Biochimica et Biophysica Acta, 775 (1984) 291–296
Elsevier

BBA 72203

DECREASE OF TRANSPORT OF SOME POLYOLS IN SICKLE CELLS

C.T. CRAESCU [a,*], R. CASSOLY [b], F. GALACTEROS [a] and C. PREHU [a]

[a] *INSERM U.91, Hôpital Henri Mondor, 94010 Créteil and* [b] *Institut de Biologie Physico-chimique, 13, rue Pierre et Marie Curie, 75005 Paris (France)*

(Received March 26th, 1984)

Key words: Erythrocyte membrane; Polyol transport; Non-electrolyte transport; Sickle cell; Kinetics

This paper reports the results of kinetic studies on the inward net-flux of small non-electrolytes (ethylene glycol, glycerol and erythritol) in sickle cells as compared to normal erythrocytes. Net transport rates were evaluated by turbidimetric measurements for ethylene glycol and glycerol and by hematocrit monitoring for erythritol. A 2-fold and 4-fold reduction in the permeability coefficient for ethylene glycol and glycerol, respectively, were found in sickle cells as compared to normal erythrocytes. In contrast, no significant changes in erythritol transport kinetics were observed. The dependence of glycerol permeability on temperature, pH and oxygenation is the same in both types of cells. A significant correlation was observed between glycerol permeability and cell density only for sickle cells. The results indicate that irreversible modifications of membrane proteins, responsible for the glycerol and ethylene glycol transport, do occur in sickle cells.

Introduction

In contrast to the structural and functional properties of hemoglobin S which are now well documented [1], the membrane abnormalities of the sickle cells are far from being coherently understood. The abnormal intracellular hemoglobin S induces, by a direct and/or an indirect effect, cumulative damages in the membrane structure, with physiological consequences [2].

The study of membrane-mediated transports in red blood cells can give information not only on a functional cellular aspect, but also, on the structural membrane support of these processes.

In sickle cells, the first to be studied was the cationic transport (Na^+, K^+, Ca^{2+}), in connection with the problem of cellular dehydration [3]. The changes in cationic content of sickle cells are associated with an increased passive cation transport [4–6], a reduced Na^+/K^+ pump [7] and an increased Gardos effect [8,9].

An increased transport of Cl^-, SO_4^{2-} [10] and pyridoxal phosphate [11] anions was also reported.

The characterization of the possible membrane lesions responsible for these changes in permeability is especially difficult to establish since the electrolyte transport can also depend on the metabolic energy reserve as well a on the presence of cytoplasmic regulators [9]. In order to overcome these difficulties, we choosed to study the net-flux of three non-electrolytes: ethylene glycol, glycerol and erythritol.

This paper reports the results of these studies and offers some possible explanations for the effects observed in sickle cells as compared with normal erythrocytes.

* To whom correspondence should be addressed.

Abbreviations: AA cells, hemoglobin A-containing red blood cells; SS cells, hemoglobin S-containing red blood cells; PCMB, *p*-chloromercuribenzoate.

Materials and Methods

Reagent grade glycerol and ethylene glycol were obtained from Merck and erythritol from Sigma. The osmolality of solutions was measured from freezing-point depression with a Roebling osmometer.

The blood was collected by venipuncture in a EDTA-containing isoosmolal medium; red blood cells were immediately separated by centrifugation (10 min at $3000 \times g$) and subsequently washed three times in phosphate-buffered solution at 4°C.

All experiments, except when specified, were performed at room temperature (23 ± 2°C).

Membrane permeability for ethylene glycol and glycerol was measured by turbidimetry [12]. Briefly, 1 ml of a dilute suspension of washed red blood cells in phosphate-buffered saline was rapidly mixed with 2 ml of a 300 mM solution of the permeant and the absorbance at 640 nm was continuously recorded on a Carry 118 spectrophotometer (in the blank cell 2 ml of distilled water was added to the cell suspension). The linear decay of the absorbance in the initial part of the experiment (Fig. 1) gives a measure of the time constant of the exponential increase in volume. For slow penetrants like glycerol or ethylene glycol it is possible, starting from this time constant (τ), to compute the permeability (P) according to the relation [13]:

$$\tau \cdot \pi_m = \frac{\pi_m(b-a)+(1-b)}{P \cdot A/V_0} + \frac{(1-b)\sigma^2}{P \cdot A/V_0}\frac{\pi_s}{\pi_m} \qquad (1)$$

where $(1-a)$ is the fraction of cell water allowable for the penetrant (the glycerol and ethylene glycol may dissolve in 96% of the cell water, which represents 72% of the total cell volume); π_m and π_s are the normalized osmotic pressure for the impermeants and permeants, respectively; A/V_0 is the cell surface/volume ratio at isotonicity; $(1-b)$ is the fraction of cell water, osmotically active, determined as in Ref. 14 by the microhematocrit measurements, and σ is the reflection coefficient for the permeant. V_0 was evaluated with a Coulter Counter S Plus II.

Erythritol transport was studied by microhematocrit measurements of red cell suspension (~ 30% hematocrit) in phosphate-buffered saline with an initial concentration of 150 mM erythritol [15]. The sample was gently shaken in a water bath thermostated at 37°C throughout the experiment. The continuous increase in hematocrit reflects the kinetic of the erythritol inward transport.

Fractionation of red blood cells was made by centrifugation in a stractan density gradient, according to Ref. 16. The pH values of the phosphate-buffered saline in the range 5.8–8.2 were established by changing the mixing ratio of the acidic and alkaline phosphate solutions; the red blood cell suspensions were equilibrated for 30 min in each buffer.

Results

Fig. 1 represents a typical recording, absorbance versus time, of the kinetics of the inward glycerol transport for AA and SS cells. At least 60% of the total absorbance variation is linear.

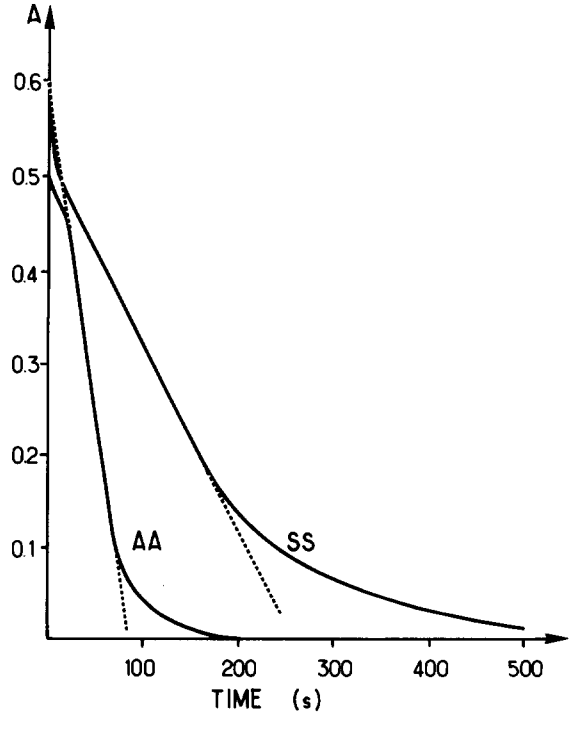

Fig. 1. Typical recordings in glycerol transport experiments on AA cells and SS cells. Absorption (A) versus time after mixing the cell suspension (0.5% hematocrit in phosphate-buffered saline) with the glycerol solution (200 mM, final concentration). The initial, linear, variation of the absorbance (interrupted lines) was used for the permeability determination.

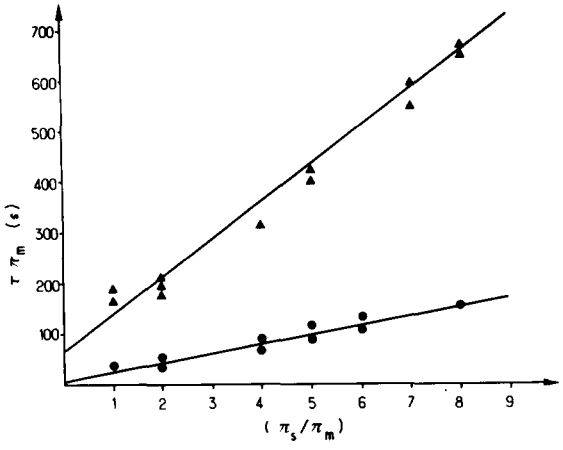

Fig. 2. The linear relationship between the modified time constant $\tau \cdot \pi_m$ and the osmotic ratio π_s/π_m for glycerol penetration in AA (▲) and SS (●) cells. The correlation coefficients for the two regression lines are $r = 0.96$ and $r = 0.98$ for AA and SS cells, respectively.

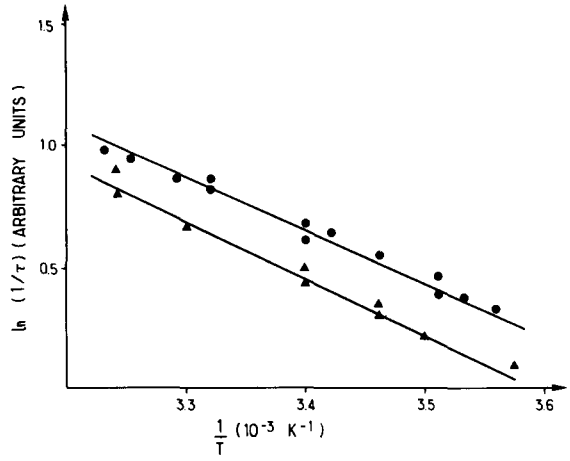

Fig. 3. Arrhenius plot for the temperature dependence of glycerol permeability in a AA (●) and SS (▲) cell sample. From the slope of the regression lines a temperature coefficient of 4.40 kcal/mol and 4.7 kcal/mol for AA and SS cells, respectively, was calculated.

Taking into account that changes in cell volume are linearly related to the logarithm of light transmission [12] the reciprocal of the initial slope of the curves in Fig. 1 represents the time constant (τ) of the exponential increase of cell volume. As results from Eqn. 1, for a constant π_m, the plot of $\tau \cdot \pi_m$ versus π_s/π_m must be a straight line. This was the case for both types of cells (Fig. 2). Using the slopes of the two lines together with the cell parameters $a = 0.31$, $\sigma = 0.92$ [13] permeability values of $P = 2.22 \cdot 10^{-6}$ cm/s and $P = 0.49 \cdot 10^{-6}$ cm/s for the AA and SS samples, respectively, were derived from Eqn. 1. For most of the samples we computed the glycerol permeability at a single permeant concentration (0.2 M) using the complete form of Eqn. 1. For 12 normal subjects we found a mean value of $P = (2.87 \pm 0.59) \cdot 10^{-6}$ cm/s and for 14 homozygous SS patients a mean value of $P = (0.73 \pm 0.20) \cdot 10^{-6}$ cm/s. The values found for AA cells are in good agreement with the literature data [17]. For SS cells, the mean glycerol permeability is 4-times lower.

Using the same Eqn. 1 and $\sigma = 0.81$ [13], we determined the ethylene glycol permeability, at an outside concentration of 0.2 M, for six normal blood samples and seven sickle cell samples. The mean values were $P = (8.5 \pm 2.4) \cdot 10^{-6}$ cm/s and $(4.28 \pm 0.76) \cdot 10^{-6}$ cm/s, respectively. The permeability of sickle cells was significantly ($P < 0.05$) lower than in normal erythrocytes. From the measurements of hematocrit versus time we found a mean half-time of $t_{1/2} = 4 \pm 0.5$ min ($n = 4$) for the erythritol transport in normal erythrocytes. The same mean value was found for four sickle cell samples (not shown).

In order to characterize further the glycerol permeability differences between AA and SS cells, we varied some physico-chemical parameters of this transport in both types of cells.

Temperature dependence

Glycerol permeability was measured at different temperatures in the range 4–39 °C; a small temperature-dependent pH increase of phosphate-buffered saline was noted in this interval (the linear temperature coefficient was 0.0072 pH units/K). For two AA patients we obtained a temperature coefficient of 4.40 and 5.20 kcal/mol, respectively, and for two homozygous SS patients we obtained 4.70 and 5.47 kcal/mol (Fig. 3). At 1 mM glycerol concentration Carlsen and Wieth [17] found an activation energy of approx. 10 kcal/mol. Our values are similar to the temperature coefficients for water filtration [13,18] or diffusion [19], or for methylurea diffusion [20] in human erythrocytes.

Apparently, there was no significant difference between the temperature coefficients of the AA and SS erythrocyte permeability for glycerol.

Oxygenation state

In order to study the effect of hemoglobin S polymerization on the membrane transport properties we determined the glycerol permeability of SS cells, at two temperatures (7 °C and 37 °C) in oxy and deoxy conditions. The deoxygenation was performed by purging with humidified nitrogen the cell suspension in a tonometer and finally adding a small quantity of dithionite (0.5 mM final concentration). the complete deoxygenation state was confirmed by the characteristic optical spectrum of deoxyhemoglobin in the final lysate.

The results obtained on three different samples of SS cells, indicate that complete deoxygenation does not affect the glycerol permeability of the SS cells.

pH dependence

Fig. 4 represents the variation of the relative permeability for glycerol with pH (the value at pH 7.3 taken as unity) for two AA and two SS blood samples. In evaluating the relative permeabilities at different pH values, we took into account the change in cell volume with pH [17,21–23]. An identical linear relationship betwen cell volume and pH for both SS and AA cells was observed:

$$V(\mu m^3) = V_0\,(\mu m^3) - 8.51(pH - 7.4) \qquad (2)$$

V_0 is the cell volume at pH 7.40. The points in Fig. 4 were corrected for variation in cell volume according to this formula. As is evident from the figure, the bell-shape pH dependence of glycerol permeability is a common feature for both types of cells.

Cell fractionation

By ultracentrifugation in a stractan density gradient, the red blood cells were separated according to their density. In AA cells, the glycerol permeability does not significantly vary with the cell density; in contrast, in sickle cells, where the denser fractions are related to an increasing proportion of irreversibly sickled cells, we observed a progressive decrease of membrane permeability with increasing density (Fig. 5).

PCMB inhibition

When a suspension of erythrocytes was

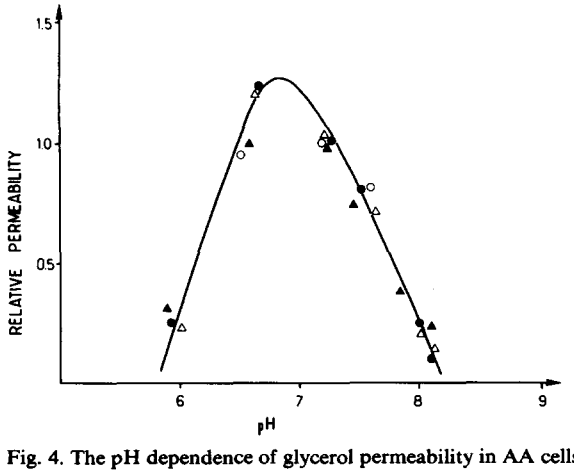

Fig. 4. The pH dependence of glycerol permeability in AA cells (●, ○) and SS cells (△, ▲); the relative values of permeability (the permeability at pH 7.3 was taken as unity for every sample) were corrected for variation in the cell volume with pH (see Eqn. 2).

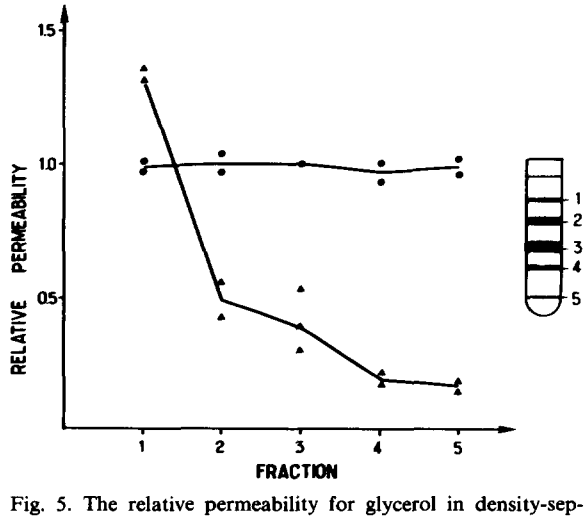

Fig. 5. The relative permeability for glycerol in density-separated fractions of an AA (●) and an SS sample (▲) (the permeability of unfractionated cells as unity). A diagram of the fractions position in the stractan gradient is also represented. No correction was necessary for the small volume variation with density. Note that the same position in the density gradient for AA and SS cells fractions does not correspond to the same density.

incubated for 30 min with 1 mM PCMB at 23°C, the glycerol permeability was approximately reduced with 30% and 20% in SS and AA cells, respectively (not shown).

Discussion

It is known that in the human red blood cells facilitated mechanisms are responsable for the transport of ethylene glycol [24], glycerol [17,24] and erythritol [15]. We determined the ethylene glycol and glycerol inward permeabilities at a single concentration of 200 mM in polyol, monitoring the light transmission changes at 640 nm as a measure of cell swelling. This simple method supposes that there is a good correlation between light transmission and cell volume changes [12]. The fact that our mean value for glycerol permeability in AA red cells ($P = 2.87 \cdot 10^{-6}$ cm/s) agrees fairly well with the value obtained by a tracer method ($P = 3.5 \cdot 10^{-6}$ cm/s) [17] confirms the validity of our experimental approach.

We have recently shown that there is no change in the inward osmotic water transport in sickle cells as compared to normal erythrocytes [25]. Thus the difference observed between glycerol and ethylene glycol transport kinetics in the two types of cells is not the consequence of modifications in the rate of the polyol-induced water transport but represents a real difference in non-electrolyte permeabilities.

The common idea about the mechanism of water and hydrophilic solutes transport is the existence of pores created by integral membrane proteins across the lipid bilayer [26,27]. Using PCMB and phloretin as transport inhibitors, it was demonstrated that water transport pathways are different from those for small non-electrolytes like urea, methylurea or glycerol [28,29].

Glycerol-facilitated transport mechanism is very sensitive to a specific inhibitor: the copper ions have no action upon erythritol transport [17] which shares a common pathway with glucose [15]. Our results showed significant changes in the transport for ethylene glycol and glycerol in sickle cells, as compared with AA cells, while erythritol transport was unchanged. This means that transport perturbations observed in sickle cells are a specific (for ethylene glycol and glycerol) and not a diffuse process.

While probably sharing a common transport pathway [24], ethylene glycol and glycerol have different susceptibilities for transport inhibitors (PCMB, phloretin) [28]; it was suggested that due to its smaller molecular radius (2.24 Å as compared to glycerol whose radius is 2.74 Å) ethylene glycol encounters less frictional resistance during the passage through the hydrophilic channel [13]. The fact that the changes reported above for glycerol in SS cells as compared with AA cells are twice those for ethylene glycol can be similarly explained on the basis of the molecular size differences. We have shown that glycerol permeability in SS cells was independent of the oxygenation state, suggesting that the impaired non-electrolyte transport is independent of the properties of the hemoglobin S inside the cells.

The pH dependence of the glycerol transport were not changed in SS cells as compared to normal erythrocytes. The bell-shaped pH dependence of glycerol permeability is similar to that described by Carlsen and Wieth [17] for AA cells. Similar dependence was found for glucose [23] and pyruvate [31] facilitated transports. For the acid inhibition of glycerol transport it was proposed [17] a competition between the permeant and the protons in the medium for an anionic binding site of the carrier. The decrease permeability at basic pH is probably due to a conformational change of the protein responsable for the transport which have titrable groups with pK values near pK 8.

A similar temperature dependences for glycerol transport in AA and SS cells means that the membrane transport for this non-electrolyte is not qualitatively different. The most probable explanation for the great reduction of glycerol transport in SS cells is the inactivation of an important fraction of specific membrane carriers in pathological cells. This conclusion is supplemented by the important decrease of glycerol permeability with cell density in SS samples (Fig. 5). The denser fractions contain a progressive percentage of irreversibly sickled cells (in our experiment from 2% to 65% in fraction 1 and 5, respectively). Irreversibly sickled cells represent the most damaged cells in SS blood [32]. The membrane abnormalities of these cells were proposed to be the result of cumulative damages

produced by repeated cycles of sickling and an increased oxidative stress [33].

Oxidative damages on the erythrocyte membrane can produce drastic effects. It was demonstrated that it is possible to decrease five times the glycerol facilitated transport in normal erythrocytes by a protoporphyrin-induced photodynamic effect [30]. This finding was correlated with the presence of cross-linking of membrane proteins which can produce strong perturbations in the integrity and function of the membrane. The membranes of SS cells are exposed to the action of endogenous free radicals whose concentration is twice that in normal erythrocytes [34]. In the same time, a considerable decrease in the efficacy of enzymatic free radical scavengers, including glutathione peroxidase, glutathione reductase and catalase was reported [35–38]. The target of the oxidative action may be equally the lipid and the protein components of the membrane. It was shown that specific, lipid-directed peroxidative action of the H_2O_2 in erythrocyte membranes does not change the carrier-mediated component of the glycerol transport [30]. This suggests that the decrease in mediated polyol transport we have observed in SS cells is determined by damages of membrane proteins (and not lipids) implicated in the facilitated transport mechanism.

References

1 Noguchi, C.T. and Schechter, A.N. (1981) Blood 58, 1057–1068
2 Clark, M.R. and Shohet, S.B. (1981) Texas Rep. 40, 417–429
3 Clark, M.R. Mohandas, N.M. and Shohet, S.B. (1980) J. Clin. Invest. 65, 189–196
4 Segel, G., Feig, S., Mentzer, W.C., Jensen, M.C., Nathan, D.G. and Shohet, S.B. (1973) in 2nd International Symposium on Membrane Permeability of Erythrocytes, Thrombocytes and Leukocytes, Vienna, June 1972 (Deutsch, E., Gerlach, E. and Moser, K., eds.), pp. 118–121, Thieme, Stuttgart
5 Glader, B.E., Lux, S.E., Muller-Soyano, A., Platt, O.S., Propper, R.D. and Nathan, D.G. (1978) Br. J. Haematol. 40, 527–532
6 Glader, B.E. and Nathan, D.G. (1978) Blood 51, 983–989
7 Clark, M.R., Morrison, C.E. and Shohet, S.B. (1978) J. Clin. Invest. 62, 329–337
8 Berkowitz, L.R. and Orringer, E.P. (1981) J. Clin. Invest 68, 1215–1220
9 Parker, J.C. and Berkowitz, L.R. (1983) Physiol. Rev. 63, 261–313
10 Hoffman, J.F. (1979) in Development of Therapeutic Agents for Sickle Cell Disease, INSERM Symp. No. 9 (Rosa, J., Beuzard, Y. and Hercules, J., eds.), pp. 3–13, Elsevier North-Holland, Amsterdam
11 Kark, J.A. and Hicks, C.U. (1981) Blood 58, 60a
12 De Gier, J., Mandersloot, J.G. and Van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 150, 666–675
13 Farmer, R.E.L. and Macey, R.I. (1972) Biochim. Biophys. Acta (1972) 255, 502–516
14 Levin, S.W., Levin, R.L. and Solomon, A.K. (1980) J. Biochem. Biophys. Methods 3, 255–272
15 Bowman, R.J. and Levitt, D.G. (1977) Biochim. Biophys. Acta 466, 68–83
16 Corash, L.M., Piomelli, S., Chen, H.C., Seaman, C. and Gross, E. (1974) J. Lab. Clin. Med. 84, 147–151
17 Carlsen, A. and Wieth, J.O. (1976) Acta Physiol. Scand. 97, 501–513
18 Vieira, F.L., Sha'afi, R.I. and Solomon, A.K. (1970) J. Gen. Physiol. 55, 451–466
19 Brahm, J. (1982) J. Gen. Physiol. 79, 791–819
20 Galey, W.R., Owen, J.D. and Solomon, A.K. (1973) J. Gen. Physiol. 61, 727–746
21 Legge, D.G. and Shortman, K. (1968) Br. J. Haematol. 14, 323–335
22 Gunn, R.B., Dalmark, M., Tosteson, D.C. and Wieth, J.O. (1973) J. Gen. Physiol. 61, 185–206
23 Brahm, J. (1983) J. Gen. Physiol. 82, 1–23
24 Hunter, F.R. (1970) Biochim. Biophys. Acta 211, 216–222
25 Craescu C.T. and Cassoly, R. (1984) Nouv. Rev. Fr. Hématol., in the press
26 Andreoli, T.E. and Schafer, J.A. (1980) in Membrane Physiology (Andreoli, T.E., Hoffman, J.F. and Fanestil, D.D., eds.), pp. 165–184, Plenum Press, New York
27 Sha'afi, R.I. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 221–256, Academic Press, New York
28 Macey, R.J. and Farmer, R.E.L. (1970) Biochim. Biophys. Acta 211, 104–106
29 Garrick, R.A., Patel, B.C. and Chinard, F.P. (1983) Biochim. Biophys. Acta 734, 105–113
30 Dubbelman, T.M.A.R., De Goeij, A.F.P.M. and Van Steveninck, J. (1980) Biochim. Biophys. Acta 595, 133–139
31 Rice, W.R. and Steck, T.L. (1976) Biochim. Biophys. Acta 433, 39–53
32 Serjeant, G.R., Serjeant, B.E. and Milner, P.F. (1968) Br. J. Haematol. 17, 527–533
33 Eaton, J., Jacob, H.S. and White, J.G. (1979) Semin. Hematol. 16, 52–64
34 Hebbel, R.P., Eaton, J.W., Balasingam, M. and Steinberg, M.H. (1982) J. Clin. Invest. 70, 1253–1259
35 Chiu, D. and Lubin, B. (1979) J. Lab. Clin. Med. 94, 542–548
36 Zimmerman, C.P. and Natta, C. (1981) Scand. J. Haematol. 26, 177–181
37 Das, S.K. and Nair, R.C. (1980) Br. J. Haematol. 44, 87–92
38 Lachant, N.A., Davidson, W.D. and Tanaka, K.R. (1983) Am. J. Hematol. 15, 1–13