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Hemolysis of Human Erythrocytes Induced by Glucose Solutions and Its Prevention

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Glucose injections are extensively employed for supplementing nutrients and body fluid. However, it was noted that when blood was mixed with 5% (isotonic) glucose solution prior to intravenous infusion, partial hemolysis was induced. In an attempt to clarify the mechanism of this hemolysis, the effect of glucose solution on human erythrocytes was studied *in vitro*. The incubation of erythrocytes with glucose below 4% produced a dramatic hemolysis and K^+ loss, and with 5% glucose a partial hemolysis was induced accompanied with remarkable wrinkling of the cell surface and transformation, probably due to partial desalination of the cells by glucose. The erythrocytes treated with 5% glucose also showed significantly increased osmotic and heat fragility. The glucose-induced hemolysis was effectively prevented by the addition of NaCl above 0.5%; this presumably reduced the release of K^+ and water from the cells.

Keywords——human erythrocyte; glucose-induced hemolysis; fragility; cell wrinkling; transformation; hemolysis prevention

Glucose injections are employed in the treatment of the circulatory inadequacies associated with the hypovolemia attending the loss of both blood and plasma and for supplementing nutrients and body fluid. Glucose, having diuretic and detoxicating actions,[1] is also used in the treatment of many kinds of diseases. Available solutions consist of various concentrations of glucose, 2.5 to 50%. Among them, 5% (0.278 M) glucose solution is extensively used for intravenous infusion itself or after mixing with various drugs and blood. However, when blood was mixed with 5% glucose solution, which is isotonic, for transfusion into a patient, partial hemolysis was noted.

In an attempt to clarify the effect of glucose solutions on human erythrocytes and to find a method of preventing glucose-induced hemolysis, we initiated a study on the effects of glucose solutions on the fragility of erythrocytes, on K^+ efflux and on the cellular morphology. The protective effect of NaCl was also examined.

Experimental

Materials——Glucose (D-(+)-glucose, anhydrous) of special grade was used throughout this experiment.

Preparation of Erythrocyte Suspension——Human blood was collected from hematologically normal adult donors, utilizing sodium citrate as an anticoagulant. The erythrocyte suspension was prepared by the same method as described in a previous paper.[2] Hematocrit value was usually $40 \pm 1\%$.

Glucose-Induced Hemolysis and Measurement of K^+ Release——A 0.3 ml aliquot of the erythrocyte suspension was added to 3 ml of glucose (2.2—8.8%) dissolved in water, and gently mixed. The mixture was incubated for 30 min at 37 °C then centrifuged at $1500 \times g$ for 3 min. The percentage hemolysis and K^+ release were determined by the methods described in a previous paper.[2]

Electron Microscopy——Erythrocytes, treated with glucose solution, were fixed with 1.5% glutaraldehyde and viewed with a Hitachi scanning electron microscope, model S-700, as described in a previous paper.[2]

Results and Discussion

Glucose-Induced Hemolysis and K^+ Efflux

A plot of percentage hemolysis against glucose concentration is shown in Fig. 1. The glucose solution was found to show hemolytic action below 4%. However, in 5% glucose solution, a partial hemolysis (about 1—3%) was observed. A prolonged incubation (1 h) of cells in 5% glucose solution produced 7—9% hemolysis. In order to estimate the effect of glucose on the permeability of the erythrocyte membrane, the efflux of K^+ from the cells was measured. The K^+ loss from the incubated cells exceeded hemoglobin liberation over the whole range, indicating that the release of K^+ from cells is faster than that of hemoglobin.

Scanning Electron Microscopic Observations

The results of scanning electron microscopy are illustrated in the photomicrographs reproduced in Fig. 2. Examination of these photomicrographs reveals that hypotonic glucose solution induced clear shape changes, as shown in Fig. 2B—D. Of particular interest is the

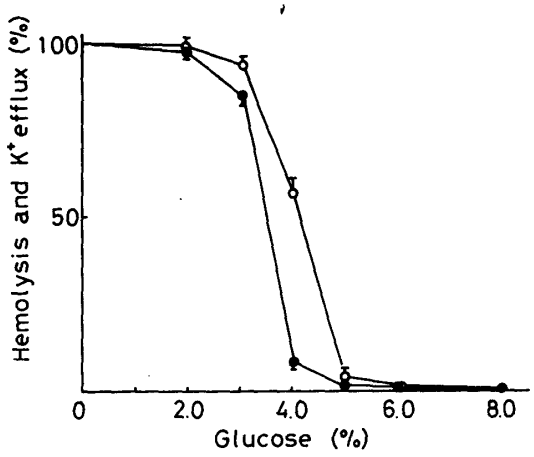

Fig. 1. Effect of Glucose Concentration on Hemolysis and K^+ Efflux

The experimental conditions are described in the text. Each point represents the mean ± S.D. of 4 experiments. ●, hemolysis; ○, K^+ efflux.

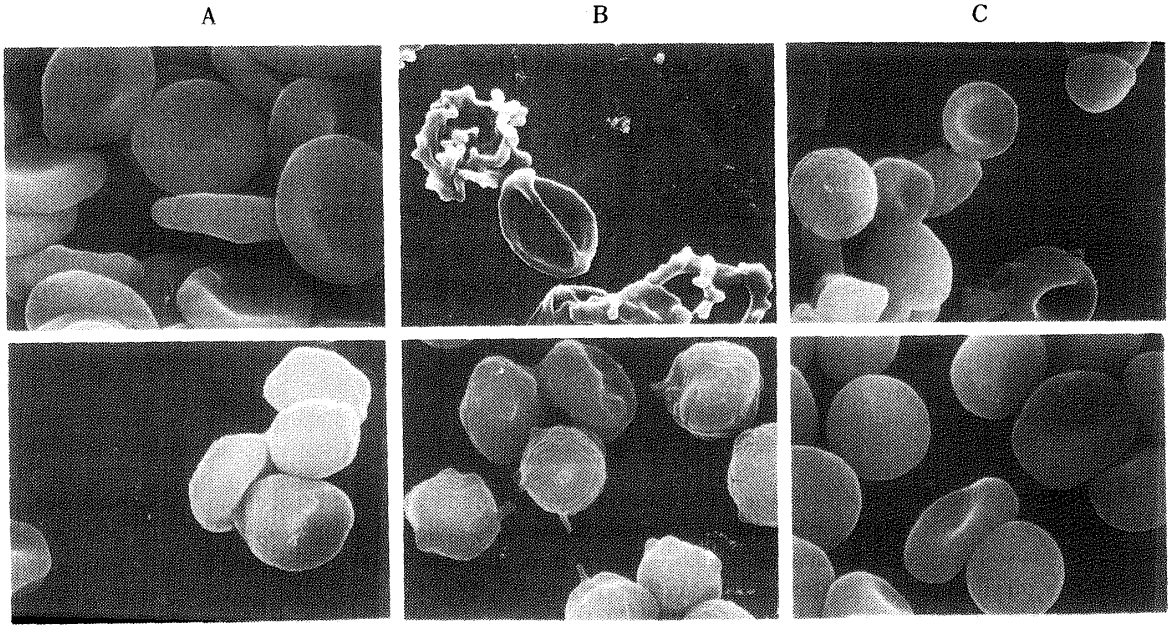

Fig. 2. Scanning Electron Micrographs of Erythrocytes Treated with Glucose Solution

A, control; B, H_2O; C, 2.5% glucose; D, 4% glucose; E, 5% glucose; F, 7% glucose. Magnification 5000×.

finding that the cells in 5% glucose became remarkably wrinkled, probably due to the partial efflux of cations induced by glucose. The alteration of the cell membrane observed in 5% glucose solution may lead to the increased fragility of the cells. The cells treated with 7% glucose solution were mostly transformed to spherocytes without hemolysis.

Fragility of Erythrocytes Treated with Glucose Solution

To compare the fragility of the cells treated with isotonic glucose solution with that of normal cells, erythrocyte suspensions were incubated in 4 and 5% glucose solution for 30 min at 37 °C, and the osmotic and heat fragility of the cells was estimated.

a) Osmotic Fragility——As shown in Fig. 3, the osmotic fragility of the cells treated with glucose at 4 and 5% was dramatically increased. It is of interest that the cells exposed to glucose underwent hemolysis even in 0.9% NaCl.

b) Heat Fragility——Glucose-treated cells were exposed to heat (40—55 °C) for 10 min and the hemolytic percentage was measured. The results is shown in Fig. 4. The fragility of the cells treated with glucose was significantly increased compared with that of the control cells.

These results indicated that erythrocytes treated with isotonic glucose showed increased

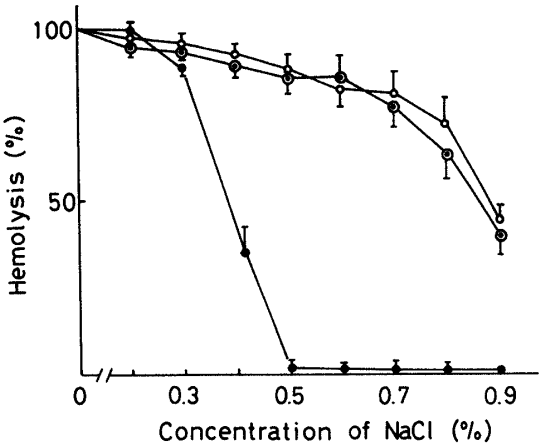

Fig. 3. Osmotic Fragility of Erythrocytes Treated with Glucose Solution

The cells were incubated in glucose solution at the indicated concentration for 30 min at 37 °C and then centrifuged. A 0.3 ml aliquot of the cell suspension was added to 3 ml of hypotonic NaCl solution and the mixture was incubated for 30 min at 37 °C, centrifuged, and subjected to the measurement of hemolysis. Each point represents the mean ± S.D. of 4 experiments. ●, isotonic NaCl–phosphate buffer, pH 7.4; ⊙, 4% glucose; ○, 5% glucose.

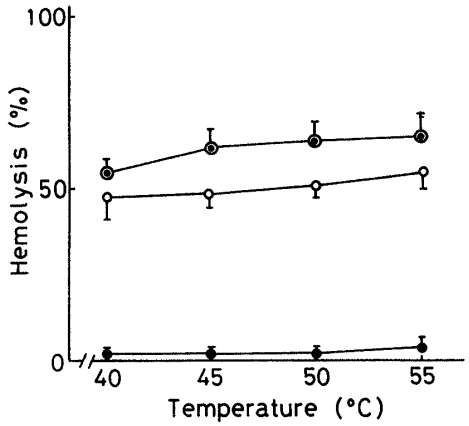

Fig. 4. Effect of Temperature on Hemolysis of Erythrocytes Treated with Glucose Solution

The cells were treated as described in Fig. 3 and a 0.3 ml aliquot of the cell suspension was added to 3 ml of isotonic NaCl–phosphate buffer, pH 7.4. The mixture was incubated for 10 min at the indicated temperature. ●, isotonic NaCl–phosphate buffer; ⊙, 4% glucose; ○, 5% glucose.

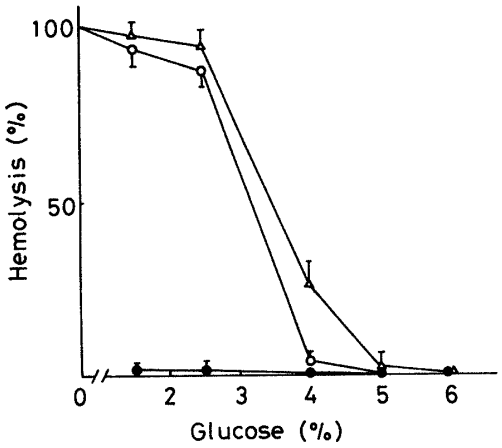

Fig. 5. Effect of NaCl Concentration on Glucose-Induced Hemolysis

Experimental conditions are described in the text. Each point represents the mean ± S.D. of 4 experiments. △, H_2O; ○, 0.3% NaCl; ●, 0.5% NaCl.

Table I. Effect of NaCl Concentration on K^+ Efflux Induced by Glucose Solution, and Ionic Strength and Depression of Freezing Point (D.F.P.) of the Incubation Medium

NaCl (%) in glucose solution	2.5% glucose		5% glucose		Ionic strength
	K^+ efflux (ppm)	D.F.P.	K^+ efflux (ppm)	D.F.P.	
0	114.2±13.3	0.285	48.1± 5.3	0.519	0.004
0.1	110.1±12.7	0.338	33.0± 3.7	0.572	0.032
0.2	109.8±11.1	0.391	24.4± 6.4	0.625	0.048
0.3	94.8±10.6	0.443	4.5± 2.0	0.677	0.063
0.4	80.2± 8.4	0.495	1.8± 0.8	0.729	0.079
0.5	1.7± 0.6	0.548	1.3± 0.7	0.782	0.094
0.7	1.1± 0.7	0.653	1.3± 0.6	0.887	0.126
0.9	1.2± 0.5	0.758	1.2± 0.4	0.992	0.157
H_2O	115.2±12.0	—	115.2±12.0	—	—

The incubation medium consisted of 3.0 ml of glucose solution containing NaCl and 0.3 ml of erythrocyte suspension in 0.9% NaCl–phosphate buffer. The ionic strength (μ) was calculated by using the equation $\mu = (1/2)(C \cdot X^2 + A \cdot Y^2)$, where C and A are the cation and anion concentrations and X and Y are the valences, respectively. The depression of freezing point was calculated based on that of 5.05% glucose solution being 0.52. Each value represents the mean±S.D. of 4 experiments.

fragility and lytic sensitivity.

The process of hemolysis induced by hypotonic glucose solutions can be explained as follows: 1) membrane expansion occurs as a result of the decrease in ionic strength in the external solution, 2) consequently formation of minipores in the membrane occurs, permitting the direct egress of cations such as K^+, 3) enlargement of the minipores to holes results in the release of hemoglobin. In 5% glucose solution, the egress of cations proceeds gradually, while glucose above 5% prevents the efflux and keeps the cell volume below a critical value by providing an external osmotic force, as shown in the case with sucrose.[3] It is recognized that the shrinkage induced by lead is caused by a decrease of the osmotic content of red blood cells due to a net efflux of K^+ that is not accompanied by Na^+ uptake.[4]

Protective Effect of NaCl on Glucose-Induced Hemolysis

In order to assess its protective effect against glucose-induced hemolysis, NaCl was added to the glucose solution and the percentage hemolysis was measured. As shown in Fig. 5, NaCl at 0.5% completely prevented the hemolysis induced by 1.5—5% glucose and NaCl at 0.3% largely prevented 5% glucose-induced hemolysis. This phenomenon is well explained by the concept that NaCl added to glucose solution effectively prevents the release of K^+ and water from the cells; K^+ efflux from cells was clearly prevented by the addition of NaCl at 0.4—0.9%, as shown in Table I.

The ionic strength and the depression of freezing point of incubation medium containing glucose are also shown in Table I. A consideration of the contributions of ionic strength and osmotic pressure (as depression of freezing point) to the protection, however, does not permit one to draw a definite conclusion about the nature of the protection, although both factors may be important.

On the basis of the results presented here, we concluded that isotonic (5%) glucose solution partly hemolyzed erythrocytes and the cells treated with 5% glucose showed increased osmotic and heat fragility, probably due to the partial desalination of the cells by glucose. The 5% glucose-induced hemolysis was effectively prevented by the addition of NaCl above 0.4%, and the hypotonic hemolysis by glucose below 5% was also prevented by NaCl at the final concentration of 0.5%.

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