URINARY METHYLMALONIC ACID EXCRETION

To the Editor:

Norman, Martelo and Denton (Blood 59:1128-1131, 1982) claim that by using a more sensitive MMA assay they have been able to identify all the patients with cobalamin deficiency from a sample of 1118 patients. Further, they imply that current techniques would probably have missed at least 4 patients they were able to diagnose.

None of the claims appear to be substantiated by the data. Assay methods using gas chromatography preceded by ether extraction are able to detect MMA excretion of less than 1 mg in 24 hr, that is, a concentration of 0.5-1 μg MMA/ml. This is sufficiently sensitive for clinical purposes and is not very different in sensitivity to the assay used by the authors.

Secondly, none of the 27 patients have been proven to have megaloblastic anemia (marrows were not reported) nor to have pernicious anemia. Three, indeed, have normal serum cobalamin levels and four a normal MCV. Apart from a decline in MMA in one patient after treatment, we are not told whether these patients responded to treatment with B12. The incidence of neuropathy among the 27 patients is far higher than would be anticipated in a random collection of patients with pernicious anemia.

A common cause of a low Schilling test result is an incomplete urine collection, particularly among elderly patients. Unless the result of the Schilling test is corroborated by measurement of plasma radioactivity, there is no guarantee of its reliability. Abnormal Schilling tests corrected by additional intrinsic factor have been considered, alcoholism probably being the most important. It is not clear how a patient with early pernicious anemia would have come to light in this survey, since such patients may not excrete excessive amounts of MMA, even after stressing the pathway by a dose of oral valine.

Thus, the clinical material used by the authors was not such as to enable them to make a meaningful assessment of their assay method.

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REFERENCES


To the Editor:

We are thankful to have an additional opportunity to emphasize the importance and advantage of cobalamin deficiency detection through urinary methylmalonic acid (MMA) quantitation.

Although we did not specifically criticize previous studies of urinary MMA quantitation in our article, we did indicate that they may have lacked the specificity and sensitivity necessary to accurately distinguish patients with slightly elevated MMA. In the study by Chanarin et al., British Journal ofHaematology 25:45, 1973, a diethyl ether extraction was performed and then the internal standard was added. Therefore, this procedure does not correct for loss of MMA during extraction. They used octanoic acid as the internal standard, an eight-carbon monocarboxylic acid, to quantify a four-carbon dicarboxylic acid (MMA), and therefore, solubility, chemical reactivity, and chromatography may be different. They do not specify the amount of MMA added during a recovery experiment, therefore, their limits of detection are not known. In addition, data for the standard curve, within-day precision, and day-to-day precision are not included to help accurately assess the reliability of their method. Urine contains over 500 organic acids, of which several may have the same retention time as MMA when assayed by gas chromatography. In our opinion, mass spectrometry is the only currently available method that can accurately quantitate low levels of urinary MMA.

In our study, 22 of the 27 patients described are known to have had a megaloblastic bone marrow. Patients 2, 11, 18, 20, and 21 did not receive a bone marrow examination. All of the 27 patients after cobalamin therapy showed normalization of their hematologic system and usually some improvement in their neurologic presentation.

Patient 11 had a recent myocardial infarction so the bone marrow was omitted, but after cobalamin injection, he expressed a feeling of well being within 24 hr and normalization of his hematocrit from 38.8 to 42.5. The Schilling test stages III and IV helped determine that patient 15's cobalamin malabsorption was caused by the unusual combination of pernicious anemia and bacterial "blind loop" syndrome. The urinary MMA level of patient 16 decreased from 461 to 49.8 μg MMA/ml 2 days after cobalamin therapy and was 3.4 μg MMA/ml 2 mo later. Patient 18 at diagnosis showed obvious hypersegmented poly. One month after cobalamin therapy, the level of MMA was 1.0 μg/ml. At presentation, patient 18 could not walk without considerable help, but after 5 mo of cobalamin therapy was able to walk up to 3 hr unassisted. Patient 21, 11 yr prior to diagnosis, had a major problem with tingling in hands and feet along with leg weakness and muscle aching. Two years prior to diagnosis, stomach films showed gastric mucosal atrophy and pernicious anemia was suggested but blood tests (Hct 37.1, MCV 109) were not pursued. Prior to the MMA test, blood was slightly macrocytic with a large number of polymorphonuclear leukocytes with 5 and 6 lobes.

The Schilling test at our Medical Center is considered very reliable and in cases where incomplete urine collection was made, the results were declared invalid, as noted in our article. A normal MCV does not eliminate the possibility of cobalamin deficiency and the hazard of a falsely normal serum cobalamin value is a well recognized fact in clinical laboratories. For example, patient 2 initially gave a serum cobalamin level of 118 pg/ml. However, prior to cobalamin therapy a month later, during the time period of our MMA assay, a repeat value of 415 pg/ml was obtained. The high incidence of neuropathy shown in our study illustrates the need for a
Urinary methylmalonic acid excretion [letter]

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