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Quantitative determination of unconjugated bilirubin in serum of human

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Abstract
Unconjugated bilirubin (UCB) is a powerful antioxidant and cytoprotectant, but at pathologically high amounts, it also induces apoptosis and cytotoxicity. To assess the function of UCB concentrations in redox control, Accurate measures of UCB concentrations in cells, fluids, and tissues are necessary for the prevention of bilirubin encephalopathy, atherosclerotic diseases, and cancer. In the present study, we evaluated a sensitive method for tissue UCB calculation.
Losses of UCB after extraction were compensated for by the use of mesobilirubin as an internal standard. Recovery rates tended to be 75%. The cutoff for detection was 10 pmol UCB/g of moist tissue. There was a 2.5% variance. When utilized to evaluate UCB concentrations in human tissues, this method yielded UCB values that were directly comparable to current methods and correctly recognized very low tissue bilirubin concentrations (40 pmol UCB/g tissue) in non-jaundiced.

Keywords: Bilirubin, method of determination, unconjugated, diazo

Introduction
Intracellular heme oxygenase and biliverdin reductase work together to make unconjugated bilirubin (UCB) from heme, with both enzymes playing a significant role in the stress response. In normal and modestly raised quantities, UCB acts as an antioxidant and cytoprotectant [1, 2], but larger amounts caused by impaired absorption, conjugation, and/or overproduction (such as hemolysis) can be neurotoxic. The quantity of unbound UCB (Bf) in the plasma *in vivo* or in the tissue culture media *in vitro* is associated with both its antioxidant properties and its toxicity to neurons (astrocytes and other cells) [3, 4]. Plasma Bf is essential for regulating intracellular UCB concentrations, which in turn dictates Bf's cytotoxicity, due to the rapid diffusion of UCB across cell membranes [5].
It is challenging to predict intracellular UCB levels from plasma Bf data, though, because UCB's oxidation, conjugation, and export from cells by membrane ABC transporters all impact intracellular levels [6].
Bilirubin is found in very small amounts in healthy tissues and biological fluids, has a strong affinity for proteins, is sensitive to light and oxygen, and breaks down rapidly in both acidic and alkaline solutions [7, 8].
These methods are not practicable for the general population due to the commercial unavailability of radiolabeled bilirubin or antibilirubin antibodies. More importantly, they understate tissue UCB levels since the pigment from the tissues is not fully removed.
The most often used methods include extracting UCB from tissues using a neutral or acidic pH chloroform/methanol solution, then measuring the results with a direct spectrophotometer or a diazo.
A diazo method for UCB in serum determination is presented in the current study. Precision may be attained by rapidly and simply preparing the sample, extracting the UCB, and then employing mesobilirubin (MBR, Fig. 1) as an internal standard (IS).

Material and Methods
Biological material
Fresh serum was collected on heparin tubes from 20 infants, both term and preterm, and submitted to the hilla hospital's medical analysis laboratory.

Methods
technique using the Biomaghreb reagent's DMSO activator, which is manually read (spectrophotometer).

Principle of Determination
Bilirubin is converted to azobilirubin using diazotized sulfanilic acid as part of the diazoreaction method (14), which was created by Malloy-Evelyn and modified by Walters *et al*., 1970. The two fractions found in serum are conjugated bilirubin/direct bilirubin (DB) and bilirubin-glucoronide.

Working style
Table 1 provides an illustration of the approaches' operational mode.
Components of the biomaghreb reagent include sodium nitrite (20 mmol/L), sulfanilic acid (30 mmol/L) + Hcl (150 mmol/L), and sulfanilic acid + HCL + DMSO (7 mol/L).
To assess the impact of the incubation duration on the accuracy of the tests, two spectrophotometer readings were taken at time T1= 05 min and T2=10 min for DB and T1 = 10 min and t T2= 15 min for TB.

Statistic Evaluation
The findings are displayed using M SD, and software is used for statistical analysis:
After confirming that the data are normal using IBM SPSS and Microsoft Excel to display the graphical representations, the ANOVA test is used to compare the concentrations.
Dimethylsulfoxide (DMSO) was from Applichem (Darmstadt, Germany).

It was feasible to produce an aqueous bilirubin solution with 0.005-3.00 mg per 100 ml of DMSO by mixing 3 mg of bilirubin with 10 ml of 0.1 N NaOH, forming an aqueous bilirubin solution, then adding 1 to 5 ml of that solution to 99 to 95 ml of buffer pH 7.4. After then, the buffer was changed to its final amount of 100 ml. Unless otherwise stated, bilirubin in buffer solution was dissolved and either moderately swirled in a beaker or inverted twice to three times in a graduated cylinder. If it was necessary to mix bilirubin in buffer, a magnetic stirrer was used as instructed, in the dark, at room temperature (24 2°), at a set rate, and for a set period of time. Unless otherwise stated, bilirubin was spectrophotometrically evaluated at wavelengths between 330 and 800 nm and completed within 10 minutes of the bilirubin's initial dissolution.

Results

Absorbance change of unconjugated bilirubin serum

The breakdown of unconjugated and patients' serum bilirubins was investigated after reaction. The decrease in absorbance at 450 nm, where the highest absorption of unconjugated bilirubin dissolved in chloroform was seen, is shown in Fig. 1. The unconjugated bilirubin decreased in absorbance at a faster pace than the patients' serum, varying between specimens. In 24 hours, the unconjugated bilirubin's absorbance dropped by around 45%.

Conclusion

When the blood bilirubin level is more than 3 mg/dL, aundice is seen. Hereditary non-hemolytic unconjugated hyperbilirubinemias, such as Gilbert syndrome and Crigler-Najjar syndrome types I and II, are caused by mutations in the UGT1A1 gene that affect bilirubin conjugation. Gilbert syndrome is characterized by moderate unconjugated hyperbilirubinemia, with bilirubin levels below 5 mg/dL, and UGT1A1 enzyme activity that is 10 to 30% of normal. The presence of more thymine-adenine (TA) repeats in the TATAA box region of the UGT1A1 gene promoter causes a reduction in UGT1A1 activity.

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