Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 839–850

Complex Analyte-Dependent and Analyte-Independent Interferences with Conjugated Bilirubin in the Enzymatic Phenol-Aminophenazone Peroxidase (PAP) Method for Creatinine Determination

By *C. D. Eng, R. Delgado* and *M. H. Kroll*

Clinical Pathology Department, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, USA

(Received February 22/July 14, 1993)

Summary: Although bilirubin interferes with the enzymatic assays for creatinine, neither a consensus of the degree of interference nor the mechanism has been established. Using multiple regression analysis, we demonstrate that the interference is negative and caused by both analyte-dependent and analyte-independent mechanisms. Furthermore, the correlative model includes terms non-linear with respect to creatinine. In the kinetic creatinine phenol-aminophenazone peroxidase method, there are analyte-dependent and analyte-independent mechanisms at work. The multivariate equation is: Crea' = 0.9879 Crea − 0.4524 Bili − 0.000828 Crea × Bili + 2.094 × 10^{-7} Crea^2 × Bili + 5.0 (Crea' = measured creatinine (μmol/l), Crea = true creatinine (μmol/l), and Bili = conjugated bilirubin (μmol/l)). The endpoint mode was affected less than the kinetic mode and exhibited different relationships in which two models describe the interference equally well. One is strictly analyte-dependent: Crea' = 0.9991 Crea − 0.00203 Crea × Bili + 2.390 × 10^{-6} Crea^2 × Bili − 1.464 × 10^{-9} Crea^3 × Bili + 3.261 × 10^{-13} Crea^4 × Bili − 9.9. The other is a complex combined analyte-dependent and analyte-independent: Crea' = 0.9834 Crea − 0.00680 Crea × Bili + 2.477 × 10^{-7} Crea^2 × Bili − 3.233 × 10^{-7} Crea × Bili^2 + 0.4652 Bili − 0.000458 Bili^2 + 12.2. These models are valid for creatinine concentrations up to 2200 μmol/l (24.9 mg/dl) and bilirubin up to 660 μmol/l (38.6 mg/dl). The interference increases with increments of either bilirubin or creatinine. In addition, we found that unconjugated bilirubin interferes differently from conjugated bilirubin in degree and mechanism. Model building, contour plots, surface plots, and possible mechanisms are discussed. We propose multiple regression analysis as the proper way to evaluate interferences because analyte-dependence can be easily missed by simple regression analysis. True creatinine concentrations can be estimated despite the interference from conjugated bilirubin. Other phenol-aminophenazone peroxidase methods may be similarly affected.

Introduction

Bilirubin is a well known negative interferent of the *Jaffé* method. There is some concern as to the degree of susceptibility for the phenol-aminophenazone peroxidase (PAP) method since compared with the Jaffé method in some studies (1, 2), the enzymatic assays for creatinine demonstrated less susceptibility to bilirubin and more as reported in others (3, 4). Moreover, published works suggest that conjugated bilirubin interferes more than unconjugated bilirubin in enzymatic assays (5, 6).

Our approach to interference studies is to derive a correlative equation by multiple regression analysis (7). The equation will contain terms which can be called analyte-dependent or analyte-independent. Analyte-independence is a term in which the degree of interference depends upon only the concentration of the interferent (8, 9). An example of this is pseudohyponatraemia is proportional only to the volume displacement caused by lipids. Another example is alkaline phosphatase, which when measured on the SMAC, is interfered by methotrexate because it ab-