DETERMINATION OF DESFERRIOXAMINE-BOUND IRON IN URINE

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An iron-chelating agent, desferrioxamine, was recently used in the treatment of a case of accidental ferrous sulphate intoxication in a European female child.[1] The amount of iron excreted in the urine was determined by a modification of the rapid incremental method of Beale, Bostrom and Taylor[2] for serum iron. This method involves dilution of serum with a glycine buffer of pH 1·9. At this pH, the iron in transferrin is liberated and addition of bathophenanthroline results in the formation of a red iron-phenanthroline complex. Beale *et al.* have demonstrated that the iron-phenanthroline complex is most stable at pH 1·9.

This present paper describes some of the difficulties encountered in the determination of urinary iron in the presence of this special chelating agent, desferrioxamine, and the methods used to overcome them. The difficulties are a consequence of interesting competition between 2 chelating agents in different redox states.

EXPERIMENT AND RESULTS

It was first established that this method could be used to measure iron in urine as well as in serum. The iron present in a normal urine was determined before and after addition of a known amount of ferrous ammonium sulphate (Table I). This added iron was readily measured with the bathophenanthroline reagent. In a comparison of the optical densities of identical quantities of iron-phenanthroline complex in urine and in serum, similar results were obtained.

TABLE I. DETERMINATION OF IRON IN URINE AFTER ADDITION OF FERROUS AMMONIUM SULPHATE

	Iron measured μG/100 ml. urine
Urine alone	39
Urine + ferrous ammonium sulphate (500 μg/100 ml. urine	552
Iron recovered	513

In order to measure desferrioxamine-bound iron in urine, it was necessary to free the iron from the chelate, which hampered formation of the iron-phenanthroline complex. This was achieved in 2 ways.

Firstly, in view of the high degree of specificity of desferrioxamine for ferric iron, the ferric iron attached to desferrioxamine was converted to ferrous iron by addition of a reducing agent, sodium metabisulphite. About 15 mg. were added to the glycine-buffered urine in the cuvette immediately before the bathophenanthroline reagent. Maximum colour development owing to the formation of iron-phenanthroline complex then occurred after 6 hours, whereas in the absence of a reducing agent it had not reached completion after 14 hours (Fig. 1). The percentage recovery of iron added to urine in the presence of desferrioxamine was 85% when a reducing agent was used. This demonstrates that reduction of ferric iron to ferrous iron favours chelation with bathophenanthroline. In addition, the stability of the ferrioxamine is much less at pH 1·9 than at pH 7·0.

Secondly, the desferrioxamine was destroyed by dry ashing of the urine. The ferric iron released was then measured after reduction with sodium metabisulphite. Maximum colour development occurred within only 1 hour, owing presumably to the fact that there was no desferrioxamine to compete with the

bathophenanthroline reagent. Both of the above procedures were used to measure desferrioxamine-bound iron in the first 24-hour urine collection of the patient (Table II).

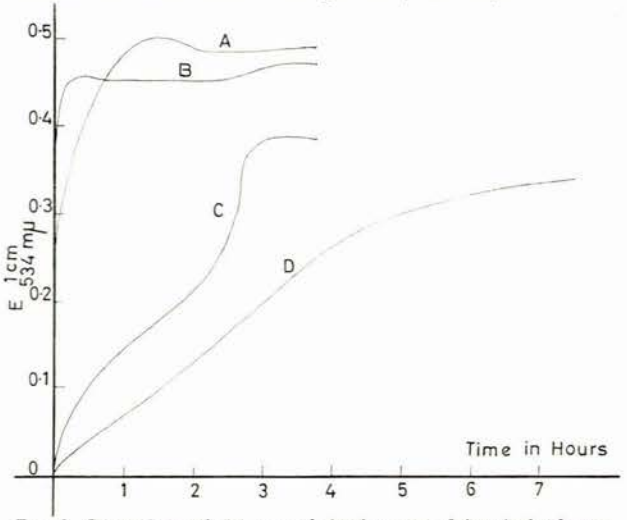

Fig. 1. Comparison of the rate of development of iron-bathophenanthroline complex in 4 aliquots of a normal urine containing 500 μg. of added ferrous ammonium sulphate per 100 ml. urine. A=urine + iron. B=urine + iron + sodium metabisulphite. C=urine + iron + desferrioxamine + sodium metabisulphite. D=urine + iron + desferrioxamine.

TABLE II. MEASUREMENT OF DESFERRIOXAMINE-BOUND IRON IN URINE

	Iron measured mg./24-hr. urine
Without preliminary ashing	9·02
With preliminary ashing	9·02

DISCUSSION

The ferrioxamines, a group of iron-containing metabolites with growth-stimulating properties for a number of micro-organisms, have been isolated from the *Streptomycetes*.[3] These are reddish-brown complexes from which the trivalent ferric iron may be removed and desferrioxamines formed. Desferrioxamine B is the chief product of CIBA's *Streptomyces* strain and has the following structural formula:

It has a high degree of specificity for ferric iron and will bind 9·3 G of iron per 100 G of reagent. It is very stable at pH 7·0 and readily water soluble. It readily removes ferric iron from haemosiderin and ferritin.[4] Nielsen,[5] using radioactive ferrioxamine and radioactive iron, has shown by electrophoresis that transferrin is unable to take up iron which is chelated to desferrioxamine, but that desferrioxamine has a limited ability to remove iron bound to transferrin.

The measurement of ferrioxamine-bound iron in urine with a bathophenanthroline reagent is probably dependent on several factors. Bathophenanthroline reacts preferentially with ferrous iron and the complex is most stable at pH 1·9. In contrast, desferrioxamine forms a complex with ferric iron and this is most stable at pH 7·0. However, neither pH change nor the presence of a reducing agent cause instantaneous release of iron from ferrioxamine. Desferrioxamine and bathophenanthroline thus seem to act as antagonistic iron chelates, the bathophenanthroline being the stronger at a pH of 1·9 and in the presence of a reducing agent. After destruction of the desferrioxamine by dry ashing of the urine and conversion of ferric iron to ferrous iron, the iron can be rapidly measured with bathophenanthroline.

CONCLUSION

Ferrioxamine-bound iron can be measured in the urine of a patient suffering from accidental ferrous sulphate poisoning and treated with desferrioxamine, by a modification of the rapid incremental method of Beale, Bostrom and Taylor. By conversion of the ferric iron to ferrous iron, maximum colour development with bathophenanthroline occurred within 6 hours. Preliminary dry ashing of the urine followed by conversion of the ferric iron to ferrous iron enabled maximal colour development to occur within 1 hour.

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REFERENCES

1. Shapiro, N. and Barbezat, G. O. (1964): S. Afr. Med. J., **38**, 461.
2. Beale, R. N., Brostrom, J. O. and Taylor, R. F. (1962): J. Clin. Path., **15**, 156.
3. Bickel, H., Gaumann, E., Keller Schierlein, W., Prelog, V., Vischer, E., Wittstein, A. and Zahner, H. (1960): Experimentia (Basel), **16**, 129.
4. Bannerman, R. M., Callender, S. T. and Williams, D. L. (1962): Brit. Med. J., **2**, 1573.
5. Nielsen, J. B. (1963): Acta med. scand., **173**, 499.