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Note

Simple estimation of urinary methylmalonic acid by isotachophoretic analysis

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It has been known that patients with vitamin B_{12} deficiency have increased excretion of methylmalonic acid in their urine[1–5]. The quantitative estimation of methylmalonic acid in urine has been carried out by thin-layer chromatography[6]. However, this method is time-consuming owing to the complicated pretreatment of the samples.

A new simple and rapid method for detecting urinary methylmalonic acid has been devised. This isotachophoretic method[7–9] has several advantages over previously described techniques.

EXPERIMENTAL

The normal urinary samples were obtained from laboratory personnel. The samples from patients with B_{12} deficiency were obtained from Takamatsu Prefectural Hospital. The samples were kept frozen if not analyzed immediately.

An aliquot of 10 ml of normal urine and urinary samples to which 2 mg/10 ml of authentic methylmalonic acid had been added, were acidified to pH 1.0 with 0.5 ml of 10 *N* sulphuric acid. A 50-ml volume of diethyl ether was then added, shaken vigorously for 2 min and extracted twice. The upper phase was placed in an evaporating dish and evaporated under reduced pressure. The residue was dissolved in 0.5 ml of ethanol and 4 μl immediately used in the isotachophoresis for quantitative estimation of methylmalonic acid.

The capillary apparatus used was a Shimadzu IP-1B isotachophoretic analyzer[10,11] (Shimadzu Seisakusho, Kyoto, Japan). The separations were carried out in a capillary tube (20 cm × 0.5 mm I.D.) maintained at a constant temperature of 20°. The migration current was 125 μA. The leading electrolyte consisted of 0.01 *M* hydrochloric acid and β-alanine (pH 3.1). The terminating electrolyte was 0.01 *M* glutamic acid. The chemicals used were analytical grade.

RESULTS AND DISCUSSION

The purpose of this experiment was to develop a simple and rapid method for determination of urinary methylmalonic acid by isotachophoresis. It was possible to detect 0.5 μg of methylmalonic acid by using the isotachophoretic analyzer.

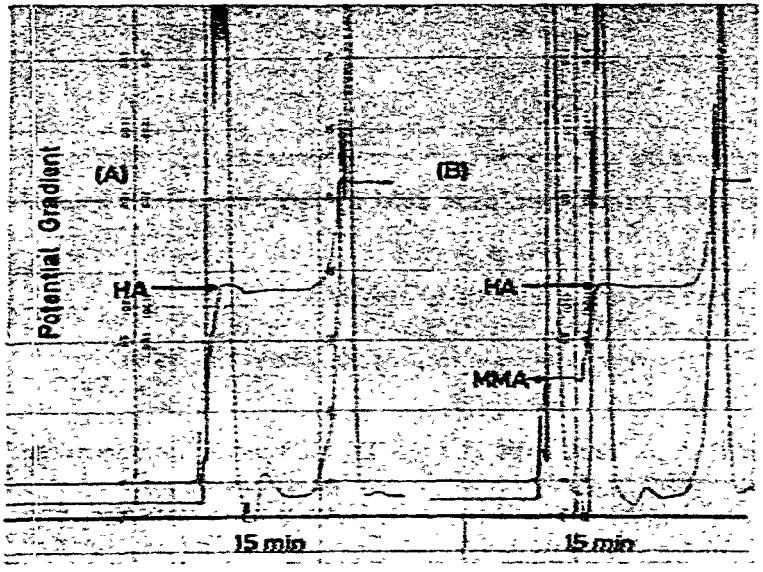

Fig. 1. Isotachophoretic runs of methylmalonic acid from normal human urine (A) and normal human urine + methylmalonic acid (2 mg/10ml) (B). The leading electrolyte was 0.01 *M* hydrochloride and β-alanine pH 3.1 and the terminator was 0.01 *M* glutamic acid. Migration current was 125 μA. Chart speed, 20 mm/min; temperature of electrolyte, 20°. HA = Hippuric acid; MMA = methylmalonic acid.

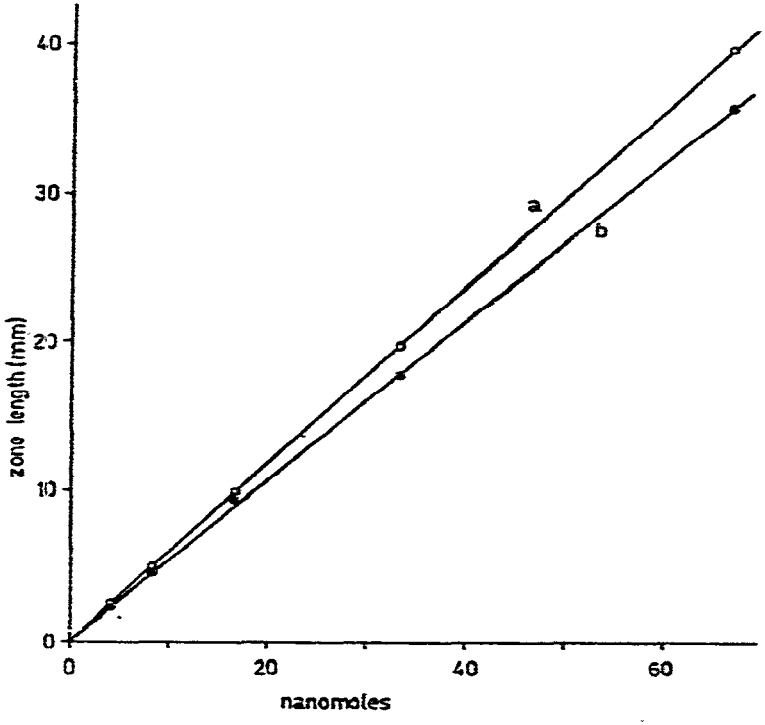

Fig. 2. Standard curves of authentic methylmalonic acid (a) and methylmalonic acid extracted from urine (b). Conditions as in Fig. 1. Chart speed, 10 mm/min.

Methylmalonic acid in normal subjects could not be detected clearly by our method, but it was possible to detect methylmalonic acid (2 mg/10 ml) added to normal urine (Fig. 1B) and in urine extracts obtained from patients receiving vitamin B_{12} therapy. The patients excreted 4.5 mg/24 h methylmalonic acid into urine, the normal level[6].

Standard curves of different concentrations of methylmalonic acid are shown in Fig. 2. The slope of the curve for authentic methylmalonic acid was linear and reproducible (Fig. 2a). The slope of the curve obtained after extracting methylmalonic acid from urine (Fig. 2b) was slightly less than that in Fig. 2a. However, it was still linear and could be utilized for the quantitative estimation of methylmalonic acid in the urine sample.

In several experiments, 92–95% of the methylmalonic acid added to normal urine was recovered by this method. The patients with vitamin B_{12} deficiency excrete a mean 212 mg/24 h of the acid into urine[6]. Therefore, with our method it is easy to detect urinary methylmalonic acid from patients with this disorder. The method is very simple and rapid compared with the thin-layer method[6], and very useful for screening patients with B_{12} deficiency.

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