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MICROBIOLOGICAL ASSAY OF FOLATE

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Folic acid is a B group vitamin found in a variety of foods. Its principal function is the transfer of single-carbon atoms in reactions essential to the metabolism of several amino acids and to nucleic acid synthesis and hence, in cell division. Thereby, its deficiency is clinically expressed in tissues with high rates of cell turnover.

Deficiency of folic acid is known to cause serious birth defects, low birth weight and pre-term delivery in children. In adults, while causing megaloblastic anaemia, it has been associated with coronary heart disease, dementia and depression.

While most foods are rich in folate, as much as 50-90% can be destroyed by improper processing and storage of foods. This leads to an inadequate dietary intake. Deficiency also occurs in decreased intestinal absorption (eg: chronic diarrhoeas), increased requirements (eg: pregnancy), alcoholism, deficiency of enzymes, increased excretion (eg: in renal dialysis) and as an effect of drugs (eg: cytotoxic drugs).

In this study we hope to analyse the serum and red cell folate and serum vitamin B12 levels of patients with megaloblastic anaemia and analyse folate concentrations in food articles likely to be the major folate contributors, prepared by different methods.

Folic acid levels will be assessed using a microbiological technique. This technique makes use of the fact that folic acid is an essential nutrient for the organism *Lactobacillus casei* (ATCC 7469). The organism was obtained in the lyophilized (freeze dried) form, from ATCC, U.S.A. This technique measures 'free' folate [defined as folate available to the micro-*organism L. casei* without conjugase treatment]. The total folate level is assessed after treating the sample with conjugase to break down the polyglutamates that cannot be utilized by the organism. (Chicken pancreas is the source of conjugase).

Rehydration of the organism was done using a microinoculum broth and it was cultured both in broth and in agar. The sample to be measured was added to the assay medium which is devoid of folate but has all other necessary nutrients for the organism. The organism was allowed to grow in it for 24 hours at 37^0C and according to the amount of folic acid in the sample, the growth of the organism differed. The turbidity of the contents of the tubes was measured at 660nm and folate present was determined using a standard curve established before.