

(14)

BILIRUBIN CO-TRANSPORTS PROTONS ACROSS MEMBRANES**Richard Wennberg¹, David Zakim², David Deamer³.**

Dept. of Pediatrics, U. of Washington¹, Dept. of Medicine, Cornell University², Dept. of Zoology, U. California, Davis³

There is conflicting evidence regarding the ionic state of bilirubin (B) in plasma and the nature of bilirubin-membrane interaction. If B binds as an anion to the outer surface of phospholipid bilayers, we reasoned that protonation of B would be a prerequisite for B entry into the hydrophobic core of the bilayer and that protons (H⁺) would be released as B emerged at the inner surface. We examined whether B would translocate H⁺ across phospholipid bilayers using small unilamellar vesicles (SUVOs) composed of either sphingomyelin (SM) or egg phosphatidylcholine (PC), each containing a pH sensitive fluorescent dye, pyranin, in 5 mM ATTM buffer. Various concentrations of B were added to SUVOs, initial pH 8.0 (in and out), while monitoring changes in internal pH. The B absorbance spectrum following addition of SM-SUVOs was also analyzed.

Results: 1) Addition of B (0.5 to 20 μ M) to SUVOs produced a marked decrease in internal pH (7.8-6.9) At pH 8.0(out), the ratio of H⁺ (moles transported in) to B (moles added) approached 1.0 as the concentration of B approached zero. Assuming equal distribution on inner and outer surfaces of the bilayer, it is concluded that B transports 2 H⁺ per B molecule and that nearly all B is bound as a dianion to the bilayer surface, leaving only miniscule amounts remaining in solution or within the membrane core. 2) the entire reaction of outer surface binding, insertion, and inner surface binding was too rapid to be analyzed and would require stop-flow instrumentation. 3) Addition of bovine serum albumin reversed H⁺ translocation entirely with PC SUVOs and partially with SM SUVOs, indicating a lower binding affinity for PC-B. 4) Surface binding of B was associated with a red shift in light absorbance with a maximum difference (aqueous vs. bound B) at 480 nm. At pH 8.0, the binding constant for SM-B was estimated to be 10^6 M⁻¹, requiring about 220 SM molecules for each high affinity unit.

Clinical Implications: The high affinity transport property of phospholipids could accommodate hepatic uptake of B without the need for a specific B transporter, but uptake efficiency would depend on the phospholipid composition of the membrane. Proton translocation could possibly contribute to B induced mitochondrial dysfunction