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SHORT COMMUNICATION

The Influence of Glucose and Saccharose on Haemolytic Action of $HgCl_2$ in Relation to the Ionic Strength of the Incubating Medium

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Summary

The haemolytic action of $HgCl_2$ (0, 15 mmol.l^{-1}) was studied in relation to the ionic strength and concentration of glucose and saccharose in incubating medium. Blood from 94 donors, aged 19-46 years were used in our experiments. In relation to the ionic strength the haemolytic action was characteristic with two maxima of haemolysis. The first at low ionic strength and second one at the high. Both maxima in solutions containing saccharose were significantly diminished in glucose. These facts show a negative influence of saccharose on the haemorheological properties of the erythrocyte membrane.

Key words

Erythrocytes – Haemolysis – $HgCl_2$ – Glucose – Saccharose – Ionic strength

Water fluxes through the erythtrocyte membrane are complicated processes, which are bound to specific membrane constituents-water channels-located in bound 3 and 4.5 (Solomon 1986, Solomon et al. 1986). They are a constant component of integral membrane proteins and carry out osmotic water transfer through the membrane. Besides this, water penetrate through the membrane bilayer in spaces of the connection between lipids and integral proteins (Lawaczek 1988). The reason for this opinion is the fact that some mercury compounds can block approximately 90 % of osmotic water permeability (Benga 1988). After this blockade only water penetration through the lipid bilayer remains. However, we have sparse information about the influence of some sugars on the visco-elastic properties of membrane proteins.

In the present experiments we studied the influence of glucose and saccharose on the stability of some membrane proteins which form its visco-elastic properties. The haemolytic action of $HgCl_2$ was studied under the conditions of changing ionic strength and different amount of glucose and/or saccharose, present in the incubating medium.

In *in vitro* experiments the blood from the group of 94 healthy donors was used. The age of donors was between 19-46 years. 15 μl of blood were added to the 3 ml of the incubating medium. The samples were incubated for 60 min at 37 °C. The incubating medium was isotonic and contained different concentration of glucose and NaCl (solution I) and saccharose and NaCl (solution II). The concentration of NaCl (ionic strength) in both incubating media was as follows (in mmol.l^{-1}): 0.0; 15.4; 30.8; 46.2; 61.6; 77.0; 92.4; 107.8; 123.2; 138.6; 142.5; 146.3; 150.2 and 154.0. Besides this, solution I contains glucose in the concentrations (in mmol.l^{-1}): 287.0; 258.3; 229.6; 200.7; 172.2; 143.5; 114.8; 86.1; 57.4; 28.7; 21.5; 14.4; 7.2 a 0.0.

On the other hand, solution II contained saccharose in the concentrations (in mmol.l^{-1}): 300.0; 270.0; 240.0; 210.0; 180.0; 150.0; 120.0; 90.0; 60.0; 30.0; 22.5; 15.0; 7.5 a 0.0. The concentration of $HgCl_2$ in each medium was 0.15 mmol.l^{-1}. After incubation the samples was centrifuged at 700 g. The haemolysis was evaluated spectrophotometricaly at 540 nm and expressed as the haemolytic rate in relation to haemolysis in distilled water.

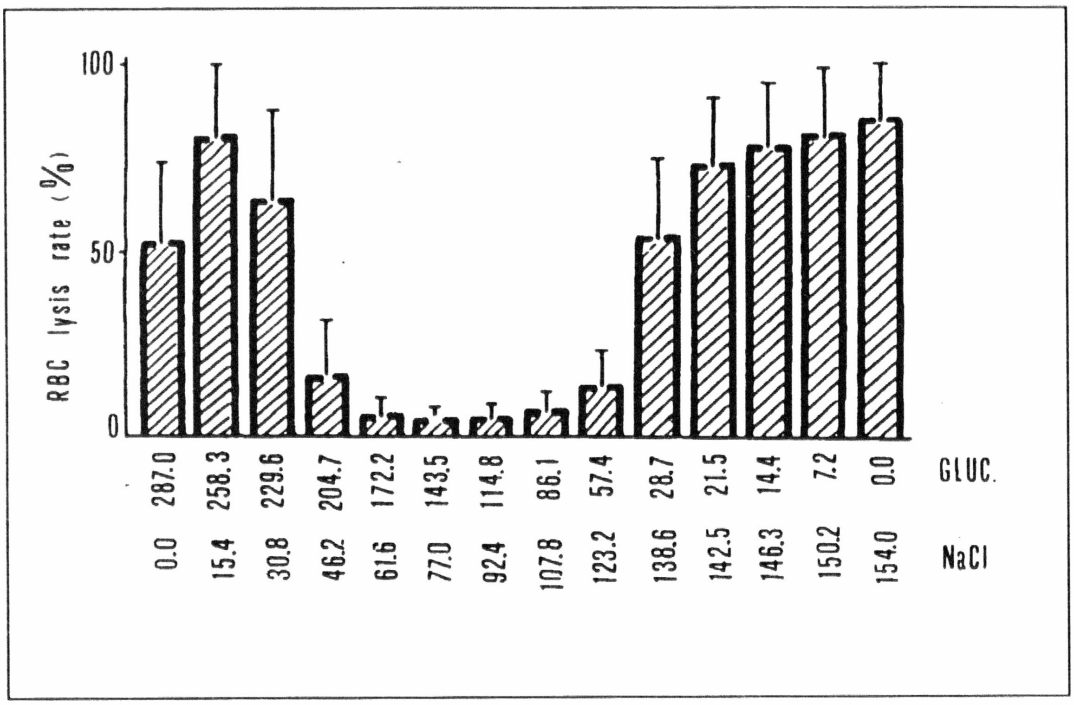

Fig. 1
Influence of glucose on the haemolytic action of $HgCl_2$ in relation to the ionic strength in the human red blood cells. *Ordinate:* the rate of haemolysis (%). *Abscissa:* concentrations of NaCl (ionic strength) and glucose.

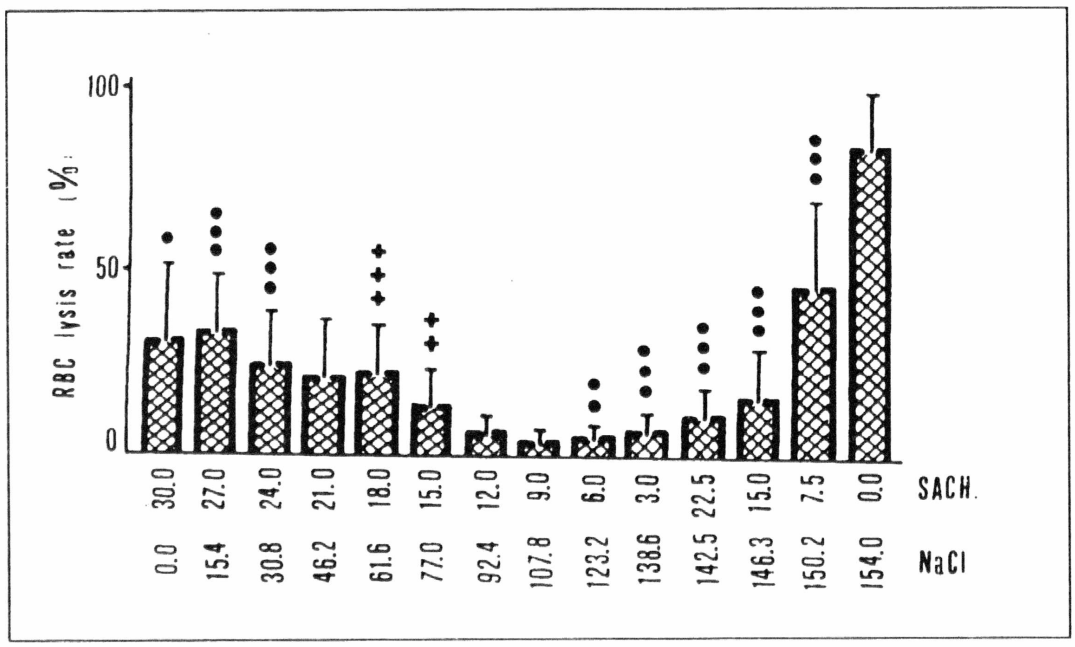

Fig. 2
Influence of saccharose on the haemolytic action of $HgCl_2$ in relation to the ionic strength in human red blood cells. *Ordinate:* the rate of haemolysis (%). *Abscissa:* concentrations of NaCl (ionic strength) and saccharose. Decrease of haemolysis: . $p<0.05$; .. $p<0.01$; ... $p<0.001$ Increase of haemolysis: + + $p<0.01$; + + + $p<0.001$, Vertical bars: ± S.D.

The results of haemolysis in different concentrations of glucose and ionic strength can be seen in Fig. 1. There are two maxima of haemolysis. The first in low ionic strength (15.4-30.8 $mmol.l^{-1}$) and the second at high ionic strength (138.6-154.0 $mmol.l^{-1}$). Minimum haemolysis was observed between 61.6-123.2 $mmol.l^{-1}$ NaCl.

Fig. 2 shows the haemolytic action of $HgCl_2$ under the conditions when glucose in the incubating medium was replaced by saccharose. In comparison with the haemolytic effect in glucose, we can see diminished haemolysis of both haemolytic maxima in saccharose. The significant lower haemolysis rate was found in isotonic saccharose alone ($p<0.05$) and further in 15.4 and 30.8 $mmo.l^{-1}$ NaCl ($p<0.01$). On the other hand, in concentrations from 61.6 to 77.0 $mmol.l^{-1}$ NaCl, haemolysis was higher than that found in glucose ($p<0.05$; $p< 0.01$ respectively). Very low haemolysis values were found between the ionic strength of 123.2 to 150.2 ($p<0.05$; $p< 0.01$).

When water permeability was blocked by inactivation of -SH groups using Hg^{2+} ions the passive penetration of water through the bilayer remained unchanged. The water influx under the above conditions is permanent and "colloid-osmotic" haemolysis occurs (Benga 1988). From the two maxima of haemolysis the first at low ionic strength can be considered as a consequence of spectrin dissolution and the second one at high ionic strength as a visco-elastic state of some membrane integral proteins. Under physiological conditions, saccharose does not cross the erythrocyte membrane. It seems possible that in the prehaemolytic phases, new membrane "pores" appear and saccharose can pass into the cell. On this base we can propose that changes of spectrin stability occur in the first maximum of haemolysis, and changes of visco-elastic properties in the second as a consequence of the influence of saccharose. The membrane seems to be less permeable in the presence of sacharose due to decreased membrane elasticity. The high saccharose blood levels can be taken as a negative factor influencing changes of blood micro- and macrorheology.

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Reprint Requests

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