Abstract

J Clin Pathol. 1966 Jan;19(1):17-28.

Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency.

Hoffbrand AV, Newcombe FA, Mollin DL.

OBJECTIVE: A simplified microbiological assay for determining the folate content of red cells is described. As in previously reported methods Lactobacillus casei is used as test organism but two modifications are introduced.

METHODS: First, haemolysis is carried out in water containing 1 g.% of ascorbic acid; secondly, haemolysates are not incubated before the assay. Using this assay, recovery of pteroylglutamic acid added in two different concentrations to five different whole blood samples was 97.0 +/- 1.9 S.E. % and 106.1 +/- 4.7 S.E. % respectively. The coefficient of variation of the assay was between 11.2 and 15.0%.Haemolysates were best stored deep frozen, showing no significant loss of L. casei activity for three to five months at -20 degrees C. On the other hand, non-haemolysed blood samples were best stored at 4 degrees C. when there was no loss of activity for seven to 10 days.

RESULTS: Experiments confirmed that plasma is necessary for the maximum release of red cell L. casei activity, and showed that only small amounts of plasma are necessary; folate- and B(12)-deficient plasma released slightly lower L. casei activities from red cells than did normal plasma.The red cell folate levels of 40 healthy normal subjects ranged from 160 to 640 mmug. per ml. of packed red cells. One hundred and twenty patients with subnormal serum folate levels due to idiopathic steatorrhoea, nutritional folate deficiency and Crohn's disease, partial gastrectomy, myelosclerosis, and polycythaemia vera were studied. Red cell folate levels were subnormal (range from 7 to 143 mmug. per ml.) in 40 patients with megaloblastic anaemia, the lowest levels occurring in the most anaemic patients. Subnormal red cell folate levels also occurred in 23 (29%) of the 80 non-anaemic patients. There was a good correlation between red cell folate level and severity of folate deficiency assessed by polymorph nuclear lobe counts, and, in the non-anaemic patients bone marrow morphology. It is concluded that, in the absence of B(12) deficiency, the red cell folate level is a precise guide to the severity of folate deficiency.Patients with serum folate levels less than 3.0 mmug. per ml. almost always had megaloblastic anaemia or obvious morphological changes of folate deficiency. In patients with borderline serum folate levels (3.0-5.9 mmug. per ml.) haematological changes varied widely. The degree of change correlated with the red cell folate level in these patients.The formiminoglutamic acid (Figlu) test was positive (range 20-660 mg. excreted in eight hours) in all 30 patients with megaloblastic anaemia due to folate deficiency tested and also in 17 (31%) of 54 non-anaemic patients who were folate deficient. The amount of Figlu excreted paralleled the red cell folate level in both the anaemic and non-anaemic, folate-deficient patients tested. Figlu excretion, like the red cell folate level, appeared to be a satisfactory index of tissue folate stores.In 46 patients with pernicious anaemia, the red cell folate levels ranged from 26 to 396 mmug. per ml., 29 (63%) of them having subnormal levels. The ratio of mean red cell to mean serum folate level, 13.0:1, was lower than that of normal subjects. As in folate deficiency the patients with the lowest haemoglobin concentrations had the lowest red cell folate levels. Figlu was positively excreted in 10 (59%) of 17 patients with pernicious anaemia tested, being particularly increased in those with low red cell folate levels.Reticulocytes of patients with pernicious anaemia on treatment and with haemolytic anaemia were shown to have higher folate levels than their corresponding mature cells.

CONCLUSION: It is concluded that reticulocytes in general have relatively high folate levels.