship between hematocrit and blood viscosity is typically exponential when studying the blood rheological properties into a single species, both in homeotherms (6, 3) and in heterotherms (8, 28, 20). The highest viscosity of rat blood breaks the linear pattern at environmental temperature, indicating that temperature has a lower effect on the viscosity of blood with nucleated erythrocytes. A possible explanation may be that elliptic pigeon and turtle RBCs (as opposed to the rat discoidal erythrocytes) can be better orientable to blood flow direction into the vessels, thus maintaining lower viscosity values when temperature decreases.

Rheological data obtained at a uniform hematocrit of 40% allow to state that there are not significant differences neither in aparent nor relative viscosity between rat and pigeon blood at their physiological temperatures. The viscosity at environmental temperature under these hematocrit conditions showed contrasting effects on these two homeotherm species. Whereas apparent and relative viscosity did not significantly change in the rat, a significant ($p<0.01$) decrease was detected in the pigeon. The wide difference for η_r values between these two species results enlarged by the paradoxal increase of the viscosity of the plasma at 20 °C in pigeons. In humans, the effect of low temperature on the mechanical stability of red cell aggregates has been considered as the main factor causing the increase of relative and apparent blood viscosities (16). This hypothesis may be valid for rat erythrocytes, with morphological and structural characteristics similar to human red cells, but probably is not as much applicable for blood having non-nucleated RBCs such as in the pigeon. Moreover, the well known differences in erythrocyte aggregation mechanisms between nucleated and non-nucleated red cells (1) could contribute to explain our findings.

At a given temperature, the oxygen carrying capacity and the oxygen delivery index are very similar in the three species (non significant differences were found),

in spite of the marked differences in hematocrit, blood viscosity and hemoglobin concentration. Several factors other than the well-known adjustment between hematocrit and oxygen delivery (optimal hematocrit) may explain this finding. Some authors have suggested that low perfusion pressure and stiff cells may result not limitant in ectothermic species such as fishes, amphibians and reptiles since the increase in the transit time of the erythrocytes in the capillary bed, provide an adequate oxygen delivery rate for the relative low oxygen consumption rates that these animals have (8, 11). However, animals such as birds, which have high metabolic rates and nucleated erythrocytes, must compensate the low deformability of their RBCs with higher heart mass, cardiac output and capillary density (5, 13, 14) than mammalian species of similar sizes. In addition, some studies does not have found evidence for a significant rheological adaptation to the cold in the erythrocytes from fish living at different temperatures, thus indicating that impaired RBC deformability is not a major factor influencing blood flow in fish at low temperature (12).

Our data seems to indicate that blood oxygen carrying capacity and oxygen delivery index are more affected by a reduction in blood temperature in the rat than in the pigeon. This implies a greater influence of the temperature on mammalian erythrocyte than on avian erythrocytes, probably as a consequence of structural differences between both kinds of RBCs, since non-nucleated erythrocytes have a lower mechanical stability than nucleated ones, which have more developed cytoskeletic structures.

In summary, our results indicate that body temperature could affect the rheological behaviour of rat blood by increasing whole blood viscosity during tissue hypothermia. As a consequence of this, general circulatory functions, and specially oxygen transport to tissues, may be critically dependent on regional thermoregulatory control in a higher extent in the rat than in other species having nucleated red cells.

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G. VISCOR, J. R. TORRELLA, V. FOUCES y T. PAGÉS. *Hemorreología y transporte de oxígeno en vertebrados. ¿Un papel en la termorregulación?* J. Physiol. Biochem., **59** (4), 277-286, 2003.

Se estudia el efecto de la temperatura sobre la reología sanguínea en tres especies de vertebrados con diferente termorregulación y características eritrocitarias. Se encuentra que la relación fibrinógeno/proteínas plasmáticas es mayor en tortugas (20%), que en palomas (5.6%) y ratas (4.2%). Se observa mayor viscosidad plasmática a temperatura ambiente que a la corporal en rata (1,69 mPa·s a 20 ºC *vs.* 1,33 mPa·s a 37 ºC), paloma (3,40 mPa·s a 20 ºC *vs.* 1,75 mPa·s a 40 ºC) o tortuga (1,74 mPa·s a 20 ºC *vs.* 1,32 mPa·s a 37 ºC). Esto sugiere que los cambios térmicos afectan a la estructura de las proteínas y causan un ajuste de la viscosidad plasmática. La viscosidad sanguínea depende del gradiente de velocidad, de la temperatura y del hematocrito, en las tres especies. Se observa un comportamiento diferente en la viscosidad aparente y relativa entre rata y paloma a temperatura ambiente. Sin embargo, la capacidad transportadora de oxígeno por la sangre parece más afectada por una reducción de la

temperatura sanguínea en ratas que en palomas. Ambos hallazgos apuntan a un mayor efecto térmico sobre los eritrocitos de mamífero que en los nucleados, indicando diferente sensibilidad térmica y estabilidad mecánica. La comparación entre las tres especies revela que la viscosidad aparente sanguínea a temperatura fisiológica en homeotermos presenta una relación lineal con el hematocrito. A temperatura ambiental, la sangre de ratas mostró una viscosidad aparente mayor de la esperada por su hematocrito, indicando un mayor impacto del descenso de temperatura sobre la viscosidad sanguínea. Esto sugiere que la hipotermia regional debida a la exposición al frío puede alterar la reología de la sangre de los mamíferos en mayor medida que en otras especies de vertebrados con eritrocitos nucleados y, en consecuencia, afectar la función circulatoria y el transporte de oxígeno.

Palabras clave: Viscosidad sanguínea, Hemorreología comparada, Eritrocitos, Vertebrados, Termorregulación.

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