

we reported here, we overcome this problem by using the filters. We have not observed any late-eluting peaks that would interfere with the subsequent chromatogram nor any reduction in column life attributable to sample contamination. Moreover, the results we obtained with the amperometric detector are comparable with those of the coulometric detector.

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### Hydroxocobalamin and Sodium Thiosulfate Interfere Negatively with Measurement of Creatine Kinase Activity

To the Editor:

Hydroxocobalamin (OHCO) is able to combine with cyanide to form cy-

anocobalamin (vitamin B<sub>12</sub>). Because of this property, OHCO is used by clinicians with sodium thiosulfate as an antidote in cyanide poisoning (Cyanokit®; Laboratoires Anphar-Rolland, Lyon, France) (1). Administration of this drug in high concentrations (serum concentrations up to 500 μmol/L and 1.3 g/L for OHCO and sodium thiosulfate, respectively) leads to a red coloration of biological fluids, which can interfere with determination of some biochemical analytes such as bilirubin (2).

In one patient treated with Cyanokit, we observed different results in serum creatine kinase (EC 2.7.3.2; CK) activity assessed by Kodak Ektachem 700® (Eastman Kodak, Rochester, NY) and Hitachi 737® (Boehringer Mannheim, Mannheim, Germany) analyzers. The activities measured by these analyzers were 673 and 1016 U/L, respectively (reference range, 20-110), whereas CK activity in a previous sample (taken a few hours before the administration of Cyanokit) was 1225 U/L.

To investigate this discrepancy, we examined the interference of OHCO and sodium thiosulfate on CK activity measured by these two analyzers and by a manual assay performed according to the recommendations of the French Society of Clinical Biology (3). All three methods in the first step convert creatine phosphate and ADP to creatine and ATP by using *N*-acetyl-cysteine-reactivated CK. According to the manufacturer's literature for the Kodak CK slide, ATP phosphorylates glycerol to α-glycerophosphate in the presence of glycerokinase; oxidation of α-glycerophosphate to dihydroxyacetone phosphate by α-glycerophosphate oxidase results in formation of hydrogen peroxide, which, in the presence of peroxidase, oxidizes a leuco dye precursor to form a dye, the rate of dye production being monitored by reflectance spectrophotometry at 670 nm. In the manual method (for which we used CK-Granutest® reagents; Merck Clevénot, Nogent sur Marne, France), ATP phosphorylates glucose to glucose 6-phosphate in the presence of hexokinase; oxidation of glucose 6-phosphate to gluconate 6-phosphate by glucose-6-phosphate dehydrogenase results in reduction of NADP<sup>+</sup> to NADPH + H<sup>+</sup>, the absorbance of which is measured at 340 nm. The reaction is initiated by creatine phosphate after preincubation of serum with *N*-acetyl-cysteine. The Hitachi assay is an adaptation of the manual procedure and involves a unique reagent (Enzyline CK-NAC® Optimisé 100; Biomérieux, Marcy-l'Étoile, France), the reaction

being initiated by the addition of serum.

Using the three methods, we compared CK activities in three pools of normolipemic nonicteric sera with high CK values (1300-1500 U/L), to which we had added increasing concentrations of OHCO (from 125 to 5000 μmol/L), sodium thiosulfate (from 0.3 to 13.3 g/L), and a mixture of both in the same proportions as supplied in Cyanokit. As shown in Fig. 1, OHCO interfered negatively in CK activity determinations by all three methods. In the presence of 500 μmol/L OHCO, the mean interference was -27%, -29%, and -21% in Kodak, Hitachi, and manual methods, respectively. The presence of sodium thiosulfate in high concentrations (up to 13.3 g/L) did not affect CK activity determined by methods measuring NADPH + H<sup>+</sup>

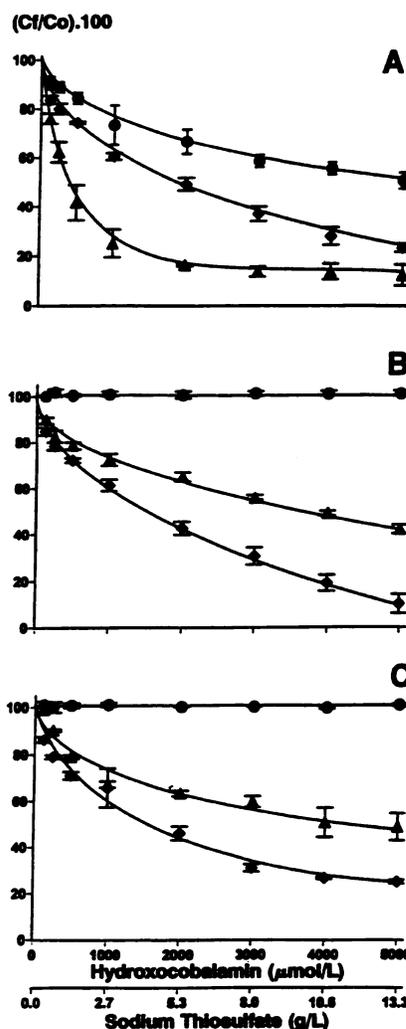


Fig. 1. Interference from hydroxocobalamin (◆), sodium thiosulfate (●), or both (▲) in the three methods: (A) Ektachem 700, (B) Hitachi 737, (C) manual method.

Cf, analyte concentration in samples with hydroxocobalamin and (or) sodium thiosulfate; Co, analyte concentration in control samples.

ormation (manual and Hitachi methods), but produced a negative interference in the Kodak assay, reaching -21% at a concentration of 1.3 g/L. In the Kodak assay, the simultaneous presence of OHCO and sodium thiosulfate led to a cumulative negative interference of -52%. Surprisingly, the combination of the two compounds resulted in a partial correction of the interference of OHCO in both manual and Hitachi methods, so that CK activity was underestimated by ~20% at the combined concentrations of 500  $\mu$ mol/L for OHCO and 1.3 g/L for sodium thiosulfate (17% and 23% for manual and Hitachi methods, respectively).

Next, we compared by regression the results for six sera with various CK activities (200–1300 U/L) to which we added OHCO (500  $\mu$ mol/L), sodium thiosulfate (1.3 g/L), or both. The linear regressions of CK activity values with (y) and without (x) the added interferences gave:

1) For the Kodak assay, OHCO =  $0.72x + 7$  ( $r = 0.998$ ), sodium thiosulfate =  $0.82x - 3$  ( $r = 0.998$ ), OHCO +

sodium thiosulfate =  $0.48x + 7$  ( $r = 0.997$ ).

2) For the Hitachi assay, OHCO =  $0.74x + 5$  ( $r = 0.999$ ), sodium thiosulfate =  $0.99x + 7$  ( $r = 0.998$ ), OHCO + sodium thiosulfate =  $0.80x - 2$  ( $r = 0.999$ ).

3) For the manual method, OHCO =  $0.78x$  ( $r = 0.998$ ), sodium thiosulfate =  $1.02x - 6$  ( $r = 0.998$ ), OHCO + sodium thiosulfate =  $0.85x - 3$  ( $r = 0.995$ ).

These results suggest that OHCO could interfere in the reactivation process of CK and (or) in the conversion of creatine phosphate and ADP to creatine and ATP, steps that are identical in the three methods. Consequently, administration of Cyanokit could lead to misleading serum CK values, which might be clinically relevant for some patients. In view of the pharmacokinetics of OHCO (plasma half-life, 4–6 h), blood sampling for measurement of serum CK activity with Kodak or Hitachi methodologies should be delayed at least 24 h after the administration of Cyanokit.

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