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Funding Source: NSF-REU Program in Biosystems Modeling and Analysis

Development of easy-use cortisol detector: DTNB, blue squaric dye, and choline oxidase sensitivity to acetylcholinesterase

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Cortisol, an adrenal hormone released during stressful “fight or flight” situations increases blood glucose levels but also affects other physiological systems of the body such as the cardiovascular, reticular, and limbic systems (Bhagavan 256). Despite its physiological importance, cheap and reliable methods of cortisol measurement for household or field use, such as LifeScan’s One Touch Sure Step® for glucose, have not yet been developed. One potential means of determining cortisol concentrations is through the use of an ELISA assay with acetylcholinesterase. The aim of this study was to determine which method of detection, acetylthiocholine with detection reagents 3-Carboxy-4-nitrophenyl disulfide (DTNB), or 1,3-Bis [4-(dimethylamino)-2-hydroxypentyl]-2,4,-dihydroxy-cyclobutenediylium dihydroide (blue squaric dye), or acetylcholine with choline oxidase in combination with LifeScan’s One Touch Sure Step, was most sensitive and most probable for a future tool. Acetylcholinesterase, derived from rabbit blood plasma, was used to remove the acetyl group from thiocholine and choline. The change in absorbance of DTNB at 410 nm, blue squaric dye at 650 nm and the change in concentration reading on the One Touch Sure Step® glucose meter were used as measurements of sensitivity for DTNB, blue squaric acid, and choline oxidase, respectively. The K_m , or steady state constant, was utilized to distinguish the three systems. The blue squaric dye ($K_m = 0.05 \mu\text{M}$) was more sensitive a detector than either the DTNB ($K_m = 2.14 \mu\text{M}$) or the cortisol oxidase (K_m still currently being studied). While the blue squaric dye was the most sensitive, the cortisol oxidase thus far has the most potential for easy usage as a cheap, household instrument for measurement is already available. Future developments on a cortisol instrument must consider both sensitivity of the instrument as well as its convenience and reliability as a household and field instrument.