

This Week's Citation Classic

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Hoffbrand A V, Newcombe B F A & Mollin D L. Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. *J. Clin. Pathol.* 19:17-28, 1966.

[MRC Group for the Investigation of Megaloblastic and Sideroblastic Anaemias and Dept. Haematol., Postgraduate Med. Sch., London, England]

A method of assay of red cell folate was established using a microbiological assay. Red cell folate was then shown to be an excellent guide to tissue folate stores except in certain well-defined circumstances. [The SCJ® indicates that this paper has been cited in over 265 publications since 1966.]

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"When I joined David Mollin's laboratory at the Royal Postgraduate Medical School, London, in 1963, the assay of serum folate microbiologically using *Lactobacillus casei* had recently been established. Studies by Victor Herbert¹ in the US and Alan Waters² in Mollin's laboratory had shown that folate in serum was not always an accurate measure of the degree of folate deficiency since very low results could be obtained in many patients without any haematological evidence of the deficiency.

"A number of groups in Israel, Sweden, Canada, Holland, and Britain had already reported assays of whole blood folate using *L. casei* or *Streptococcus faecalis* but the results were not consistent or reproducible. It was clear, however, that most of the folate in blood was in the red cells, but, unlike folate in serum, red cell folate was largely in a conjugated (polyglutamated) form and had to be deconjugated before assay. Some workers added a deconjugating enzyme (folate conjugase), e.g., from chick pancreas; others relied on the conjugase present in plasma.

"I joined Beverley Newcombe, who had worked with Waters to determine the conditions necessary to achieve a maximum folate concentration in whole blood. Early ex-

periments showed that distilled water was preferable to buffers for release of folate from red cells. As with the serum folate assay, it was necessary to add ascorbic acid to protect the folate from oxidative destruction in subsequent autoclaving. We showed that one in ten dilution of whole blood in distilled water containing one gram ascorbic acid per 100 ml gave a haemolysate with optimal release and stabilisation of folate. By happy coincidence, this ascorbate concentration lowered the pH of the haemolysate to approximately 4.6 which is optimum for the conjugase in plasma to deconjugate folate polyglutamates in red cells to microbiologically active monoglutamates.

"The presence of Barbara Anderson in the same laboratory, who had determined the optimum conditions for the serum vitamin B₁₂ assay using *Euglena gracilis*,³ ensured that the new assay would be fully tested by reproducibility and recovery experiments. It was then shown that the red cell folate was an excellent guide to tissue folate stores except in patients who had recently been transfused or had raised reticulocyte counts, or had vitamin B₁₂ deficiency. In both vitamin B₁₂ and folate deficiencies, the most anaemic patients had the lowest red cell folate levels, implying that the degree of depletion of tissue folate stores determined the severity of anaemia in both deficiencies.

"The assay became standard in studies of folate deficiency and metabolism. Moreover, the results in the paper indicated that the red cell folate assay was in general the best test for folate deficiency. It formed a cornerstone for subsequent studies⁴ on folate deficiency in a wide variety of diseases. The fact that the paper has been so widely quoted is largely due to the determination of Mollin to leave no loose ends before publication, either in studies of the method or studies of the significance of the results obtained. The assay remains a standard diagnostic test for tissue folate status."

1. Herbert V. The assay and nature of folic acid activity in human serum. *J. Clin. Invest.* 40:81-91, 1961.
2. Waters A H & Mollin D L. Studies on the folic acid activity of human serum. *J. Clin. Pathol.* 14:335-44, 1961.
3. Anderson B B. Investigation into the *Euglena* method for the assay of vitamin B₁₂ in serum. *J. Clin. Pathol.* 17:14-26, 1964.
4. Chanarin I. Folate in blood, CSF and tissues. *The megaloblastic anaemias*. Oxford: Blackwell Scientific, 1979. p. 187-97.