In 1962, Victor Herbert showed that folate deficiency develops in man on a low folate diet (less than 5 µg folate/day, daily dietary requirement 100-200 µg folate/day) and megaloblastic anemia results.

A low serum folate less than 3 ng/ml, seen after 3 weeks of a low folate diet, reflects failure of folic acid ingestion or absorption long before definite signs and symptoms of overt deficiency appear: hypersegmentation of polymorphonuclear leukocytes, low red cell folate, macroovalocytosis, megaloblastic marrow and anemia.

The time needed to manifest florid folate deficiency on an inadequate folate diet depends upon the diet, prior tissue folate stores and iron status but it is roughly 3-6 months.

Alcohol has many effects on folate metabolism. Excess alcohol consumption is often associated with a poor (folate deficient) diet and malabsorption. Alcohol accelerates the induction of megaloblastic anemia in subjects on a low folate diet. It antagonizes the hematopoietic response to folate (75 µg/day) in folate-deficient alcoholics, but its effect can be overcome either by larger doses of folic acid or by cessation of alcohol ingestion. Alcohol also induces an acute fall in serum folate which is rapidly reversible on withdrawal of the alcohol and is not correlated with depletion of folate stores. This acute fall in serum folate is not due to an assay artefact and may be due to the interference by alcohol with the delivery of folic acid from stores to the peripheral circulation. While the exact mechanism of alcohol's effect on folate storage and release by the liver is still under investigation, alcohol may produce a reversible sequestration of folate in the hepatocyte (as intracellular polyglutamate) at the expense of its release into bile as the methylenetetrahydrofolate monoglutamate for recirculation to tissues. The significance of alcohol-induced changes in folate binding and folate binding protein to the delivery of folate to tissue stores is unclear. Thus, the relationship of alcohol with folate deficiency and megaloblastic anemia is multifaceted.

Megaloblastic changes are not detected following ingestion of alcohol in the absence of folate deficiency: well-nourished alcoholics with good folate intake do not show megaloblastic changes and decreased folate absorption is seen with alcohol only when there is established folate deficiency.

The hematologic assessment of alcoholic patients is of clinical significance since folic acid deficiency is common in malnourished (but not in well-nourished) alcoholics. The question is when and how to evaluate an alcoholic for folate deficiency. Laboratory tests which have been used include hypersegmentation, macroovalocytosis, bone marrow examination, serum folate and red cell folate, but the dietary history is key.

Hypersegmentation

The evaluation of hypersegmentation (neutrophil lobe average greater than 3.5) is not done routinely, even though it can be useful if done carefully.

Sensitivity: Lindenbaum and Nath reported that only 6/357 patients failed to show hypersegmentation (i.e., 1 or more 6-lobed poly/100 polys) in megaloblastic anemia and that hypersegmentation of polys persists during folate therapy for about 2 weeks.

Specificity: Hypersegmentation of polys is not specific however, since it can occur as a congenital anomaly and on chemotherapy.
Macrocytosis

**Sensitivity:** The sensitivity of the MCV for the diagnosis of megaloblastic anemia is not documented, although the MCV is typically elevated and macroovalocytes are seen. The MCV may be normal, especially when other diseases such as iron deficiency, thalassemia minor, alcohol-related sideroblastic anemia and anemia of chronic disease are present, in which case a dimorphic smear is seen.

**Specificity:** Macrocytosis is not a useful indicator of folate deficiency since round macrocytosis in the absence of hypersegmentation is usually not due to folate deficiency. It is common in liver disease, in marked reticulocytosis, in hypothyroidism, in malignancy and with antimetabolite use.

There is a so-called "macrocytosis of alcoholism" that is common in well-nourished alcoholics and which usually consists of round and not oval macrocytes, an MCV below 110 and no hypersegmented polys. By definition, it is not associated with folate deficiency.

In summary: a high MCV is common without folate deficiency and although macrocytosis is one of the commonest abnormalities in alcoholics, it is not necessarily related to folate deficiency. The higher the MCV is, the more likely it is that the macrocytosis is due to folate (or B₁₂) deficiency. At Yale about half of patients with MCV greater than 115 had folate or B₁₂ deficiency. 75% of those with MCV greater than 125 had these deficiencies. Of patients with MCV greater than 115 who did not have these deficiencies, half had proved liver disease, usually alcoholic or were alcoholics with probable but unproved liver disease.

Megaloblastic Marrow

Megaloblastic changes include erythroid hyperplasia, nuclear-cytoplasmic dissociation in red cell precursors and giant metamyelocyte and band cells.

**Sensitivity:** Bone marrow evaluation is subjective and megaloblastic changes may be masked in iron deficiency.

**Specificity:** Red cell megaloblastic changes are seen not only in folate and B₁₂ deficiency but also in refractory macrocytic anemias, preleukemic states, AML and erythroleukemia. In these, however, hypersegmented polys and giant bands and metamyelocytes are not seen in the bone marrow. Their absence rules out significant folate deficiency.

A bone marrow is an invasive procedure costing $100 and it is probably not practical in the general management of the millions of alcoholics in this country.

Serum Folate

**Sensitivity:** In megaloblastic anemia, due to folate deficiency, serum folate is typically low although it may be normal because of a very recent dietary intake. A fasting specimen is recommended. 40% of the Seattle skid road hospitalized patients studied by Eichner had megaloblastic anemia but only 23% had low serum folate.

**Specificity:** Serum folate levels reflect low folate absorption from diet and may be low several weeks or months before tissue deficiency develops and hematopoietic changes occur. They are thus not evidence of tissue deficiency. High serum levels of alcohol produce low serum folate concentrations acutely. Dilantin produces low serum folate
because it lowers folate absorption by inhibiting deconjugation of polyglutamate to monoglutamate. Reduced serum folate is also reported in approximately 10% of patients with \( B_{12} \) deficiency.

**Method:** Most clinical studies used the Lactobacillus casei method for measuring folate. Various radioisotopic dilution assays are in current use but are incompletely studied, with reference ranges invented and probably invalid.\(^{13}\)

In summary: serum folate measurements are not useful in alcoholic patients.

**RBC Folate**

**Sensitivity:** RBC folate is increased in iron deficiency, where there is an apparent failure of folate utilization,\(^{16}\) hence it may fail to be reduced when there is combined folate and iron deficiency. It may be normal in folate deficiency when anemia is slight and it is also obscured by red cell transfusion.

**Specificity:** In the assessment of body folate status, rbc folate is less affected by acute dietary folate changes than is serum folate, and so in one sense is more specific. However, red cell folate is low in 60% of patients with \( B_{12} \) deficiency\(^{17}\) so that a low rbc folate cannot be interpreted without a \( B_{12} \) level.

**Method:** The few clinical studies used the rbc L. casei folate assay. The validity and efficacy of the current assays and their so-called reference ranges have not been proven, so that their sensitivity and specificity are unknown.

In summary: clinical approach to an alcoholic patient should include a dietary history and the best course in suspected folate deficiency is to improve the diet and stop alcohol. Alcohol withdrawal and hospital diet lead to reticulocytosis in 3-10 days in folate deficient alcoholics with correction of anemia within 2 months. After checking for anemia and hypersegmentation in an alcoholic patient with high MCV, a therapeutic trial of folate (100 \( \mu g/\)day for 7 - 10 days, so as not to treat \( B_{12} \) deficiency, total cost 3 cents) could be begun, since toxicity of folate is not apparent.

Failure to respond with increased reticulocytes to this treatment in 6 - 10 days could indicate:

- wrong diagnosis
- failure to take folate, continued drinking
- iron deficiency, anemia of chronic disease, infection or inflammation.

Indeed, the possibility of combined nutritional deficiencies must not be overlooked.

Pursuit of the diagnosis of \( B_{12} \) deficiency must be a clinical decision.

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REFERENCES


