

Determination of Blood Concentrations of Urea and Potassium Cyanate that Reduce Mechanical Hemolysis

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Abstract

Mechanical hemolysis can be reduced in vitro by treating blood with urea or potassium in concentrations of 300 mg and 4 mg/100 ml blood respectively.¹ These concentrations have also been used clinically in treating hemolytic disease.²⁻⁵

Introduction

The present study was undertaken to determine in vitro the optimal blood concentrations of urea or potassium cyanate which minimize the hemolysis induced by mechanical trauma to human red blood cells.

Materials and Methods

Human blood not more than two days old was used and was divided into three parts, two for treatment with urea or cyanate and the third was used as the control. Graded concentrations of urea and potassium cyanate in 5% invert sugar were prepared under sterile conditions and mixed with the blood. Only fresh urea solution not more than one week old, stored at 4°C was used within 2

hours of warming to room temperature. The concentrations were tested as indicated in Table 1.

Each concentration was tested in six experiments. To obtain equal volumes in all three test systems, 5% invert sugar was added to the part of the blood used as the control. From each part of the blood five 10 ml plastic test tubes were filled with blood excluding all air. Each test tube contained one glass bead 3 mm in diameter. All test tubes

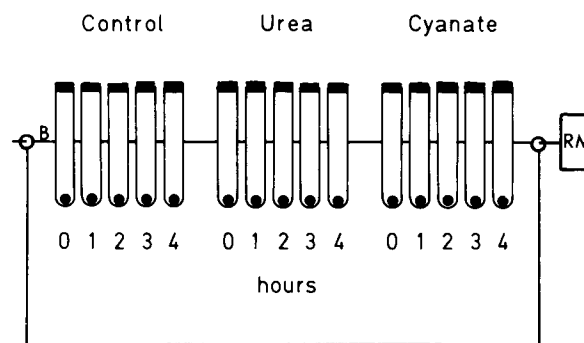


FIGURE 1. Schematic model for producing mechanical hemolysis: Blood filled test tubes containing a glass bead (black dot) mounted on a horizontal bar (B) which is connected to a rotating motor (RM).

Table 1

Drug	Concentration (mg/100 ml blood)									
Urea	20	50	100	150	250	300	400	600	800	1000
Potassium cyanate	0.01,	0.05,	0.1,	0.3,	0.5,	1.0,	5.0,	10		

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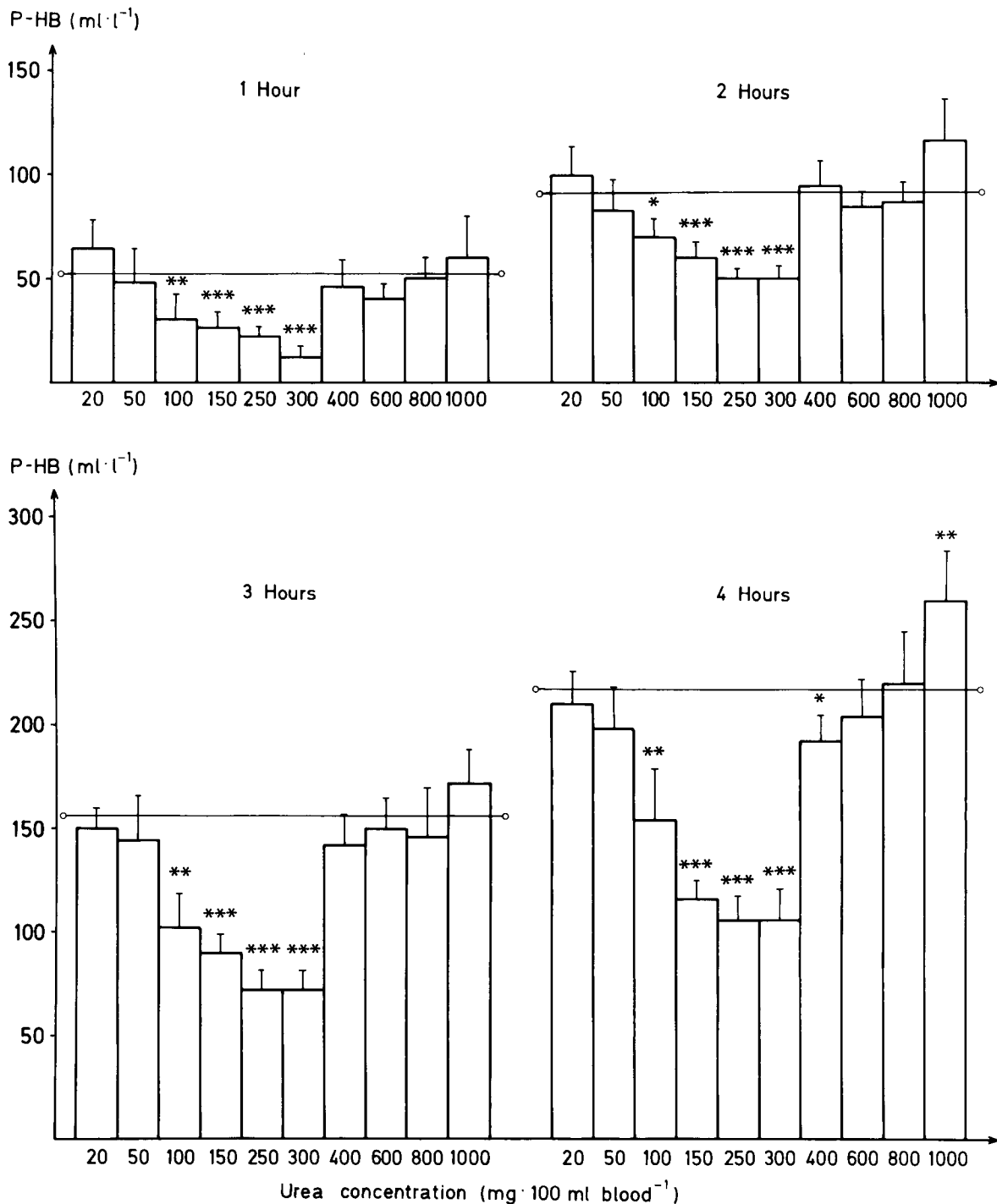
Submitted 5/83.

Revised 1/84.

Accepted 2/84.

were mounted on a horizontal bar that was rotated at 60 turns per minute during a period of four hours causing the glass bead to fall in a column of blood, producing mechanical hemolysis. Just before the

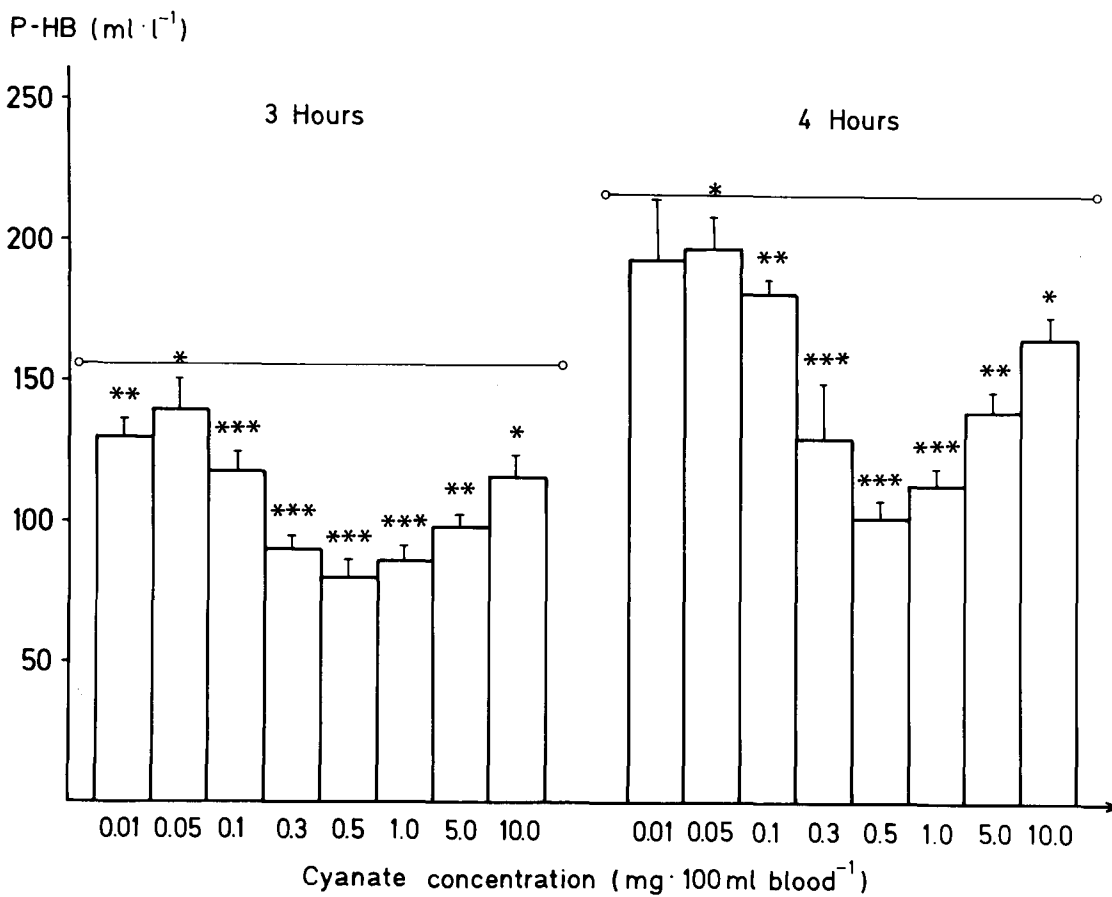
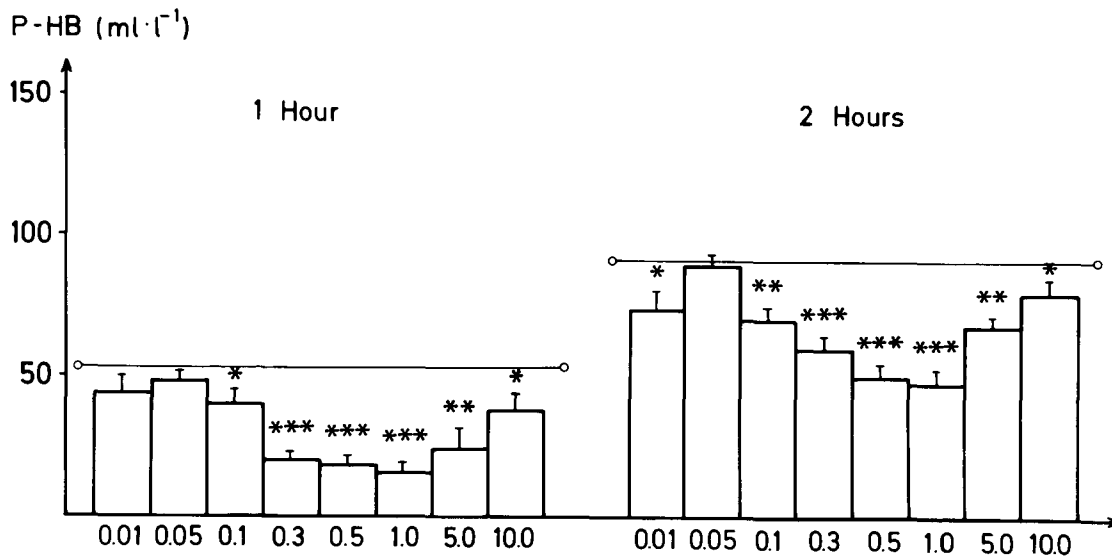
start of rotation and at hourly intervals one test tube from each part was removed to determine the plasma hemoglobin (P-HB) (Figure 1). The paired t-test was used for statistical analysis.



FIGURES 2A AND 2B. The effect of urea at different blood concentrations on the amount of plasma hemoglobin (P-HB) released by mechanical trauma.

0 — 0 = Average mean P-HB in the control groups.

* = P<0.05, ** = P<0.01, *** = P<0.001



FIGURES 3A AND 3B. The effect of potassium cyanate at different blood concentrations on the amount of plasma hemoglobin (P-HB) released by mechanical trauma.

0 — 0 = Average mean P-HB in the control groups.

* = P<0.05, ** = P<0.01, *** = P<0.001

Results

Urea

At concentrations of 100, 150, 250 and 300 mg urea/100 ml blood there was a significant reduction of P-HB compared to the controls. This effect was maximal with the latter two concentrations reducing the mean hemolysis during four hours by 51% (Figure 2^{a&b}). A similar protective effect was also noted with a concentration of 200 mg urea/100 ml but only four observations were recorded due to a mishap during the experiment (broken test tubes). Twenty and 50 mg urea/100 ml blood did not give any significant reduction of hemolysis. Nor had 400, 600 and 800 mg urea/100 ml blood any effect. At 1000 mg urea/100 ml blood there was increased hemolysis.

Cyanate

Potassium cyanate concentrations of 0.1 to 10 mg/100 ml blood reduced hemolysis significantly. The effect was maximal at a cyanate concentration of 0.5 and 1 mg/100 ml blood, reducing the mean hemolysis at the end of the experiments by 54 and 48%, respectively. The protective effect was progressively lessened as the cyanate concentrations increased. At a cyanate concentration of 25 mg/100 ml blood there was significantly more hemolysis than in the controls (Figure 3^{a&b}).

Conclusions

Urea and cyanate were shown to reduce mechanical hemolysis in in-vitro models using human blood. The blood concentrations of urea and cyanate that showed a reduction in hemolysis compared to controls, were found to be in the range of 100 to 300 mg/100 ml blood for urea, and 0.1 to 10 mg/100 ml for cyanate. The best protective effects were observed at 250 to 300 mg and 0.5 to 1.0 mg/100 ml blood for urea and cyanate respectively.

Discussion

Preliminary tests showed that the control group used in this study produces a plasma hemoglobin around 200 mg/100 ml blood after four hours which is comparable to the hemolysis produced by pumps and tubing used in extracorporeal circulation.⁶

Mechanical trauma causes the red cell wall to stiffen before the cell hemolyzes.⁷ At protective concentrations urea could possibly act to reverse the action of trauma by increasing the red cell membranes plasticity and therefore deformability. Increased hemolysis in the experiments using a urea concentration of 1000 mg/100 ml blood is probably due to relatively mild direct toxic effect on the red cell membrane.⁸ The direct toxic action of urea at 6 g/100 ml on the red cell membrane causes the membrane to spontaneously fragment.⁸ In clinical doses urea has been given safely for treating cerebral edema.⁹ Mechanical trauma to this blood causes hemolysis more easily.

Urea in solution has been shown to isomerically transform slowly into cyanate. This is both temperature and time dependent, and significant amounts can be detected at room temperature after one week but none when stored below 5°C for four weeks.^{10,11} Therefore any protection to the red cells afforded by the use of fresh urea solutions in this study should be independent of any action of cyanate.

Cyanate and urea both react with cellular proteins.^{2,12,13,14} This suggests that these two agents act in a more or less similar fashion when reducing hemolysis. Cyanate induces irreversible inhibition of lipid synthesis in the red cell membrane, leading to damage and a shortened life span.¹⁵ This damage is reduced at cyanate concentrations below 120 mg/100 ml. The tendency to hemolyse in these experiments at 25 mg/100 ml is possibly due to the trauma to the red cells worsened by cyanate induced changes in the cell membrane.

Red cells are known to withstand hemolysis in a hyperosmotic solution of 500 mosm/L.⁸ The high urea concentrations in the present experiments (1000 mg/100 ml blood) gave a blood osmolarity of 470 ± 10 mosm/L, suggesting that hyperosmotic action is not the only mechanism of the red cell destruction.

Blood trauma causing the activation of hematologic responses occurs as soon as blood comes into contact with the foreign surfaces of an extracorporeal system. These responses and the subsequent effects they have on other organs make total cardiopulmonary bypass (CPB) limited to about five or six hours.¹⁶ Previous reports and reviews have given detailed account of the forces required to damage the red cell and the unavoidable

trauma that this cell undergoes during CPB.^{6,17-21} Hemolysis increases severely during CPB. It is also present in a mild low grade chronic state in the patients with normally functioning artificial heart valves²²⁻²⁴ and increases the potential for thrombo-embolic events.⁶

It appears that we have now reached a point when various CPB techniques, and sophistication in the manufacture of various devices, find it difficult to prevent further damage of the blood and its cells.¹⁷ This has led to a rethinking of the strategy in preventing or reducing trauma effects. For example, the use of prostacyclin in inhibiting the platelet response is one method.^{25,26} The red cell's integrity is vital for the normal function of the microcirculation and therefore it is only appropriate that these cells warrant some form of protection in order to reduce complications from damaged blood and its replacement.^{27,28}

In these studies urea and cyanate increased red cell resistance to trauma by about 30%. If this were theoretically added on as a potential for extension of maximum CPB time (~6 hours), one could speak in terms of a gain of two hours allowing maximum CPB to be extended to eight hours. However, the damage to the rest of the blood systems and organs would probably make these durations of total CPB too toxic a "dose" of CPB for the patient.

The combined effect of the blood response to trauma and exposure to unphysiological surfaces has been suspected to result in a general systemic inflammatory state. This may be related to post-operative clinical events including pulmonary, renal, cerebral and clotting disorders after CPB.²⁹⁻³⁵

The body is endowed with a natural response to every insult and the best we can hope to achieve is to minimize the effects. We have been slow to understand this in extracorporeal and artificial implant technology but may now be on the verge of possibly controlling the damaging effects, which have contributed to the morbidity and mortality of cardiac surgery over the past 30 years.

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