

THE KINETICS OF HEMOLYSIS AS AN INDICATOR OF SPHEROCYTOSIS

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Spherocytosis is a genetic disease characterized by specific morphological changes in erythrocytes. Their fragility causes them to lyse when passing through microcapillaries. The changing shape and breaking of erythrocytes can be evaluated by the transmittance of a cell suspension. Transmittance as a function of time for cells under osmotic stress can be used to retrieve parameters possibly related to membrane strength and/or permeability. Properly designed stopped-flow experiments allow both parameters to be evaluated. Plasma membrane permeability can be measured when osmotic stress induces cells swelling without lysis. Subsequent experimenting in the lytic range of osmotic stress provides information concerning membrane strength. This simple set-up has been tested as a diagnostic tool in detecting selected blood diseases. The effect of permeability change on kinetic parameters was studied by changing the content of cholesterol in the plasma membrane and by blocking membrane porines. Changes of the plasma membrane and associated structures were evaluated with respect to measured kinetic parameters. By altering lipid bilayer composition, eliminating the glycocalix and disrupting the cytoskeleton, membrane permeability and strength were affected. The hemolytic propensity of blood from healthy volunteers and patients with spherocytosis was compared. Erythrocytes from sickle cell population have a more fragile plasma membrane, this being reflected by alterations in all measured kinetic parameters. Presented results show that the kinetic measurement of cell swelling and hemolysis can be used as a reliable, fast and inexpensive diagnostic tool.