Oxalic Acid in Biology and Medicine

A. Hodgkinson

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A. HODGKINSON
MRC Mineral Metabolism Unit
The General Infirmary
Leeds

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as enzymic decarboxylation or reaction with uranium-4-(2-pyridylazo)-resorcinol (Mayer *et al.*, 1963; Baadenhuigsen and Jansen, 1975).

Several authors have omitted both the separation and washing stages by measuring the change in concentration of calcium in the supernatant fluid, before and after precipitation and calculating the oxalate concentration from the difference between the two calcium values, (Giterson *et al.*, 1970; Fraser and Campbell, 1972; Menachè, 1974; Koehl and Abecassis, 1976). Some authors have added a known amount of oxalate as well as calcium, to the sample in order to improve recoveries and reproducibility (Fraser and Campbell, 1972; Koehl and Abecassis, 1976). However, the normal urine values reported by these authors are generally rather low and include some zero values, suggesting that serious losses may be occurring in some samples (see Table 6.2). One potential source of error, particularly at low oxalate concentrations, is the need to measure accurately, small changes in a relatively high concentration of calcium (Baadenhuijsen, 1975).

In a further attempt to overcome losses during precipitation, we have utilized the strong tendency of calcium oxalate to co-precipitate with calcium sulphate at pH 7·0; quantitative precipitation was further encouraged by the addition of ethanol (Zarembski and Hodgkinson, 1965b; Hodgkinson and Williams, 1972). Some minor modifications of our procedure have been suggested by Husdan *et al.* (1976).

Several authors have utilized the very low solubilities of thorium IV cerium III euorpium III and lead II oxalates (Table 2.4) to isolate oxalic acid from other interfering substances, but with varying success. Suzuki (1934, 1936) and Kamiya *et al.* (1937) precipitated oxalic acid from blood as the cerous salt at pH 2·3 and estimated the oxalate in the precipitate idiometrically. They reported normal values ranging from 2 mg/100 ml to 4 mg/100 ml but these were soon recognized as being much too high.

Pernet and Pernet (1965) precipitated oxalic acid from serum or plasma as lead oxalate, after adding a known amount of oxalate. The precipitated oxalate was reduced to glyoxylate and estimated colorimetrically with phenylhydrazine. A mean value of 288 μg of anhydrous oxalic acid/100 ml was reported for normal human blood, a figure which is probably much nearer to the normal value (see Chapter 6).

Europium nitrate was introduced by Vittu and Lemahieu (1965) for the determination of oxalate in urine. Inorganic phosphate interfered with the precipitation and was first removed by the addition of magnesium chloride and precipitation as magnesium ammonium phosphate. Since magnesium ions also interfere with the precipitation of europium oxalate, the addition of excess magnesium chloride must be avoided and it is