(DCCD)[13] had practically no effect on thiamine uptake. A slight activation by 0.1 mM DCCD may probably be explained by the prevention of dissipation of the electrical potential through the membrane[14]. When the concentration was raised to 2 mM, an apparently nonspecific inhibitory effect could be observed similar to the reported case of aerobic proline uptake[15]. $HAsO_4^{2-}$ is known to decrease the intracellular ATP level[15]. As shown in all 3 strains in the figure, 10 mM Na_2HAsO_4 could completely inhibit the uptake of thiamine.

The fact that the Mg^{2+}-ATPase-less mutant of *E. coli* did not show any lowered thiamine uptake aerobically, and also the finding that about 50% of the activity was inhibited by anaerobic conditions suggests that the H^+ gradient across the membrane can be established by this ATPase-less mutant (probably defective in the F_1 fraction). The possibility is also supported by the fact that H^+ gradient has been detected in Mg^{2+}-ATPase defective mutants of *E. coli*[14,16]. The uptake activity shown above is expressed as the total amount of accumulated thiamine. The step of phosphorylation does not exert any serious influence on the rate of uptake because about the same level of uptake is observed in both *E. coli* 70-23 and its phosphorylation defective mutants[17].

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The effects of two neutral polymers on the geometry and deformability of the human erythrocyte[1]

L.S. Sewchand, S. Leuchter, R.E. Lovlin, J.S. Beck and S. Rowlands

Faculty of Medicine, University of Calgary, Calgary, Alberta (Canada T2N 1N4), 18 January 1979

Summary. Polyvinylpyrrolidone and dextran decrease cellular deformability. Changes in volume do not wholly account for the changes which imply a stiffening of the plasma membrane. The effects differ from those induced by charged macromolecules.

Adsorption of macromolecules onto the human red cell membrane (RCM) causes changes in cellular area and volume[2], in cellular deformability[3,4] and in cellular aggregation[5-7]. 2 neutral polymers, polyvinylpyrrolidone (PVP) and dextran (Dx) are readily adsorbed onto the RCM[8-10], and induce aggregation[10-12]. This study indicates that they also change cell geometry and deformability.

Methods. Cells from a finger-prick were suspended (v/v concentration ~1% to minimize rouleau formation) in Tris-HCl-buffered Ringer solution (pH 7.40±0.02, 310±2 mOsm) containing PVP (360,000 daltons) or Dx (70,000 daltons). Cells falling towards a glass coverslip on an inverted microscope with oil immersion optics were photographed when seen on-edge and the magnification was measured by a stage micrometer. Using the criterion of Ponder[13,2] the profile of each cell was drawn on an enlarged photograph. With the method recently described by Beck[14], the volume and area of each cell was calculated from measurements of diameter and of maximum and minimum thickness. Deformability is operationally defined by our method[15] which is to measure the pressure, averaged over at least 50 cells, needed to suck a cell within 1 sec into a micropipette of diameter approximately 2 µm. The treated cells are standardized against untreated cells from a control subject whose cells, in turn, have been standardized against a small normal population as described by Schachar et al.[15]. For all measurements differences between mean values were assessed by Student's t-test and regarded as significant for $p < 0.01$. In the next section each quoted result is accompanied by the SE of the mean value.

Results and discussion. For each of a number of concentrations of the 2 polymers, between 60 and 160 cells were photographed, their profiles measured and their areas and volumes calculated[14]. Up to a concentration of PVP of 3 $g \cdot l^{-1}$ none of the changes in the measured or calculated values reached statistical significance by our above-stated criterion. At a concentration of 5 $g \cdot l^{-1}$ the mean minimum thickness of the cells had increased from a control value of 1.30±0.010 µm to 1.47±0.012 µm, a change of 13%. Maximum thickness increased 3.6%, from 2.51±0.012 µm to 2.60±0.021 µm while volume increased 6.1% from 104.2±1.1 µm to 110.6±1.7 µm. Changes in diameter and area of the cells did not reach statistical significance, nor did variation in any of the values as the concentration of PVP was further increased from 5 $g \cdot l^{-1}$ to 15 $g \cdot l^{-1}$.

Dx at 5 $g \cdot l^{-1}$ caused a similar increase in cellular volume (8.5%) from a control value of 104.2±1.1 μm^3 to 113.1±1.8 μm^3, but the changes in maximum and minimum thicknesses of the cells differed markedly from those in PVP. The change in minimum thickness (2.1%) did not reach statistical significance while maximum thickness increased from 2.51±0.012 µm to 2.79±0.016 µm a change of 11%. As concentration of Dx was increased 10-fold there were no further significant changes. The increase in cell volume in

both PVP and Dx is intriguing in view of Jay's finding that albumin had the reverse effect[2]. Jay also reported a significant decrease in surface area.

Several methods are in use for assessing cell deformability: cell filtration through materials of known porosity (Teitel[16]) the rate of centrifugal packing (Sirs[17]) and the micropipette technique[15,18]. These various methods have not been correlated; ours reflects the cell's ability to change rapidly its geometry as it must to traverse fine channels in the microcirculation and spleen. By our method, deformability decreased uniformly and significantly as polymer concentration increased. The mean pressure required to draw cells into a micropipette increased from a control value of 21.4 ± 0.04 kPa to 22.6 ± 0.28 kPa (5.6%) as the concentration of PVP was raised to 5 $g \cdot l^{-1}$ and increased further to 23.8 ± 0.29 kPa (11.2%) at a concentration of 7 $g \cdot l^{-1}$. The increase in pressure implies a decrease in deformability as we defined it above. In Dx the mean pressure increased from a control value (using a different pipette) of $23.6 + 0.04$ kPa to 24.8 ± 0.37 kPa (5.1%) at a Dx concentration of 5 $g \cdot l^{-1}$ and continued to increase uniformly up to 27.9 ± 0.41 kPa (18.2%) as concentration rose to 50 $g \cdot l^{-1}$. An increase in volume alone decreases deformability[19]. The observed increase in volume plateaued at a polymer concentration of about 5 $g \cdot l^{-1}$ but deformability continued to decline as concentration increased. This was particularly marked for Dx at concentrations up to 50 $g \cdot l^{-1}$ and it implies a stiffening of the membrane in addition to the change in volume. The change in volume is not an artefact. The osmolarity of the suspending medium changed by less than 1% with concentration change and the refractive index of the medium increased by less than 0.05% relative to that of water at concentrations beyond which changes in volume were not observed. In any case an increase in refractive index is expected to decrease the apparent volume as measured optically.

From these preliminary studies we conclude that the interaction of neutral polymers with the RCM is complex and different from that of other macromolecules[2-4]. Both PVP and Dx cause an increase in volume, the former by a swelling in the region of the 'dimple' and the latter by swelling of the rim. Both polymers decrease the deformability of the cell but more than can be accounted for by the change in volume. Some molecular rearrangement must be involved in a stiffening of the membrane. Such a molecular change may be in the peripheral membrane protein. Spectrin appears to influence red cell shape[20-22] and there is evidence that spectrin interacts with integral membrane glycoproteins which are exposed to the suspending medium[22-24].

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Immunoglobulin synthesis by cord blood lymphocytes

W.B. Pittard, III, Kathleen Bill, D.M. Epstein and S.H. Polmar

Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University, School of Medicine, Cleveland (Ohio 44106, USA), 13 November 1978

Summary. The IgG, IgA and IgM synthesis by adult peripheral blood and cord blood lymphocytes incubated alone and with pokeweed mitogen was quantitated. The cord blood lymphocytes produced no immunoglobulin even with mitogen stimulation while the adult peripheral blood lymphocytes responded to the mitogen with a significant ($p < 0.04$) increase in immunoglobulin production.

Pokeweed mitogen (PWM) induces polyclonal immunoglobulin synthesis in vitro by adult peripheral blood lymphocytes (PBL)[1]. In contrast, this mitogen has been shown qualitatively to have minimal effect on immunoglobulin synthesis by lymphocytes derived from cord blood[2]. Quantitation of immunoglobulin synthesis by cord blood lymphocytes (CBL) has not been reported. Therefore, we prepared PWM response curves with CBL's and simultaneously cultured adult peripheral blood and cord blood lymphocyte preparations with PWM. Immunoglobulin synthesis was then quantitated with radioimmunoassays.

PBL's and CBL's were isolated using the ficoll-hypaque method[3] from 20-30 ml of heparinized blood drawn from 6 separate placentas (cord blood) and 6 separate adult donors. A differential was performed on dried smears using nonspecific esterase staining[4] to facilitate cellular identification and counting was performed with a hemocytometer.

2 CBL response curves to PWM (Gibco) were prepared using mitogen concentrations starting with 0.1 ml of undiluted mitogen followed by serial dilutions in media to 1:80. In addition to the 2 CBL response curves, 6 PBL and 6 CBL culture sets were prepared. The sets included 1. lymphocytes alone, and 2. lymphocytes plus PWM. PWM was added at a concentration previously determined to give maximal stimulation of PBL's (0.1 ml of 1:5 mitogen dilution) in preliminary studies. Each culture contained