Short communication

The Effect of Temperature on the Age-dependent Stability of Rat Erythrocyte Membrane

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During postnatal development functions of organ and tissue undergo significant qualitative changes. These changes affect also membrane fluidity. Membrane fluidity can be regarded as one of the decisive factors which determine the membrane stability, deformability and permeability. It also significantly influences the electrical characteristics of membranes.

Important characteristics of erythrocytes with regard to their passage through microcirculation are elasticity and deformability. These characteristics are influenced by two components, structural and dynamic. The structural component is connected with the stability of the spectrin-actin complex (Schmid-Schönbein et al. 1986a, b). The dynamic component is connected with the metabolic activity of erythrocytes (Nakao et al. 1960; Brewer 1974). In addition, erythrocyte membrane fluidity is also strongly influenced by temperature. It is generally assumed that in homoiothermic animals biological membranes have optimum fluidity within range of 37—38 °C (Fox 1975). Under patological conditions however, the temperature may drop below this range or, more frequently, exceed it. This is also associated with changes in osmotic hemolysis which decreases with increasing temperature due to the increased fluidity of the lipoid bilayer. The break point for human erythrocytes is around 25 °C i.e. close to the transition temperature of phospholipids (Constantinescu et al. 1987). In a previous work we could observe that brilliant cresyl blue (BCB) causes disintegration of erythrocytes in vitro in isotonic enviroment. This process is characterized by gradual changes in the shape of erythrocytes from discocytes to disintegrating spherocytes. At a constant temperature (37 °C) the disintegration was age-dependent (Nicák 1986).

The aim of the present study was to gain more insight on the influence of physico-chemical factors on the permeability of erythrocyte membrane. The effect of temperature on erythrocyte disintegration in isotonic NaCl solution both in the presence and absence of BCB was investigeted using erythrocytes from animals of different stages of postnatal development.

Erythrocytes were obtained from Wistar rats aged 21, 42, 90—105, 340

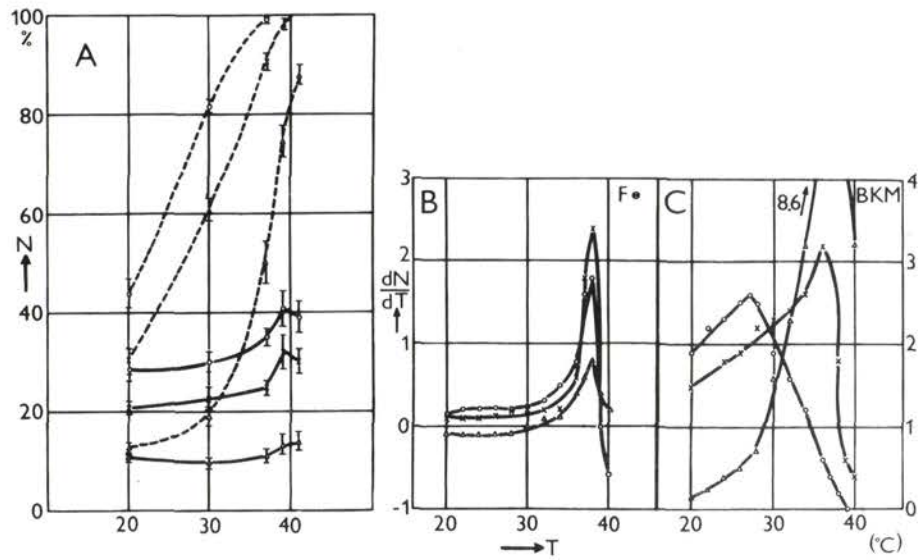

Fig. 1.A: Temperature-dependent disintegration of erythrocytes from 21 days old rats in isotonic NaCl solution in the absence (full line) and in the presence of BCB (dashed line). *B:* Graphic derivation of erythrocyte disintegration curves in isotonic NaCl solution. *C:* Graphic derivation of erythrocyte disintegration curves in isotonic NaCl solution in the presence of BCB; *N:* % of disintegration, *T* = temperature; triangles — disintegration after 2 hours of incubation, crosses — disintegration after 4 hours of incubation, circles — disintegration after 6 hours of incubation

—360, and 690—720 days. Blood samples were taken by incision of the tails into heparinized test tubes.

Blood (25 μl) was mixed with 4.975 μl of isotonic NaCl solution (154 mmol/l, pH 7.4), and BCB hydrochloride was added to the experimental series. The control tubes were prepared in the same way but omitting BCB. The pH of both experimental and control solution was adjusted to 7.4 with 0.1 mol/l HCl or NaOH.

The solutions were incubated in a thermostat at five different tempereratures: 20 °C, 30 °C, 37 °C, 39 °C, and 41 °C for 6 hours. RBC numbers were determined in Bürker chamber immediately after the preparation of the samples and after 2, 4, and 6 hours of incubation. Initial RBC numbers were taken as 100%. Twenty parallel samples were determined after incubation at 20 °C, 30 °C, and 37 °C, and 16 parallel samples after incubation at 39 °C and 41 °C each. The results were statistically analysed by *F* and *T* test.

Fig. 1 shows results for 21-days old rats.

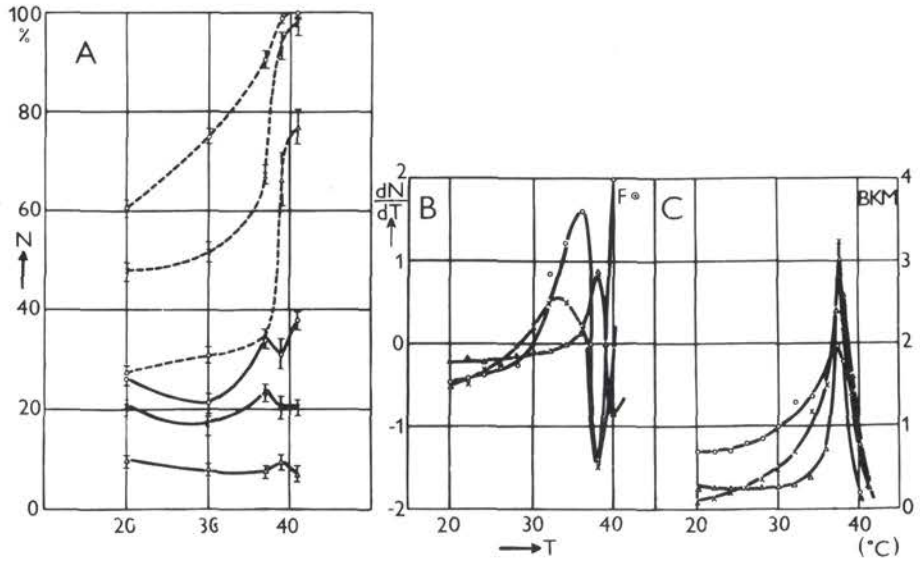

Fig. 2. Temperature-dependent disintegration of rat erythrocytes: 42 days old animals. For symbols see legend to Fig. 1.

A non-linear enhancement of erythrocyte disintegration in isotonic NaCl solution was observed at 2, 4, and 6 hours with a maximum at 37 °C (Fig. 1*A*). There is a sudden drop in disintegration after exceeding this temperature with a minimum at 39 °C. Within temperature interval between 39 °C and 41 °C disintegration is slightly enhanced after 2 hours, more enhanced after 4 hours, and a significant increase is observed after 6 hours of incubation.

Erythrocyte disintegration was significantly enhanced in the presence of BCB over the entire range of temperatures studied, and was total at 37 °C, 39 °C, and 41 °C after 4 and 6 hours.

Important information can be derived from the inflexion points obtained by graphical derivation of the curves (Batuner an Pozin 1956). The derivation curves (Fig. 1*B* and 1*C*) were constructed from the curves in Fig. 1*A* and show maximum control disintegration (NaCl only) at 37 °C and minimum at 39 °C for all time intervals. Inflexion points for this solution are at 36 °C and 38 °C. In the presence of BCB the inflexion points depend on the incubation time: after 2 hours it is 38 °C, after 4 hours 37 °C, and after 6 hours 30 °C.

Fig. 2 shows the temperature dependent disintegration of erythrocytes for 42 days old rats. The maximum disintegration in isotonic NaCl solution (Fig. 2*A*) occurs after 4 and 6 hours at 39 °C. The curve shows no extremes after 2

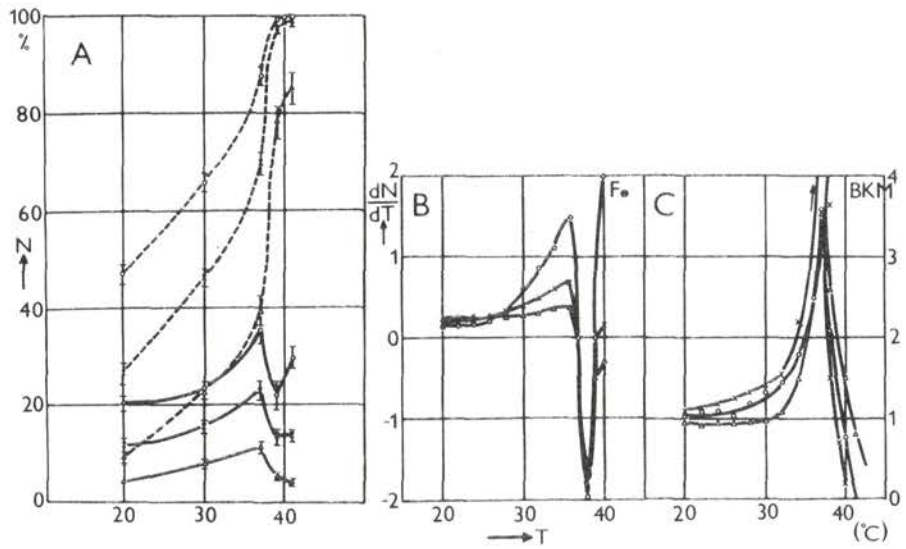

Fig. 3. Temperature-dependent disintegration of rat erythrocytes: 90—105 days old animals. For symbols see legend to Fig. 1.

hours only slight by exhanced disintegration at 39 °C and 41 °C. The disintegration is significantly smaller at 37 °C for all incubation times. The inflexion points after 2, 4, and 6 hours are at 38 °C (Fig. 2*B* and 2*C*).

In the presence of BCB disintegration is greatly enhanced with increasing temperature, and is characterized by S-shaped curves without any extremes. The inflexion points depend on time of incubation, similarly as it was the case with 21 days old rats: after 2 hours the inflexion points is at 38 °C, after 4 hours at 36 °C, and after 6 hours at 27 °C (Fig. 2*C*).

Fig. 3*A* shows the results obtained for 90—105 days old animals. A non-linear increase in erythrocyte disintegration in isotonic NaCl solution appears within 20—37 °C for all time intervals studied. Maximum is reached at 37 °C followed by a sudden drop to approximately the same level as observed at 20 °C. At 41 °C disintegration is slight by enhanced after 2 h and an intense increase is observed after 6 hours of incubation.

In the presence of BCB erythrocyte are disintegrated rapidly over the entire range of temperatures used. Total disintegration is reached at 39 °C and 41 °C after 4 and 6 hours. The obtained curves are also S-shaped.

The derivation curves constructed from the curves shown in Fig. 3A show maximum disintegration in the control solution after 2, 4, and 6 hours of

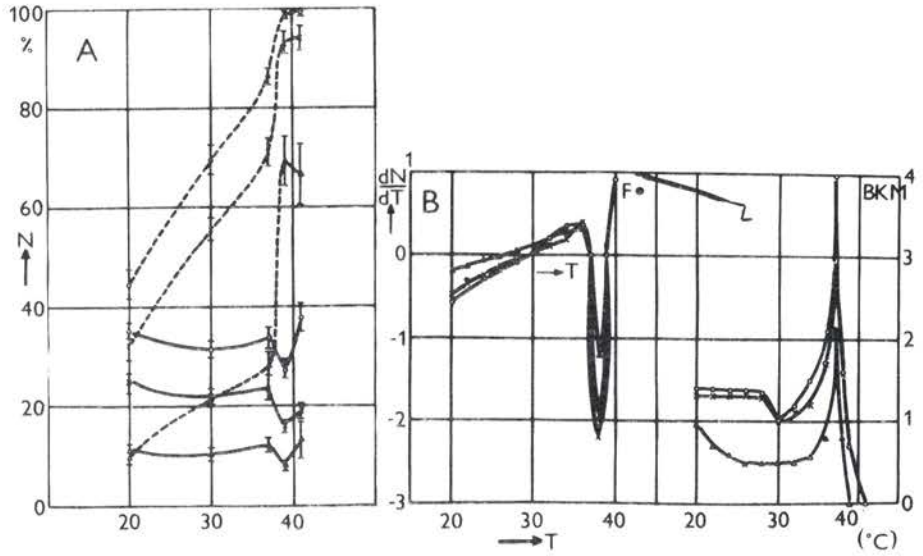

Fig. 4. Temperature-dependent disintegration of rat erythrocytes: 340—360 days old animals. For symbols see legend to Fig. 1.

incubation at 37 °C and minimum disintegration at 30 °C (Fig. 3*B*). The inflexion points after 2, 4, and 6 hours of incubation are at 37 °C (Fig. 3*C*).

The respective values for 340—360 days old rats are shown in Fig. 4.

For this age groups, erythrocyte disintegration in isotonic NaCl solution after 2, 4, and 6 hours is temperature dependent showing high values at 37 °C. It drops significantly on exceeding this temperature and reaches a minimum at 37 °C. In the temperature interval between 39 °C and 41 °C disintegration is again rapidly enhanced.

In the presence of BCB disintegration is nonlinear over the entire range of temperature and approaches the absolute value at 39 °C and 41 °C only after 6 hours of incubation.

The derivation curves (Fig. 4*B* and 4*C*) show inflexion points in the control solution at 36 °C and 38 °C and at 30 °C and 37 °C in the presence of BCB.

The results for 690—720 days old rats are shown in Fig. 5.

In isotonic NaCl maximum disintegration after 2 hours of incubation was observed at 39 °C (Fig. 5*A*); after 4 and 6 hours alike maximum disintegration was at 37 °C and minimum at 30 °C. Another local minimum appears at 39 °C.

In the presence of BCB disintegration is enhanced with temperature, the

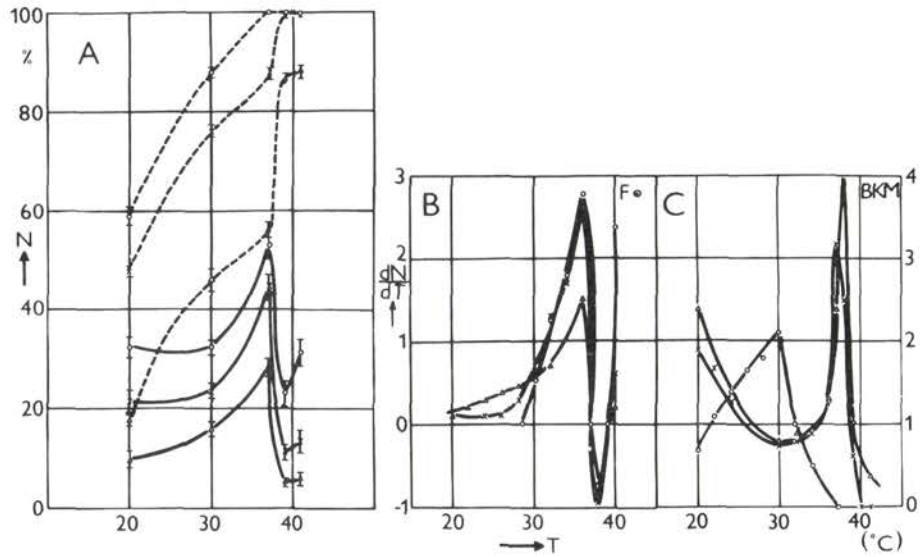

Fig. 5. Temperature-dependent disintegration of rat erythrocytes: 690—720 days old animals. For symbols see legend to Fig. 1.

curves are S-shaped. Disintegration spreads only slowly in the temperature interval of 20 °C—37 °C after 2 hours of incubation, it sharply increases and reaches 80% at 41 °C. After 4 hours of incubation it increases slowly in the temperature range between 20 °C—30 °C, at higher temperatures it spreads rapidly reaching complete disintegration at 41 °C. After 6 hours of incubation it spreads rapidly over the whole range of temperature investigated.

The inflexion points after 2 hours of incubation in NaCl solution are at 38 °C and 40 °C (Figs. 5B and 5C), after 4 hours at 33 °C and 38 °C, and after 6 hours at 36 °C and 38 °C. In the presence of BCB the inflexion points are at 37 °C and 38 °C.

Three independent parameters determined the disintegration of erythrocytes in the present study: temperature, age of the animals and duration of incubation. Temperature influenced erythrocyte disintegration in dependance on the incubation solution used.

On exceeding a certain temperature, discontinuous changes in the mobility of molecules and their parts in biological membranes occur, i.e. phase transition from gel to liquid crystal state occurs. Apart from this, discontinuities in membrane transport and/or multiple temperature breaks also occur. One of the

reasons for enhanced erythrocyte disintegration in the presence of BCB at temperature above 37 °C could be the membrane fluidity which is assumed to reach maximum values at 37 °C—38 °C on warm blooded animals. Another reason could be the temperature break-points.

The disintegration-temperature curves differ in their patterns depending on the presence or absence of BCB. In pure isotonic NaCl solutions disintegration was temperature dependent, reaching extremes at 37 °C and 39 °C and showing inflexion points at 36 °C and 38 °C. In the presence of BCB erythrocyte disintegration increased with the incrreasing temperature without extremes, and inflexion points after 6 hours of incubation were at 37 °C—38 °C for all age categories.

Disintegration of rat erythrocytes in isotonic NaCl solutions observed in our experiments is similar to osmotic hemolysis of human erythrocytes, in which maximum hemolytic activity was observed at 37 °C (Constantinescu et al. 1987).

It may be concluded based on the above results that the rat erythrocyte membrane undergoes qualitative changes during postnatal development, which significantly influence the biophysical characteristics of the red blood cell membrane.

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