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THERMAL TRANSITIONS OF RED BLOOD CELL DEFORMABILITY

CORRELATION WITH MEMBRANE RHEOLOGICAL PROPERTIES

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Red blood cell deformability has been studied by the initial filtration flow rate as a function of temperature. The well-known transition at 49–50°C (probably due to spectrin denaturation) is shown. Another transition is demonstrated around 18°C (the cell becomes stiffer below this temperature range). The erythrocyte membranes prepared by a mild dialysis technique have the same deformability as intact erythrocytes at room temperature; they also show the same low-temperature transition. No such transition has been found for hemoglobin solutions of viscosity 30 g · dl $^{-1}$. It is interesting to compare these results with those obtained by other methods which measure the properties of natural or artificial lipid membranes and which also demonstrate a thermal transition at 15–20°C. Therefore, the deformability of intact normal erythrocytes seems to depend mainly on the rheological properties of the membrane.

Introduction

Above 49–50°C, many biophysical and biochemical properties of the erythrocyte change. The cell rheology also shows a sudden transition at this temperature, above which cell fragmentation occurs [1,2]. Many authors have demonstrated a dramatic reduction of the erythrocyte deformability in the 49–50°C range [3–7]. This phenomenon has been used to alter the erythrocytes in order to trap them in the spleen for isotope scanning [8].

A low-temperature (15–20°C) transition of natural or artificial membranes has been described by some authors [9–20]. These results are still controversial [21–25]. No low-temperature transition of the erythrocyte deformability has yet been described. Therefore, we wish to present a deformability study in the 4–50°C temperature range, made on intact erythrocytes and on their ghosts.

In this study, the deformability is defined as the ability of the erythrocyte to filter through the narrow 5 μm pores of a Nuclepore membrane. The relation between filterability and deformability has already been discussed [26–29]. Some filtration results, however, have been obscured by secondary phenomena (erythrocyte settling and aggregation, filter clogging by aggregates and white blood cells, etc.). We have used a novel method which is independent of these factors (as a first order of approximation) so that, as will be discussed, the results are relevant only to the rheological properties of the individual erythrocytes.

Experimental

Filtration measurements

The filtration measurements were made with the initial flow rate method as described elsewhere [30].

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The apparatus, called Hemorheometer (available from IMH, 2 Allée du jardin de la Cure, 95470 St. Witz, France) has been modified in order to perform temperature-controlled measurements (Fig. 1). An upper plastic block is composed of a central capillary (vertical glass tubing, 6 cm long, 2.5 mm diameter), surrounded by water circulating from a thermostat. The capillary is terminated by a conical section under which is situated the Nuclepore membrane (diameter 13 mm; pore diameter 5 μm). A miniature thermocouple is set on the conical section so as to measure the liquid temperature, just above the membrane.

The central capillary can be filled by either a erythrocyte suspension or the suspending medium (buffer). Two level detectors situated at the top part of the capillary and separated from each other by 9 mm, can actuate then stop an electronic chronometer when the meniscus of the liquid passes in front of them during the filtration procedure. The time lapse thus measured is proportional to the flow rate and therefore to the global fluidity of the liquid being filtered. When this liquid is the suspension, the time lapse will be called t_s; for the buffer, it will be called t_b. A lower plastic block holds the membrane, under which a positive pressure of about 10 cm H_2O can be applied, thus preventing the hydrostatic pressure flow. By suddenly releasing this overpressure (by means of a three-way cock), the filtration starting time is thus precisely known. A simple pumping and metering device gives this positive pressure.

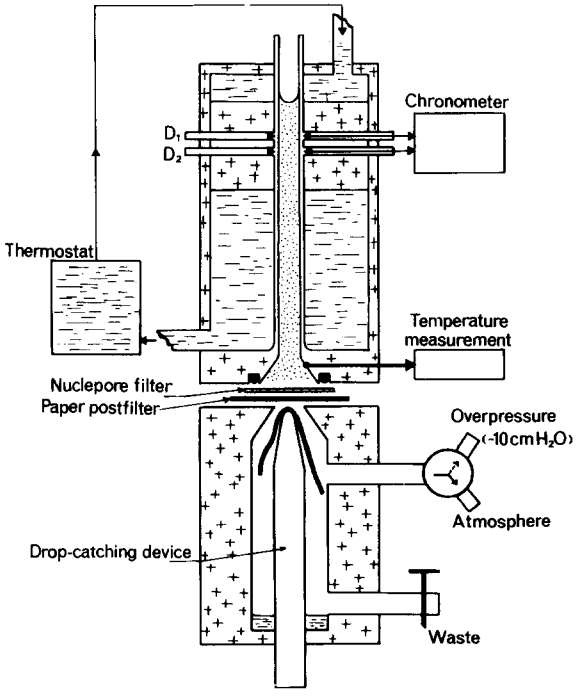

Fig. 1. Schematic drawing of the filtration head of the 'Hemorheometer' modified for temperature control.

The total volume of the glass tubing is about 1 ml; the volume giving the measured time lapse is about 50 μl. Therefore, the time lapse is proportional to the initial flow rate, as a first order of approximation. In this work, we will express our results as a rigidity index, IR, defined as follows:

$$IR = \frac{t_s - t_b}{t_b} \cdot \frac{100}{H}$$

where H is the haematocrit. High IR values indicate low erythrocyte deformability.

We have compared the suspension temperature given by the miniature thermocouple to the circulating water temperature. In these measurements, about 5 min was allowed for thermal equilibration (temperatures increasing from 4°C to 50°C). We have found that the two readings were similar (the maximum deviation is about 2% for the high-temperature range and negligible for the low-temperature range). As this temperature correlation is satisfactory, in the subsequent experiments only the circulation water temperature was taken into account (increasing values, 5 min equilibration at each temperature). The temperature stability is ± 0.1°C.

In order to evaluate any membrane thermal dilatation artefact, the viscosity of distilled water was determined as a function of temperature and compared to the values obtained by classical methods (see Table F-51 in Ref. 31). The results are shown in Fig. 2 (in order to obtain the experimental viscosity values, the membrane is calibrated with water at 25°C). As the experimental points coïncide with the theoretical values, we conclude that the pore geometry changes due to thermal variations are negligible.

As previously shown [30,32], the rigidity index is independent of the haematocrit, in the 0–10% range. This result indicates that, as defined above,

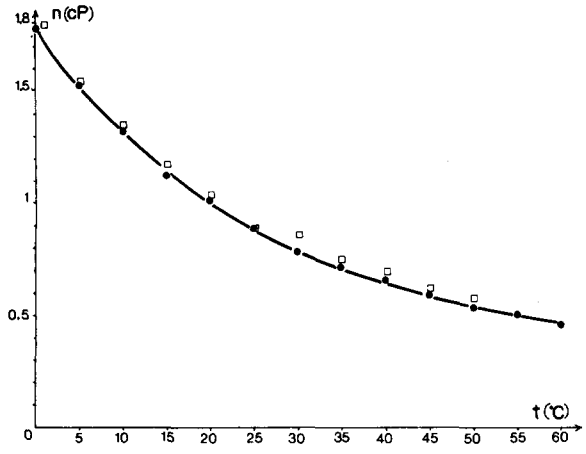

Fig. 2. Viscosity of distilled water at different temperatures measured (□) with the Hemorheometer (the calibration is done at 25°C). Correlation with the values (●) deduced from empirical law [31].

IR is a parameter which depends only on the average individual erythrocyte deformability property and not on the cells' interactions. This conclusion has been developed elsewhere [33]. Furthermore, we have shown that, from this index and two hematocrit determinations, a significant average transit time through one pore could be deduced (Koutsouris, D., Hanss, M. and Skalak, R., unpublished data).

Preparation of erythrocyte suspensions

The erythrocyte suspension is prepared without any washing of erythrocytes, according to the previously described technique which gives low white blood cell counts [30]. The usual [30] isotonic suspending medium (buffer 1) is a Tris-HCl buffer (pH = 7.4) with added NaCl, KCl, $CaCl_2$ and glucose at physiological levels and a slight amount of human serum albumin (0.5 g/l; Sigma No. A-2386 Albumin, Human Fraction V). The buffer is always prefiltered through a 0.45 μm Millipore membrane before any preparation of suspension. The suspension haematocrit is about 8%; its exact value is measured by a conductimetric method [34] as the microcentrifuge technique has very poor precision at such low values, and as the indirect methods through the mean cell volume measurements may be unreliable, for they depend on the deformability [35].

Preparation of ghost suspension

Usually erythrocyte ghosts are prepared according to the method of Dodge et al. [60]. However, a milder technique, similar to the one described by Usami and Chien [36], has been used for this work, as we wanted intact ghosts, with as few membrane fragments as possible.

The blood samples are collected from normal volunteers in 'Venoject' tubes (anticoagulant: heparin) and used in the following 1 h. Two 10 ml tubes are filled with the blood and centrifuged (10 min; $600 \times g$). The middle part (1.5 ml) of each packed cells column is collected and 6 ml of 50% haematocrit erythrocyte suspension (in buffer 1) are prepared. This suspension is put into a cellophane dialysis tubing and a 3h dialysis at room temperature against 200 ml of a hypotonic (30 mosM) buffer 1 (decreased NaCl concentration) is performed. Afterwards, the suspension is centrifuged (conical 10 ml tubes; volume adjustments with hypotonic buffer 1; 4°C, 20 min; $10\,000 \times g$). After the centrifugation, the supernatant is eliminated and replaced by isotonic buffer 1 and another similar centrifugation is performed (after resuspending the membranes). At the end of this second centrifugation, the supernatant is collected and discarded; 0.4 ml of membranes is very slowly (1 min) aspirated in a 1 ml syringe and diluted in buffer 1 in order to obtain a final 8% volume concentration ghost suspension. Exceptionally, some ghost preparations have been washed up to five times.

Preparation of hemoglobin solution

Outdated blood-bank samples are washed twice with NaCl 2 M ($600 \times g$ centrifugation); the supernatant is thrown away and the cells are broken through three or four freezing and thawing procedures (using liquid nitrogen); a known quantity of pure NaCl is added so as to obtain a 2 M final concentration. The resulting solution is stirred for 2 h in the cold (4°C). A $1700 \times g$ centrifugation is then performed (at 4°C) for 40 min. The supernatant is collected and vacuum-concentrated, then dialyzed against a 0.15 M KCl solution. This allows the preparation of pure hemoglobin solutions at a concentration of about 31.5 g/dl, as controlled through spectrophotometric measurements.

Viscosity measurements

An automated Ubbelohde capillary viscometer is used (Viscomatic MS, FICA HS830) at different temperatures between 10 and 35°C. Though the viscosity of hemoglobin solutions becomes highly concentration-dependent above about 30 $g \cdot dl^{-1}$ [37,38], the viscosity has been measured at a single concentration, $c = 29.6$ g/dl, which is very near the value inside the normal erythrocyte [29]. At each temperature, the viscosity of a given solution is obtained by averaging ten flow measurements for the hemoglobin (Hb) solution and for the KCl solution. The density correction at each temperature is made by means of a Sovec Inc., 02D densimeter. The viscometer is calibrated with a 0.14 M KCl solution for the different temperatures. The results are given as a relative viscosity, $\eta_{rel} = \eta_{Hb}/\eta_{KCl}$, and a reduced viscosity, $\eta_{red} = (\eta_{rel} - 1)/c$, where η_{Hb} and η_{KCl} are, respectively, the viscosity for the hemoglobin and KCl solutions at a given temperature.

Expression of results

The suspension to be measured (erythrocyte or ghost) is prepared and controled as described above. The filtration apparatus is fitted with the membranes and set at the lowest temperature (4°C; 15 min equilibrium). The buffer and the suspension are put into the thermostat bath for temperature equilibration for 5 min before each different temperature setting. Permanent gentle shaking prevents erythrocyte sedimentation during this period.

The membrane is calibrated by measuring t_b; then, without changing nor moving the filter, the suspension filtration rate is determined. In our apparatus, t_b is about 0.5 s and t_s about 0.8 s for normal erythrocytes at 8% hematocrit and 25°C. The reproducibility is $\pm 1\%$ for the time-lapse measurements and $\pm 3\%$ for IR on a given blood sample or suspension.

We will use in this work the variations of IR relative to a standard value:

$$\Delta IR(\%) = \frac{IR_i - IR_0}{IR_0}$$

where IR_i is the IR value measured at temperature T_i and IR_0 is the value measured at the standard $t = 25$°C value. If the erythrocyte rheological properties had the same temperature coefficient as the buffer's (mainly the water viscosity), IR would be essentially temperature-independent (as the ratio would be constant); therefore $\Delta IR(\%)$ would be equal to zero at any temperature.

The use of a differential index, such as ΔIR, amplifies all the variations of the rheological properties. However, it can be applied only when the accuracy of the measurements is high enough, such as obtained with the Hemorheometer'.

Results

As the measurements at different temperatures are time-consuming, we have found it impossible to make filtration determinations at numerous temperatures over the whole 4–50°C range. Therefore, we have concentrated the measurements on a given sub-range for a given sample. The results for the erythrocytes are shown on Table I and summarized in Fig. 3. A sharp transition above 49°C is demonstrated. Another transition, less important but significant, is also observed between 15°C and 20°C.

In order to compare the erythrocytes and their membrane deformability, we have prepared the ghosts with a mild dialysis so that they were essentially intact. This has been verified by comparing the volume distribution curves obtained for

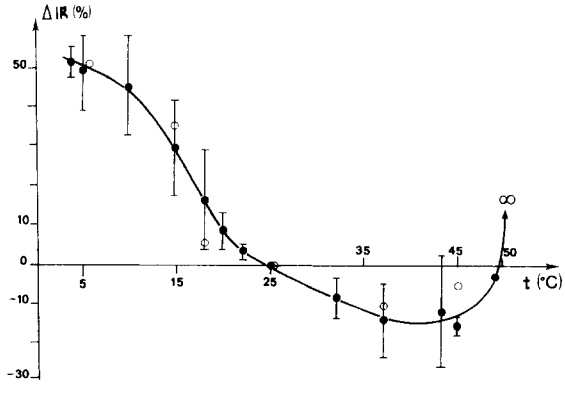

Fig. 3. Rigidity index variations (relative to the 25°C value) for erythrocytes (●) and ghosts (○). Each point is the average of all the measurements made at the temperature shown (see Table I); the standard deviation of each measurement is given by an error bar.

TABLE I

RIGIDITY INDEX (IR) OF ERYTHROCYTES AS A FUNCTION OF TEMPERATURE FOR 19 DIFFERENT NORMAL DONORS

The values between brackets are the IR variation (%) relative to the 25°C value: IR, ΔIR(%) and $\overline{\Delta IR}(\%) \pm$S.D. are the average values for a given temperature.

t (°C)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	IR	$\overline{\Delta IR}(\%)$ ±S.D.
4												15.9 (54.8)			14.5 (49.1)					15.6	51.9± 4.0
5	14.8 (53.8)	16.1 (55)			15.2 (37.8)	11.5 (49.1)	15.9 (62.2)	13.8 (34.8)			16.7 (50.5)									14.8	49.0± 9.6
10	15 (55.1)	16 (54.4)				11.8 (53.5)	15.5 (59)	12.1 (17.5)	12.7 (30)	13.4 (25.3)	15.3 (37.8)	15.5 (52.8)	14.5 (45)	15.8 (45)	14.1 (44)	13.9 (46.3)				14.3	43.6±12.1
15						11.4 (47.7)	14.9 (52.1)	11.3 (9.8)	13 (32.7)	13.7 (28)	13.5 (21.6)	12.6 (23.5)	12.2 (23.3)	13.6 (24.8)	12.1 (23.5)	11.8 (24.2)				12.7	28.3±12.1
18						11.9 (55.6)		11.7 (13.5)	11 (12.2)	11.7 (9.4)	12.1 (9)	11.5 (12.8)	11 (11.1)	12.4 (13.8)	11.3 (15.3)	11.0 (15.8)				11.6	16.9±13.7
20						9.3 (20.2)	10.7 (9)	11.3 (9.8)	10.7 (9.2)	11.1 (3.8)	11.9 (7.2)	11.2 (7.8)	10.7 (8)	12 (10.1)	10.7 (9.2)	10.2 (7.4)				10.9	9.3± 4.0
22																	11.0 (3.8)	10.7 (2.9)	11.0 (2.8)	10.9	3.2± 0.5
25	9.6	10.4	11.2	9.8	11	7.7	9.8	10.3	9.8	10.7	11.1	10.2	9.9	10.9	9.8	9.5	10.6	10.4	10.7	10.1	0
30					10.1 (−7.6)			10.2 (−1)												10.2	−4.3± 4.6
37			8.3 (−25.6)	9.3 (−4.9)	9.6 (−12.4)															9.1	−14.3±10.4
44	9.1 (−5.2)	10.3 (−1)	7.5 (−33)																	9.0	−13.1±17.3
45				7.8 (−19.9)	8.9 (−18.9)															8.4	−19.4± 0.7
49.5				10 (−3.2)																10	−3.2
50	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞											

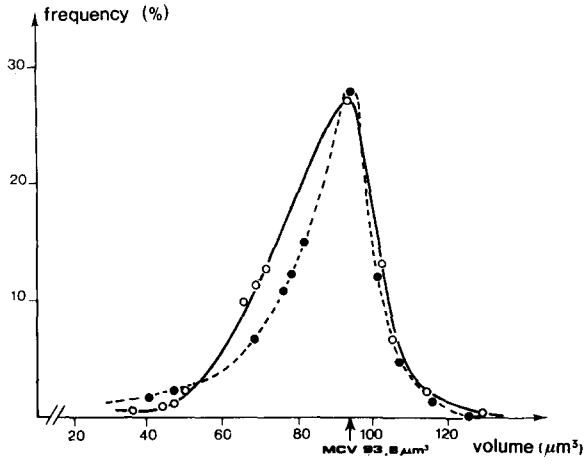

Fig. 4. Distribution curves of the erythrocyte (●— — —●) and of the ghost (○——○) volumes, as measured with a Coulter counter.

TABLE II

GHOST RIGIDITY INDEX (FOR TWO DIFFERENT SAMPLES) AS A FUNCTION OF THE NUMBER OF WASHINGS

Number of washings:	1	2	3	4	5
Sample 1	9.9	9.7	11.4	11.5	12.5
Sample 2	10.2	10.5	10.8	12.1	12.8

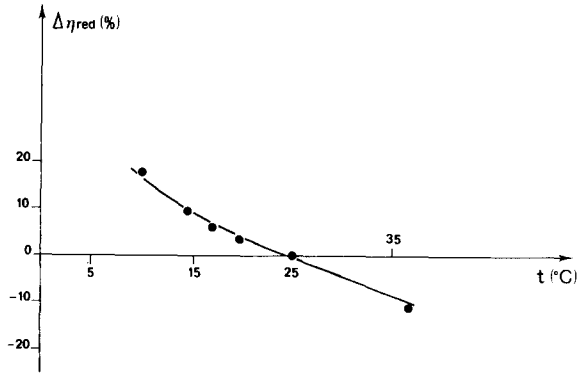

Fig. 5. Variation of the relative reduced viscosity as a function of temperature for a 30 $g \cdot dl^{-1}$ hemoglobin solution (same scale as in Fig. 3).

TABLE IV

VISCOSITY OF A 30 $g \cdot dl^{-1}$ HEMOGLOBIN SOLUTION AS A FUNCTION OF TEMPERATURE

See text for the definitions of η_{rel}, η_{red} and $\Delta\eta_{red}$.

t(°C)	η_{rel}	η_{red}	$\Delta\eta_{red}$(%)
10	7.49	0.219	+17.7
15	6.98	0.209	+8.1
17	6.71	0.196	+5.4
20	6.68	0.192	+3.2
25	6.49	0.186	0
37	5.88	0.164	+11.8

TABLE III

RIGIDITY INDEX OF GHOSTS PREPARED WITH A MILD DIALYSIS TECHNIQUE AS A FUNCTION OF TEMPERATURE

Same definitions as for Table I.

t(°C)	Number 1	2	3	$\overline{IR}$	$\overline{\Delta IR}$ (%)
6	14.2	16.5		15.4	50.0
	(55.3)	(44.7)			
15	13.6	15.4		14.5	40.9
	(48.6)	(33.1)			
18	9.9	11.9		10.9	6.2
	(8)	(4.4)			
25	9.2	11.4	11.4	10.7	0
	(0)	(0)	(0)		
37	8.1		10.7	9.4	−8.6
	(−11.1)		(6.1)		
45			10.5	10.5	−7.9
50		22			
		(−92.1)	∞		

TABLE V

RIGIDITY INDEX OF THE INTACT ERYTHROCYTES AND THEIR MEMBRANES ON SEVEN DIFFERENT NORMAL DONOR BLOOD SAMPLES (MEASUREMENTS MADE AT 25°C)

	Number							
	1	2	3	4	5	6	7	$\overline{IR} \pm$ S.D.
Erythrocytes	9.7	10.2	10.8	9.9	9.9	10.3	10.3	10.2 ± 0.36
Ghosts	9.2	11.0	11.3	10.7	12.5	10.7	11.4	11.0 ± 1.0

a given erythrocyte suspension to the one obtained with the membrane suspension. Fig. 4 shows that they are very similar (the volumes are measured with a Coulter counter model Z.B.I. with the C1000 attachment).

In a preliminary study, we have shown that the ghost rigidity index increased regularly with the number of washings (Table II). The origin of this phenomenon is not clear; a similar result has been observed for the erythrocytes [30]. Therefore, the studies on the temperature variation of the IR of ghosts have been performed on suspensions washed only twice. This experiment has been done on three different samples. The results are shown in Table III and summarized in Fig. 3.

Table IV gives the values of the η_{rel} and η_{red} of the hemoglobin solution at different temperatures. Fig. 5 shows the relative (to 25°C) temperature variations of η_{red}. A significant increase in η_{red} is demonstrated when the temperature is lowered. This could be explained by increased interactions between the protein molecules. No transition appears between 10 and 25°C.

Discussion

The erythrocyte deformability is governed by three main determinants: excess membrane area, intracellular viscosity and membrane viscoelasticity [29]. Their relative importance is usually not well known for normal erythrocytes. In particular, they depend on the measurement techniques, static methods being essentially sensitive to the membrane elastic moduli and dynamic methods being sensitive in a variable manner to the membrane and cell content viscosity.

As measured by the initial filtration flow rate method, the erythrocyte filterability is an intrinsic cellular rheological property which can be used as a deformability measurement. The results demonstrate that, on normal cells, this deformability is mainly governed by the rheological membrane properties. Indeed, when a mild hemolysis technique is used in order to obtain intact ghosts, IR is very similar for the ghosts and the original cells (Table V). Moreover, the ghost rigidity index increases with the number of washings, as is also found when measuring the erythrocyte rigidity, either after washing procedures, or after a long incubation in the buffer [30]. The temperature variations of IR for the ghosts and the erythrocytes (Fig. 3) are also very similar, which is another argument for a membrane origin of the erythrocyte deformability.

The 49–50°C transition is well known [1–7] and has been correlated with the spectrin thermal denaturation profile [7,39–41]. We cannot say yet whether the difference in the profiles for the erythrocytes and the ghosts is significant; if this were the case, it could give some insight on the interactions between the membrane interface and the intracellular content.

Our main finding is the rigidity transition in the 15–20°C temperature range which has not been found previously by most of the workers studying the erythrocyte deformability in the same temperature range. The results previously obtained by filtration techniques [3,42–45] have a much lower accuracy than ours because they use ill-defined experimental conditions (as regards the membrane plugging and the actual temperature). However, by measuring the passage time of the first 0.05 l of human or fish whole blood through 5 μm Nuclepore filters, Kikuchi et al. [46] were able to show a sharp rise in the erythrocyte rigidity below 15–18°C.

Investigation of the temperature variation of

the elongation of human erythrocyte ghosts as a shear rate using the Ektacytometer technique has recently been described [47]. No variations were reported between 10 and 45°C. Above this temperature a progressive rigidification was demonstrated. This result is, however, not comparable to ours, as it was obtained with variable incubation temperature, the deformability measurements being done at room temperature. Only irreversible temperature effects could then be shown.

The influence of temperature on erythrocyte deformability has been studied by elongation attached cells in a given shear rate between 2 to 50°C and measuring the elongation after glutaraldehyde fixation [5]. Though the measurements were performed at only five different temperatures between 2 and 48°C, the reporeted results show a significant deformability increase in the 10–25°C region.

The elongation recovery relaxation time τ between 6 and 37°C has been studied and the cell surface viscosity has been deduced assuming that the cell content viscosity had a negligible influence on τ [48]. No transition was observed in the plotted recovery time figures (if, however, the calculated surface viscosity is plotted versus temperature, a break appears in the 20°C region). The surface viscosity calculation depended strongly on the surface shear elasticity modulus results given by Waugh and Evans [49] and by Evans and Skalak [50]. In these papers, the cell elasticity was determined between 5 and 45°C by the micropipette technique. A regular decrease in the elastic area compressibility modulus was found for increasing temperatures. The same result was shown for the elastic shear modulus (which is probably the main parameter measured in filtration experiments). However, as many independent measurements must be averaged, the elasticity determinations is long and tedious so that the resulting accuracy is poor. The variation coefficient on these direct measurements was about 15% (as compared to 2% with our method). If a relative value is used, similar to ΔIR, these experimental errors become much larger than the total thermal transition amplitude which is looked for (40% as measured with our technique and expressed as ΔIR). Another factor which may obscure the comparison between the two results is the important side-effect recently reported [51]. Indeed, a strong albumin-mediated interaction between the cell membrane and the glass micropipette well has been found which may seriously disturb any intrinsic cell membrane thermal property. On the other hand, numerous authors using various methods have already shown a thermal transitions in the 15–20°C range, either on intact or sonicated ghosts or on artificial lipid membranes. Around 20°C, a sudden change in the membrane structure of intact erythrocytes has been suggested by studying the temperature dependence of the uptake of glucose [9]. A change around 19°C in the thermal viscosity curve of sonicated ghosts has been shown [10]. The band intensity at the C-H stretching frequencies in intact or sonicated ghosts and liposomes has been studied at different temperatures [11]. A sharp transition of the intensities has been demonstrated in the 0°C and also in the 17°C region.

With ESR, NMR or fluorescence probes and by X-ray studies, the same transition has often been observed [52]. By studying the width of the 129 MHz ^{31}P-NMR spectra of pronase-digested, diethyl ether-extracted ghosts, a significant transition has been shown at 20°C [12,14]. It was correlated with sphingomyelin, which would enter a gel state below 20°C. By spin-labeling intact erythrocytes with various classes of phospholipid label, a transition at 18°C has also been found, but a phosphatidylcholine origins suggested [13]. Discontinuities at various temperatures near 20°C by have been observed studying on erythrocytes the ESR spectrum of the spin label 5-nitroxide stearic acid [19]. In ghosts, two transitions in the 13.5–16.5°C and in the 37.5–40.5°C regions have been shown through studying the ESR spectrum of the 16-nitroxide stearate, which probes the hydrophobic part of the phospholipidic bilayer [53]. The same results have been obtained by others [54] with the same spin label.

Recently, through a positron lifetime study, a sudden change in the ortho-positronium annihilation rate has been demonstrated in human erythrocyte ghosts at 18–20°C [18]. In studying the role of membrane lipids in cold agglutination of human erythrocytes, a marked slope change at 16°C of the temperature dependence of an agglutination parameter (relative equilibrium association constant) has been shown [20].

However, Gottlieb and Eanes [23] have criticized the viscosity results of Zimmer and Schirmer [10], as they could not find by an X-ray diffraction study on ghosts any rigid crystalline state upon lowering the temperature to −20°C. Maraviglia et al. [25] have studied the ^{2}H-NMR spectrum of phospholipid probes incorporated into intact human erythrocyte ghosts. They conclude that the membrane keeps a liquid crystalline phase down to −5°C. But, if their results for the first moment of the spectra are re-plotted in the same relative units as IR, a significant transition appears in the 15–20°C region. Therefore, any results on the membrane fluidity thermal variation should always be discussed taking into account the experimental precision.

The origin of this transition around 15°C is presently unclear. It can be the consequence of a membrane reorganization due to a modification in the equilibrium between domains in a bilayer structure and domains in a micellar structure, as suggested recently by Hope and Cullis [55]. The rôle of the cell proteins should also be discussed.

As shown in Fig. 5, the hemoglobin viscosity does not show any low-temperature transition, even if a relative viscosity variation (comparable to ΔIR) is used. Moreover, the same transition seems to be demonstrated also for the ghosts (residual hemoglobin content of about 5% after two washings).

The possible role of spectrin in the cell membrane rheology has been discussed [56] in view of its hyperelastic properties, a shown by the elastic behavior of the membrane after large irreversible deformations. A model has been proposed in which the membrane lipid phase would behave as a pure liquid, with a zero elastic shear modulus. The semi-solid properties of the underlying protein network could then explain the permanent erythrocyte deformation which appears at the high, long-duration stresses which are used in cell elongation experiments [51]. In erythrocyte filtration experiments, the stress duration is short: typical cell transit times are in the millisecond region [30,32]. Under these conditions, the application of Evans' hypothesis may not be straightforward. Moreover, Evans and Skalak have recently [50] stressed the speculative character of the rôle of spectrin in membrane rheology.

Evidence has been given [57] that the lipid bilayer (and not the spectrin) is the main determinant of the erythrocyte ghost shape. The dependence (near a lipid transition) of the cellular membrane elastic response on the interactions between the lipids and the intrinsic proteins has been pointed out [58]. Therefore we think that the relationship between the membrane rheology and its structure is still an open matter.

In conclusion, by using a new rheological method based on the initial filtration flow rate through 5 μm pores of diluted erythrocyte suspensions, which has a significantly higher accuracy than the previous techniques and which is well temperature-controlled, we have been able to show two thermal transitions. The one between 49°C and 50°C can reasonably well be attributed to denaturation of membrane protein (probably spectrin). Another deformability transition (not as important as the first one) is observed for the first time between 15 and 20°C (higher rigidity below this transition). As it occurs for the ghosts as well as for the erythrocytes, and as by studying different properties a similar transition has been found by numerous authors on intact or sonicated cell membranes and on artificial lipid bilayers, we think that in normal erythrocytes at room or body temperature, the cell deformability depends mainly on the membrane viscoelasticity. Therefore, in pathological conditions where erythrocyte deformability is reduced, not only the hemoglobin abnormalities must be searched for, but also the membrane composition and structure (endoface and intrinsic proteins, lipids, protein and lipid interactions); indeed, clinical results have already shown alterations in the erythrocyte membrane lipid composition, in correlation with reduced deformability [59].

References

1 Ham, T.H., Shen, S.C., Flemings, E.M. and Castle, W.B. (1948) Blood 3, 373–403
2 Coakley, W.T. and Deeley, J.O.T. (1980) Biochim. Soc. Trans. 8, 110–111
3 Teitel, P. (1965) Nature 206, 409–410
4 Ham, T.H., Sayre, R.W., Dunn, R.F. and Murphy, J.R. (1968) Blood 32, 862–871
5 Williamson, J.R., Shanahan, M.O. and Hochmuth, R.M. (1975) Blood 46, 611–614

6 Deeley, J.O.T., Crum, L.A. and Coakley, W.T. (1979) Biochim. Biophys. Acta 554, 90–101
7 Nagasawa, T., Kojima, S. and Kimura, E. (1982) Jpn. J. Physiol. 32, 25–33
8 Hamilton, R.C., Alberson, T.O. and Harwig, J.S. (1976) J. Nucl. Med. 17, 1038–1043
9 Lacko, L., Wittke, B. and Geck, P. (1973) J. Cell Physiol. 82, 213–218
10 Zimmer, G. and Schirmer, H. (1974) Biochim. Biophys. Acta 345, 314–320
11 Verma, S.P. and Wallach, D.F.H. (1976) Biochim. Biophys. Acta 436, 307–318
12 Cullis, P.R. (1976) FEBS Lett. 68, 173–176
13 Tanaka, K.I. and Ohnishi, S.I. (1976) Biochim. Biophys. Acta 426, 218–231
14 Cullis, P.R. and Grathwohl, C. (1977) Biochim. Biophys. Acta 471, 213–266
15 Brahm, J. (1977) J. Gen. Physiol. 70, 283–306
16 Obaid, A.L. and Grandall, E.D. (1979) J. Membrane Biol. 50, 23–41
17 Galla, H.J. and Luisetti, J. (1980) Biochim. Biophys. Acta 596, 108–117
18 Chow, E.I.H., Chuang, S.Y. and Tseng, P.K. (1981) Biochim. Biophys. Acta 646, 356–359
19 Herrmann, A., Arnold, K., Lassmann, G. and Glasser, R. (1982) Acta Biol. Med. Germ. 41, 289–298
20 Flamm, M., Basu, M.K., Schachter, D., Bertles, J.F. and Maniatis, A. (1982) Blood 60, 340–344
21 Ladbrooke, B.D.and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–307
22 Sheetz, M.P. and Chan, S.J. (1972) Biochemistry 11, 548–555
23 Gottlieb, M. and Eanes, E.D. (1974) Biochim. Biophys. Acta 373, 519–522
24 Davis, J.H., Maraviglia, B., Weeks, G. and Godin, D.V. (1979) Biochim. Biophys. Acta 500, 362–366
25 Maraviglia, B., Davis, J.H., Bloom, M. and Westerman, J. (1982) Biochim. Biophys. Acta 686, 137–140
26 Fung, Y.C. (1980) Biomechanics: Mechanical properties of living tissues, Springer-Verlag, New York
27 Skalak, R. (1981) Scand. J. Clin. Invest. 41 Suppl., 55–58
28 Schmid-Schönbein, H. and Gaehtgens, P. (1981) Scand. J. Clin. Invest. 41 Suppl. 13–26
29 Chien, S. (1979) in Quantitative Cardiovascular Studies (Hwang, N.H.C., ed.), pp. 272–287. University Park Press, Baltimore
30 Hanss, M. (1983) Biorheology 20, 199–211
31 Hardy, R.C., Cottington, R.I. and Swindells, J.C. (1977) in Handbook of Chemistry and Physics (Weast, R.C. ed.), ÇRC Press, Baton Rouge, LA
32 Koutsouris, D. (1982) Thesis for doctorate (Biomechanics) University of Paris XIII.
33 Skalak, R., Hanss, M. and Chien, S. (1983) Biorheology 20, 311–316
34 Koutsouris, D. and Hanss, M. (1983) Innov. Technol. Biol. Med. 1, 85–94
35 Corry, W.D. and Meiselman, H.J. (1978) Blood Cells 4, 465–480
36 Usami, S. and Chien, S. (1973) Biorheology 10, 425–430
37 Ross, P.D. and Minton, A. (1977) Biochem. Biophys. Res. Commun. 76(4), 971–976
38 Cokelet, G. and Meiselman, H. (1968) Science 162, 275–277
39 Brandts, J., Erickson, L., Lysko, K., Schartz, A.T. and Taverna, R.D. (1977) Biochemistry 16, 3450–3454
40 Mohandas, N., Creenquist, A.C. and Shohet, S.B. (1978) in The red cell, pp. 453–472, Allan R. Liss, New York
41 Cassoly, R., Daveloose, D. and Leterrier, F. (1980) Biochim. Biophys. Acta 601, 478–489
42 Murphy, J.R. (1967) J. Lab. Clin. Med. 69, 758–775
43 Schmid-Schönbein, H., Weiss, J. and Ludwig, H. (1973) Blut 26, 369–379
44 Leblond, P.F. and Coulombe, L. (1979) J. Lab. Clin. Med. 94, 133–143
45 Kenny, M.W., Meakin and Stuart, J. (1981) Clin. Hemorheol. 1, 135–146
46 Kikuchi, Y., Hughes, G.M. and Albers, C. (1982) Experientia 38, 822–824
47 Heath, B.P., Mohandas, N., Wyatt, J.L. and Shohet, S.B. (1982) Biochim. Biophys. Acta 691, 211–219
48 Hochmuth, R.M., Buxbaum, K.L. and Evans, E.A. (1980) Biophys. J. 29, 177–182
49 Waugh, R. and Evans, E.A. (1979) Biophys. J. 26, 115–132
50 Evans, E.A. and Skalak, R. (1980) in Mechanics and Thermodynamics of Biomembranes, pp. 180–190, CRC Press, Baton Rouge, FL
51 Markle, D.R., Evans, E.A. and Hochmuth, R.M. (1983) Biophys. J. 42, 91–98
52 Lee, A.G. (1975) Prog. Biophys. Mol. Biol. 29, 3–56
53 Sato, B., Nishikida, K., Samuels, L.T. and Tyler, F.H. (1978) J. Clin. Invest. 61, 251–259
54 Laurent, M., Daveloose, D., Leterrier, F., Fischer, S. and Shapira, G. (1980) Clin. Chim. Acta 105, 183–194
55 Hope, M.J. and Cullis, P.R. (1981) Biochim. Biophys. Acta 640, 82–90
56 Evans, E. and La Celle P. (1975) Blood 45(1), 29–43
57 Lange, Y., Gough, A. and Steck, T.L. (1982) J. Membrane Biol. 69, 113–123
58 De Lacey, E.H.B. and Wolfe, J. (1982) Biochim. Biophys. Acta 692, 425–430
59 Garnier, M., Hanss, M. and Paraf, A. (1983) Clin. Hemorheol. 3(1), 45–52
60 Dodge, I.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119–134