Estimation of ferrioxamine in jaundiced urine

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SYNOPSIS A simple modification to Fielding and Brunström’s method for the estimation of ferrioxamine is described for use with jaundiced urine. Manufactured ferrioxamine HCl may be slightly impure, and when used for standard purposes should be rendered anhydrous and checked for iron content. Standard ferrioxamine solutions suitable for calibrating Fielding and Brunström’s method may be prepared by adding standard quantities of ferric iron to excess desferrioxamine.

A rapid and simple spectrophotometric method for the estimation of ferrioxamine in the urine was described by Fielding and Brunström (1964). At pH 2-4 the iron of ferrioxamine rapidly complexes with excess sodium sequestrene to form a colourless ferric salt; the difference in optical density between an appropriately treated aliquot of urine decolourized by sequestrene and an aliquot not exposed to sequestrene is determined and the ferrioxamine concentration obtained by reference to a standard calibration curve for ferrioxamine.

Ferrioxamine has an absorption maximum at 430 mµ; as the value for bilirubin is similar the presence of bile pigments in urine will seriously interfere with the estimation of ferrioxamine. Simple dilution of the urine has not proved a satisfactory way round this difficulty. Since the measurement of chelated iron in the urine following desferrioxamine administration has important diagnostic and therapeutic applications in liver disease, a simple procedure is reported which renders the most heavily jaundiced urine suitable for ferrioxamine estimation by the method of Fielding and Brunström.

METHOD

PRINCIPLE Bile pigment is co-precipitated as an insoluble barium complex by the addition of barium chloride to urine. Excess barium is precipitated as barium sulphate (if not removed at this stage the barium will be precipitated by the addition of phosphate buffer during the ferrioxamine estimation). The supernatant solution is then estimated for ferrioxamine by Fielding and Brunström’s method.

REAGENTS In addition to the reagents described by Fielding and Brunström the following are prepared from analytical grade chemicals using iron-free, glass-distilled water.

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plotted against extinction. The regression line is shown for standard solutions estimated by Fielding and Brunström’s method without initial BaCl₂ treatment. The results for solutions prepared in water and normal urine are indistinguishable and have been pooled. Analysis of variance indicates no significant deviation from linearity, a residual error of \( E_{450} 0.007 \), with a regression line slope of \( E_{450} 0.340/1000 \mu \text{g} \) ferrioxamine-iron/100 ml (equivalent to \( E_{450} 0.0292/\text{mg} \) ferrioxamine HC1/100 ml).

Similar results were obtained for solutions estimated after BaCl₂ treatment. Standards prepared in water, normal urine, and heavily jaundiced urine gave indistinguishable results which have been pooled. After correction for dilution a regression line slope of \( E_{450} 0.331/100 \mu \text{g} \) ferrioxamine-iron/100 ml is obtained. Comparison of this curve with that obtained for the unmodified procedure indicates 97.5% mean recovery of ferrioxamine after BaCl₂ treatment.

**DISCUSSION**

The estimation of ferrioxamine in the urine provides a simple method for monitoring iron excretion during desferrioxamine therapy, in addition to its special application in the differential ferrioxamine test (Fielding, 1965). Both these aspects are relevant in the field of liver disease. The modification described here overcomes the difficulty presented by jaundiced urine without detracting from the simplicity or accuracy of the original method; as the results are in close agreement, the additional steps may be included or omitted as necessary.

Ferrioxamine solutions have been prepared in the manner described owing to the uncertain purity of the ferrioxamine HC1 available for standard purposes. This material, freshly received in the laboratory, has been found to lose 10-7% weight on vacuum desiccation, and the anhydrous material to have a mean iron content of 7.78% (range 6.67-7.94%); the theoretical value for iron in ferrioxamine HC1 is 8.59%. A different specimen examined by Mr. P. R. W. Baker of the Wellcome Research Laboratories, Langley Court, Beckenham, Kent, had 8.1% loss on drying and the anhydrous material 7.95% iron (by wet ashing with a colorimetric finish using bathophenanthroline, a method similar to our own); similar findings have subsequently been made at CIBA Laboratories, Horsham (Burley, 1967). A calibration curve prepared from anhydrous material of this composition would be predicted to give a 7.5% overestimate of ferrioxamine, whereas the observed overestimate given by such a curve compared with the regression line in Fig. 1 has been 5.5%.

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**REFERENCES**

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