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**DIRECT EVIDENCE OF BILIRUBIN UPTAKE INTO LIVER CELLS
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Bilirubin, the product of heme catabolism, is formed in all cells and shed into the blood, where it is transported by albumin to the liver. At this level, it is taken up, glucurono-conjugated and excreted into the bile by an ATP-dependent efflux pump [1]. Uptake of bilirubin from the blood into the liver has been proposed to be mediated by OATP-2 (*SLC21A6*) [2,3], with a K_M for bilirubin of about 160 nM [3]. Later, this bilirubin transport function has been questioned [4]. Bilitranslocase is a plasma membrane organic anion carrier [5,6] that binds bilirubin with high affinity ($K_d = 2$ nM) [7]. The aim of this work was to test directly the bilirubin transport capacity of Bilitranslocase in liver cells. A cell transport assay was set up, based on the measurement of the time-dependent disappearance of bilirubin from a medium bathing a monolayer of cultured human liver cells (HepG2). The involvement of Bilitranslocase was investigated by testing the effect of an anti-sequence antibody on the kinetics of bilirubin disappearance.

The medium containing bilirubin was a simple phosphate buffered saline solution (pH 7.4). Under these conditions, its solubility is < 70 nM, i.e. high enough to saturate Bilitranslocase, but far too low to be detected by radioactive counting or conventional UV-Vis spectroscopy. The samples were therefore assayed by thermal lens spectrometry [8], a technique that enabled to measure bilirubin concentrations in the range 2-50 nM, avoiding the confounding presence of albumin. Bilirubin uptake was found to be a quite fast phenomenon, that was abolished not only by the anti-sequence anti-Bilitranslocase antibody, but also by nicotinic acid, that binds to Bilitranslocase with high affinity ($K_d = 11$ nM) at the same level as bilirubin [7]. The serine reagent Phenylmethylsulfonyl Fluoride, that binds to the bilirubin and nicotinic acid binding site of Bilitranslocase [9], abolished bilirubin uptake into cells as well. Taurocholate and digoxin, two OATP reference substrates, did not affect bilirubin uptake in our assay. Thus, Bilitranslocase is a bilirubin carrier, as also accepted by the Transport Classification Database (<http://tcdb.ucsd.edu/>).

References

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