ERYTHROCYTE FRAGILITY AND POTASSIUM EFFLUX AS AFFECTED BY TEMPERATURE AND HEMOLYZING RATE

Avinoam LIVNE
Division of Life Sciences, Negev Institute for Arid Zone Research

and

Avraham RAZ
Biology Department, University of the Negev, Beer-Sheva, Israel

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1. Introduction

Hypotonic hemolysis carried out gradually occurs at a lower salt concentration than rapid hemolysis [1, 2]. Katchalsky et al. [1] related this phenomenon to the greater extent of cellular swelling under gradual hemolysis before lysis occurs. However, Seeman et al. [2] concluded that prelytic loss of intracellular K^+, rather than the extent of swelling, accounts for the reduced osmotic fragility under gradual hemolysis. Seeman et al. also suggested that the increased K^+ leakage at elevated temperature observed by Davson [3] may explain the reduced fragility at the higher temperature. Contrary to Seeman's suggestion, our study shows that the effect of temperature on hemolysis is not associated with a modified K^+ release. The results, however, substantiate the conclusion that the prelytic release of KCl accounts for the reduced osmotic fragility under slow hemolyzing rate.

2. Materials and methods

Human or dog blood was drawn from fasting adult males. Stock suspensions of thrice wasted erythrocytes [2] containing 15% (v/v) packed cells were prepared in a solution of 150 mM NaCl. All solutions were buffered with 2 mM Na phosphate, pH 7.2. Aliquots (50 μl) of the stock suspension were mixed with 2 ml of 150 mM solutions of either NaCl or KCl in 20 ml scintillation vials and diluted to a final volume of 5 ml with hypotonic solutions (NaCl or KCl, respectively) at different hemolyzing rates. For "slow hemolysis", the hypotonic solutions were delivered to the cell suspension through capillary tubings at 0.4 ml/min with continuous mixing.

For "fast hemolysis" the mixing of the hypotonic solution with the cell suspension took place within 1–2 sec. Following an incubation of 10 min starting from the onset of dilution, the suspensions were centrifuged at 2000 g for 3 min and the supernatant analyzed for hemoglobin content (Klett-Summerson colorimeter, 54 filter) and, where indicated, for K^+ content (Unicam SP 90A atomic absorption spectrophotometer). The temperatures given in the figures refer to the entire hemolyzing procedure, including centrifugation. Fresh blood was used throughout. Solutions were made from Analar-grade salts.

3. Results and discussion

The hypotonic hemolysis studies are commonly conducted in a NaCl medium. If the reduced osmotic

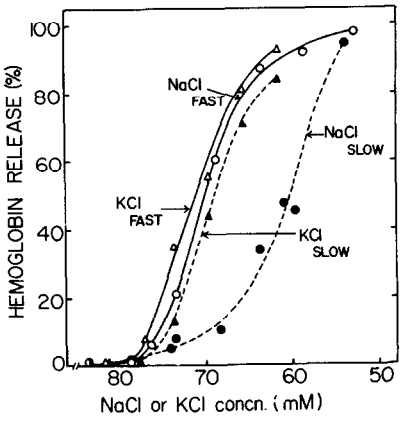

Fig. 1. Effect of fast and slow hemolyzing rates on the release of hemoglobin from human erythrocytes in NaCl or KCl solutions of different final concentrations at 20°.

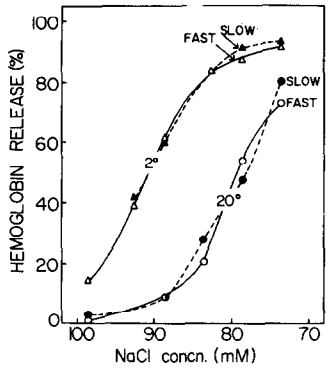

Fig. 2. Effect of fast and slow hemolyzing rates on the release of hemoglobin from dog erythrocytes in NaCl solutions of different final concentrations at 2° or at 20°.

fragility under slow hemolysis is indeed related to an increased K^+ efflux [2], it is anticipated that in a KCl medium the difference between slow and fast hemolysis will be minimized. Fig. 1 shows a marked difference between the curves of slow and fast hypotonic hemolysis of human erythrocytes in a medium of NaCl. However, when the cells are hemolyzed in a KCl medium, this difference is greatly diminished; slow hemolysis in KCl resembles the fast hemolysis

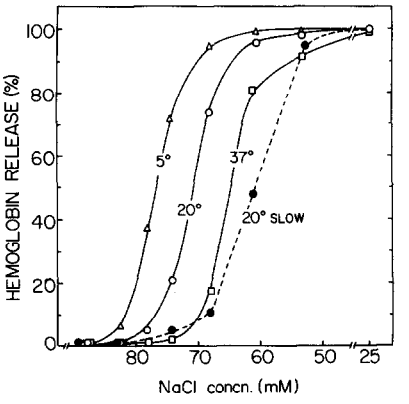

Fig. 3. Effect of temperature on the release of hemoglobin from human erythrocytes in NaCl solutions of different final concentrations. Solid line (——) fast hemolysis. Broken line (- - -) slow hemolysis at 20°.

in NaCl. It has been shown that Cl^- is readily transferred across the erythrocyte membrane [4] while the exchange of Na^+/K^+ is probably negligible [5, 6] during the 10 min incubation period used in our experiments. It is thus reasonable to conclude that the increased efflux of KCl under slow hemolysis in NaCl medium results in an osmotic adjustment and in a decreased osmotic fragility of the cells, while such an adjustment is unattainable in a KCl medium.

Dog erythrocytes contain 107 mEq/liter Na^+ and only 9 mEq/liter K^+, compared with 20 and 100 mEq/liter of Na^+ and K^+, respectively, in human red blood cells [7]. Therefore, on the basis of our interpretation of the results in fig. 1, it is expected that dog erythrocytes will hemolyse at the same concentration of external NaCl solution regardless of the hemolyzing rate. Fig. 2 substantiates this expectation at either 2° or 20°. Similar results were also obtained at 35°. The parallel experiment, using a KCl medium, is complicated by the high permeability of dog erythrocytes to K^+ resulting in lysis.

Fig. 3 shows that the higher the temperature the lower the osmotic fragility, as already reported [2]. Slow hemolysis at 20° was included in this experiment to simultaneously compare K^+ and hemoglobin release as affected by temperature and rate of hemo-

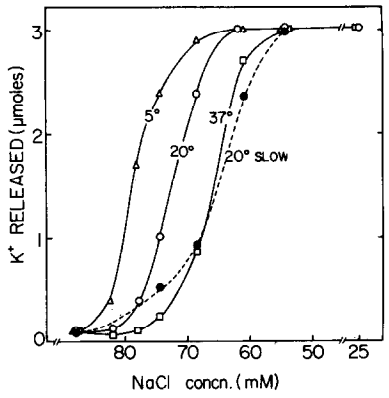

Fig. 4. K^+ release from human erythrocytes in μmoles per tube. Details as in fig. 3.

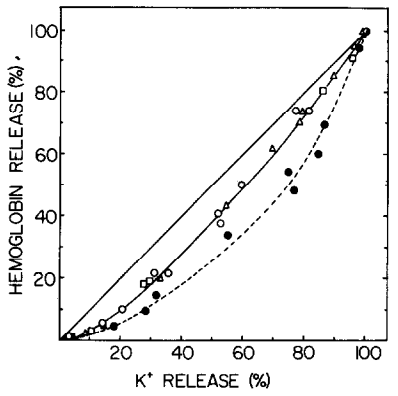

Fig. 5. Prelytic K^+ release as affected by temperature and rate of hemolysis. Upper solid line - theoretical (no prelytic K^+ release). Middle line - fast hemolysis at: 37° (□), 20° (○) or 5° (△). Broken line - slow hemolysis at 20°.

lysis. The data for K^+ release are presented in fig. 4. If, as suggested by Seeman et al. [2], excessive K^+ efflux accounts for the reduced fragility at elevated temperatures, it is anticipated that for a given level of hemoglobin release, K^+ leakage should differ accordingly at different temperatures. To enable analysis of the prelytic K^+ release, the data of figs. 3 and 4 (and of additional experiments performed under identical conditions) are replotted in fig. 5, relating K^+ release to hemoglobin release [8]. The upper straight line is theoretical, describing no prelytic K^+ release. Unlike slow hemolyzing rate which clearly results in an excessive release of K^+ (fig. 5, broken line and [2]), the different temperatures all yield essentially identical prelytic K^+ leakage (middle line). It is thus concluded that the effect of temperature on hemolysis is not associated with a modified K^+ release. In view of this conclusion, the temperature-dependent fragility of red blood cells appears to be related to the direct effect of temperature on the surface area of the erythrocyte membrane [9].

References

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