INTERFERENCE FROM CITRATE USING THE TITAN YELLOW METHOD AND TWO FLUOROMETRIC METHODS FOR MAGNESIUM DETERMINATION IN PLASMA

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Summary

This study was undertaken to learn whether or not citrate interfered with the determination of magnesium in blood plasma by the Titan yellow method and by two fluorometric methods that employ 8-hydroxyquinoline or 8-hydroxy-5-quinolinesulfonate. A series of determinations of magnesium in human serum was made by these three methods and by atomic absorption spectrophotometry, which served as a reference. The results support the conclusion that the values for magnesium analyzed in citrated plasma by these three methods represent total plasma magnesium minus the interference from citrate.

Introduction

Citrate has been found to interfere with the Mann's dye color reaction in the determination of magnesium in plasma, resulting in a reduction of the magnesium value that is directly proportional to the concentration of citrate in the sample [1]. No statements could be found regarding the possible interference of citrate using the 8-hydroxyquinoline [2] or 8-hydroxy-5-quinolinesulfonate method [3] for magnesium determination; conflicting reports were found that citrate does [4] and does not [5,6] interfere with the Titan yellow method. Therefore we have investigated the effect of citrate on the magnesium determination by these methods. Citrate does not interfere with the atomic absorption spectrophotometry determination [1], which served as a reference.

Materials and Methods

On two separate occasions blood was drawn from two different donors in dry plastic syringes and placed in test tubes containing: (1) no additive, (2) dry heparin, (3) dried heparin and the amount of dextrose found in ACD "A" treated blood, (4) and (5) dried heparin and 1.5 ml or 3.0 ml of ACD solution
"A" that had been completely dried at 50°, and, (6) and (7) dried heparin and 1.5 or 3.0 ml of liquid ACD solution "A" (hydrous dextrose 2.45 g/100 ml, sodium citrate, 2.20 g/100 ml, and anhydrous citric acid, 0.73 g/100 ml). Tubes containing 1.5 and 3.0 ml of ACD solution and 20 ml of whole blood contained ACD in half and full concentration of ACD bank blood, respectively. The blood was mixed, centrifuged for 10 min at 2000 rev/min, the serum or plasma was removed and analyzed in triplicate. Atomic absorption determinations were made on a model 303 Perkin—Elmer spectrophotometer as previously described [1]. The method of Tietz [7] was followed for the determination of magnesium using Titan yellow; readings were made on a Beckman DU spectrophotometer at 540 nm. The fluorometric estimation of magnesium with 8-hydroxyquinoline was determined by the method of Schachter [2]; readings were made on a Turner model 110 fluorometer with a 47B primary filter and a 2A-12 secondary filter. Schachter's method was followed for the estimation of magnesium with 8-hydroxy-5-quinolinesulfonate [3] using a Turner model 110 fluorometer with a 405 primary filter and a 2A-12 secondary filter.

Results

The results for the determinations are shown in Fig. 1 and described in the legend. For each method studied, the relation of each variable (no heparin (serum), dextrose, ACD "A" and dilution) was shown in relation to the heparinized plasma value, which was represented by a single bar. The magnesium value was not affected by heparin or dextrose, but it was significantly reduced when measured in ACD solution by the Titan yellow method and by the two fluorometric methods, the degree of reduction being roughly proportional to the amount of ACD solution "A" present. A greater reduction was found in plasma containing liquid solution than dry solution because of the dilution effect.

Fig. 1. These figures present, from left to right, the values for plasma or serum magnesium obtained by (1) atomic absorption spectrophotometry, (2) Titan yellow method, (3) the fluorometric method using 8-hydroxyquinoline, and (4) the fluorometric method using 8-hydroxy-5-quinolinesulfonate. The bar represents values found for heparinized plasma which were: 1.70, 2.01, 1.80, and 1.84 mequiv/l, respectively. Values above and below the plasma values are plotted on the individual graphs. It can be seen that only the dilution affected the readings by atomic absorption spectrophotometry, while ACD solution (but not dextrose) also affected the readings by the other three methods.
Discussion

Although atomic absorption spectrophotometry is replacing the chemical methods for analysis of magnesium, many fine laboratories do not yet have this relatively expensive equipment. The results of this study indicate that when magnesium determinations are required for a patient who has received recent transfusions of citrated blood, elimination of the citrate, as by ashing, would be a prerequisite for meaningful determinations by the three chemical methods tested as well as by the Mann’s dye method.

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