UNDER THE AUSPICES:

THE FIRST WORLD CONGRESS ON WATER CHANNEL PROTEINS (AQUAPORINS AND RELATIVES) CELEBRATING THE 25th ANNIVERSARY OF THE DISCOVERY OF THE FIRST WATER CHANNEL PROTEIN (LATER CALLED AQUAPORIN 1)

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SECRETARIAT:

WATER EXCHANGE IN RED BLOOD CELLS: HISTORICAL COMMENT AND NMR STUDIES

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Introduction. Water exchange across the membranes of erythrocytes (red blood cells; RBCs) in higher animals is rapid, on the millisecond time scale. The exchange is mediated mostly by proteins [1] and yet not all the exchange is via aquaporins. The most convenient approach for estimating the permeability coefficient is the so-called manganese doping method that has been championed by Benga et al. [e.g., 2]. However, a new NMR method exploits the differential splitting of the ^2H_2O resonance of human RBCs suspended in gelatin that is 'set' and then held stretched in a special device in the NMR probe [3]. The method actually measures 'heavy water' exchange that may be different due the higher mass of the deuterium atom, but the advantage is the lack of the paramagnetic dopant, Mn^{2+}[4]. While the selective advantage of high water permeability of the human RBC is still speculated upon [5], methods to measure the shape changes in this cell are advancing [6]. Such methods hold promise for accurately characterizing RBC shapes simultaneously with measuring water exchange thus probing the effect of cytoskeletal rearrangement on this characteristic of the cell.

Materials and Methods. NMR spectra were acquired on a Bruker (Karlsruhe, Germany) Avance III 400 MHz NMR spectrometer tuned to ^1H or ^2H frequencies. A Bruker broad-band probe was used for the stretched gel experiments; while for the diffusion diffraction experiments a high-field gradient (up to 10 T m^{-1}) probe was used. Human RBCs were obtained by venipuncture (PWK) under University of Sydney Human Ethics Committee approval. Other experimental details are in the cited references.

Results and Discussion. ^2H_2O exchange measured by a new ^2H NMR quadrupolar-splitting method [4] yielded an estimated permeability coefficient for human RBCs at 25^{o}C very similar to that from the "older" ^1H NMR method [2]**.** Fast diffusion-interference experiments using *q*-space analysis based on the ^1H_2O signal, record shape changes in human RBCs on the minute time scale. The shape changes are not directly related to the mean ATP concentration inside the cells. The effects of shape changes on water transport are yet to be reported.

Conclusions. Historically the first serious suggestion that water transport in human RBCs is substantially protein mediated is credited to Benga et al. [1]. New NMR methods of measuring water transport continue to be developed [4] thus enabling different experimental conditions in which to study correlations with characteristics such as cell shape and metabolic-energy status [6].

Keywords. ^1H NMR; ^2H NMR; quadrupolar splitting; stretched gel; water permeability.

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