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CORRESPONDENCE

To the Editor: Based on a valid comment received from Professor Rudi Schmid, we wish to submit an amendment to our paper on bilirubin toxicity, published in your July 2003 issue (1). Rudi has advised us that Equation 1, which we utilized to calculate the unbound concentrations (BF) of unconjugated bilirubin (UCB), is inaccurate at BF values above aqueous saturation (70 nM). The now octogenarian guru of the bilirubin field was very perceptive to detect this oversight on our part.

Equation 1, derived from Brodersen's Eq. 15 in (2), is based on a model in which unbound bilirubin (UCB) monomers are in equilibrium with monomers bound at two independent sites on albumin. This is valid when the unbound UCB concentration, BF, is below aqueous saturation (70 nM), but at BF values above saturation, errors arise due to self-association of unbound UCB monomers (3). Thus, as BF increases above saturation, oligomers and larger aggregates of UCB will constitute a progressively larger proportion of the total unbound UCB, and the monomer concentrations (BF values calculated from Eq. 1) will progressively underestimate the true total unbound UCB concentrations.

Among the studies evaluated in our meta-analysis (Tables 1 and 2 in the original paper), the error is relatively small at calculated BF values of 71 and 85 nM (4–6), which are only slightly above aqueous saturation. In addition, in the studies done without albumin , the unbound UCB concentrations are equal to the *measured* total UCB concentrations of 250 nM and 400–500 nM (7–10), without using Eq. 1 and without assumptions about states of aggregation. No toxicity was observed in any of the studies at BF values below 70 nM. Therefore, despite the inaccuracy of our calculated higher BF values, our conclusion is still valid that "UCB can impair various cellular functions of astrocytes and neurons exposed to BF near or modestly above its aqueous solubility limit, at which UCB exists as soluble oligomers and metastable microaggregates."

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