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Rapid radio-proteinbinding measurement of urine folates

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Abstract. A method is presented for the determination of urine folates by a commercial radio-proteinbinding assay widely used in clinical diagnostics for assessing plasma folate levels. Good recovery of urine folates over the range of the calibration curve was observed. Because of the speed, sensitivity and precision of this test, urine folate levels can be measured in bioavailability studies or for the assessment of folate status.

Introduction

Radiometric methods of folate determination are used above all for the analysis of plasma, serum and erythrocytes where 5-methyltetrahydrofolic acid is the main folate derivative [1]. These methods have advantages over the microbiological assay: they are faster, cheaper and unaffected by the presence of antibiotics or folate antagonists. Since folate binding proteins show different affinities to folate derivatives (especially to polyglutamates) radio-proteinbinding assays are not applicable for complex folate mixtures, as they exist in foodstuffs [2, 3]. According to published data [4] and own investigations with the microbiological and the HPLC-method [5], urine only contains folate monoglutamates. As there are no data in the literature applying radiometric methods for measuring urine folate levels, the recovery of folic acid added to urine was examined by this test and the folate concentration in urine samples of a rat bioassay was measured.

Experimental

Folate levels in rat urine were determined using a commercial radio-proteinbinding assay from Becton Dickinson (Heidelberg). This kit is based on the principle of competitive protein binding between radiolabeled folic acid and unlabeled folates deriving from the tested sample. The amount of bound [^{125}I]-folic acid is inversely proportional to the amount of free folates in the sample. Urine samples were diluted with 0.2% (wt/vol) ascorbic acid solution to appropriate concentrations (1:2 to 1:10). From two dilutions 200 µl were analysed in duplicate with the radio-assay. Values were calculated as mean ± standard deviation from these four determinations. The recovery of folic acid from urine over the range of the calibration curve was examined by adding five different folic acid standard solutions (1, 2, 4, 10 and 20 ng/ml) to diluted urine samples. From the difference between measured folate content in doted and undoted samples folate recovery was calculated.

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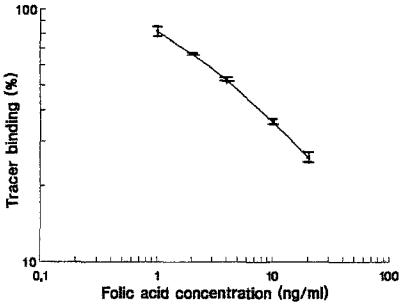

folic acid concentration (ng/ml)	recovery of folic acid (%)
1	86
2	89
4	96
10	90
20	105

Fig. 1. Calibration curve of folic acid concentration and tracer binding, and recovery of folic acid added to rat urine

Results and discussion

Figure 1 shows the obtained calibration curve of folic acid concentration and tracer binding, and the recovery of folic acid from urine over the range of the calibration curve. With increasing folic acid concentration the binding of tracer decreases. Near-linearity is obtained by double-logarithmic plotting. There is good recovery of folic acid from urine, with values between 86 and 105%.

After three weeks of depletion (rats fed diets free of folic acid) the folate contents in rat urine analysed with this radio-proteinbinding assay were 1.6 ± 0.3 µg/day. After a 14-days repletion period, in which diets with increasing concentration of folic acid were fed (0.25, 0.5, 1.0 and 2.0 mg/kg diet), a concentration-dependent increase in urine folate excretion was measured (1.8 ± 0.3, 2.2 ± 0.3, 3.1 ± 0.6, and 7.1 ± 1.5 µg/day) [5].

In conclusion, this radio-proteinbinding assay is well suited for the determination of folate concentrations in urine.

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