

Value of Estimating Methylmalonic Acid Excretion in Anaemia

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Summary: A rapid technique suitable for routine pathology laboratories has been used to estimate methylmalonic acid excretion in a 24-hour urine collection following a 10g. valine load. Levels above 40 mg./24 hours were found only in patients with vitamin B₁₂ deficiency. Patients with pernicious anaemia treated more than 24 hours before urine collection and patients with other types of anaemia had methylmalonic acid levels below 25 mg./24 hours.

This method of demonstrating vitamin B₁₂ deficiency can be applied rapidly in debilitated patients so that specific treatment can be instituted within 36 hours of admission.

Introduction

It has been established that vitamin B₁₂ is required in the metabolism of propionic acid to succinic acid, acting as a coenzyme for the conversion of methylmalonic acid to succinic acid (White, 1962). Methylmalonic acid has been shown to be excreted in small amounts in normal urine, but to be present in excess in vitamin B₁₂ deficiency (Cox and White, 1962; Barness, Young, Mellman, Kahn, and Williams, 1963). There is an overlap between the normal and abnormal ranges, but this can be abolished by giving a valine load before collecting urine (Gompertz, Hywel Jones, and Knowles, 1967).

Methods of estimating methylmalonic acid in urine have been described using gas chromatography, which is accurate but unsuitable for use in most clinical laboratories (Cox and White, 1962) and thin-layer chromatography, which is only semi-quantitative (Bashir, Hinterberger, and Jones, 1966). The development of a colorimetric method suitable for use in a routine laboratory (Green, 1968) stimulated the present study to find whether methylmalonic acid excretion would be of value in clinical situations in which a severe megaloblastic anaemia existed.

The definitive diagnosis of megaloblastic anaemia may be lengthy, yet in some clinical situations rapid treatment with the deficient haematinic is essential. Megaloblastic anaemia is usually gradual in development and often occurs in the elderly, who are slow to complain. The problem is whether to give vitamin B₁₂ or folic acid therapy. Assay of blood levels of serum vitamin B₁₂ and folate and other diagnostic procedures are relatively slow. Intrinsic factor and intrinsic factor antibody determinations can be carried out rapidly in some centres but are not abnormal in all cases of vitamin B₁₂ deficiency. The possible risk of provoking neurological symptoms encourages most physicians to treat the patient with vitamin B₁₂ before investigations are complete. When the anaemia has been due to folate deficiency we have seen the delay in response so caused to have serious consequences.

Patients and Methods

Thirty-eight patients with haemoglobin levels between 3.2 and 12.8 g./100 ml. have been studied. Thirty-seven were

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admitted to hospital with symptoms of anaemia and one with neurological symptoms of subacute combined degeneration of the cord (Hb 12.8 g./100 ml.). A marrow sample was aspirated from the sternum or iliac crest in each case. Thirty-one had megaloblastic erythropoiesis, seven were normoblastic.

Serum vitamin B₁₂ levels were assayed with *Lactobacillus leichmannii* (normal level 120-400 µg./ml.).

Serum folic acid activity and red cell folate were assayed with *L. casei* (normal serum level 5-25 µg./ml.; normal red cell level 165-640 µg./ml.).

Valine Loading.—All but five patients were given 10 g. of valine dissolved in water and flavoured with fruit juice.

Collection of Urine.—Twenty-four-hour urine specimens were collected into bottles containing 10 ml. of concentrated hydrochloric acid as a preservative. The total volumes were recorded and aliquots stored at -20° C. until the estimation was carried out.

Estimation of Methylmalonic Acid.—The method is described in detail elsewhere (Green, 1968). Acidified urine was saturated with ammonium sulphate and extracted with ethanol and ether, and the extract was purified with a strongly basic ion exchange resin. The methylmalonic acid was eluted and a colour developed with diazotized *p*-nitroaniline.

Results

Patients with Vitamin B₁₂ Responsive Anaemia.—Twenty-three patients aged 40 to 83 were studied; all had megaloblastic erythropoiesis and serum vitamin B₁₂ levels below 100 µg./ml. The Schilling test was abnormal in all those patients in whom this was performed (see Table). All patients responded to vitamin B₁₂ therapy as shown by a rise in reticulocyte count and a significant increase in haemoglobin concentration. Each of the 15 patients whose urine was collected before treatment was started had excretion rates of methylmalonic acid above 50 mg./24 hours (range 50-320 mg./24 hours). Two patients treated with vitamin B₁₂ immediately before receiving the valine and starting the 24-hour urine collection had levels of 63 and 45 mg./24 hours respectively. Urine from six patients who had been given vitamin B₁₂ more than a day before the 24-hour urine was collected had levels below 20 mg./24 hours (see Chart).

Valine Loading.—Eighteen of the vitamin B₁₂ deficient patients received valine before collection of the 24-hour urine; five were not given valine. In 12 patients who had received no therapy the excretion of methylmalonic acid in the urine 24 hours after the valine was more than 60 mg./24 hours (range 60-320 mg./24 hours). Three untreated patients who were not given valine had levels of 50 and 100 (two cases) mg./24 hours. Valine loading in two patients immediately after vitamin B₁₂ treatment gave methylmalonic acid levels of 45 and 63 mg./24 hours. Valine was given to four patients who had been treated with vitamin B₁₂ for more than 24 hours; in these the excretion of methylmalonic acid was 15 and 5 (three cases) mg./24 hours. In all the untreated cases of vitamin B₁₂ deficiency (15) the 24-hour excretion of methylmalonic acid was above 50 mg./24 hours. In the 24 hours following therapy raised levels were still obtained, but after a greater interval the excretion fell below 20 mg./24 hours.

