FOLIC ACID DEFICIENCY

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MACROCYTIC anæmia, associated with a megaloblastic erythropoiesis, glossitis, weight loss, diarrhoea, and an absence of neurological symptoms or signs and of evidence of deficiency of vitamin B₁₂ are usually accepted as the features of deficiency of folic acid or related pteroylglutamate compounds. Such an assumption is based on the clinical and hæmatological remission induced by the administration of a synthetic compound, folic acid. The term 'folic acid' also stands for the family of conjugated pterins and pteridines having 'folic' biological activity (Hutner, Nathan and Baker, 1959). The clinical picture occurs in malnutrition, in certain diseases of the small intestine, such as celiac disease, tropical sprue or jejunitis from various causes, and in pregnancy. Sometimes it follows treatment with anti-folic compounds, such as aminopterin, or with certain anti-convulsant drugs, such as primidone, phenobarbitone, epanutin or mysoline. Because of the variety of conditions in which deficiency of folic acid occurs, it is thought that it is the result of a poor intake, poor absorption, increased demand created by a disordered metabolism, or a combination of these factors.

Diagnosis of Folic Acid Deficiency

1. *Hæmatological Diagnosis*

A megaloblastic anæmia with a normal level of vitamin B₁₂ in the serum, or not responding adequately to parenteral administration of vitamin B₁₂ but with complete remission with folic acid therapy, still remains the most satisfactory conclusive proof.

2. *Microbiological Assay in Blood and Tissues*

Assays using *Streptococcus faecalis* or *Lactobacillus casei* are available and relatively simple. Results are difficult to interpret, however, as will be appreciated when considering the demonstra-

tion by Usdin (1959) of at least nine pteroylglutamate fractions, chromatographically distinct, in the blood. The microbiological activity of these fractions varies and none of them has been identified with certainty with known chemical members of the pteroylglutamate complex. The manner in which folic acid activity is released prior to assay can affect the results obtained. Moreover, Toennies and Phillips (1959), using whole blood, have shown that there are substances in red cells which release folic acid activity (F.A.A.) from the plasma.

Using *Streptococcus faecalis*, we were unable to show any consistent difference in F.A.A. between the blood and material from the bone marrow in normal subjects and in patients with deficiencies of either folic acid or vitamin B₁₂ (Cox, Matthews, Meynell, Cooke and Gaddie, 1960a). Also it must be emphasized that blood folic acid activity is spontaneously increased during active blood regeneration (Cox, Meynell, Cooke and Gaddie, 1960b). Following hæmorrhage, acute or chronic, blood folic acid activity rises for many days and falls to normal levels only when the blood picture has returned to normal. A similar phenomenon occurs following specific therapy with vitamin B₁₂ or with iron. Furthermore, in patients with clinical evidence of deficiency of folic acid an initial rise of F.A.A. falling to pre-treatment levels occurs following the administration of folic acid, only to be followed by a secondary rise in F.A.A., subsequent to the reticulocytosis, which persists for some time before falling to normal levels coincident with the restoration of the blood picture to normal. Such observations must influence the interpretation of 'resting' blood levels. We were unable to find any correlation between the blood and bone marrow content of folic acid activity (Cox *et al.*, 1960).

Recently Herbert, Baker, Frank, Pasher,

Sobotka and Wasserman (1960) have described a method of folic acid assay on serum using *Lactobacillus casei*, which, they claim, correlates with the clinical conditions of folic acid deficiency when abnormally low serum levels are found, in contrast to the findings in patients with deficiency of vitamin B₁₂. We have not been able to substantiate this claim using *Streptococcus faecalis*. The probability is that these organisms, *L. casei* and *S. faecalis*, do not measure the same thing. Much more work is necessary before diagnostic significance can be placed on the serum levels.

3. Urinary Excretion of Test Doses of Folic Acid

The urinary excretion of folic acid following parenteral (intramuscular) administration has not proved satisfactory. Some patients with apparent clinical deficiency excrete normal amounts of folic acid (Girdwood, 1956; Cox, Meynell, Cooke and Gaddie, 1958a), whilst approximately 50% of patients with pernicious anaemia had a reduced excretion (Girdwood, 1956; Cox, Meynell, Cooke and Gaddie, 1958b). Two factors need consideration in this context. The amount of folic acid necessary to saturate the 'enzymatic systems' is probably very small and less than the differences that can be readily detected in the urine. Secondly, the unsaturation of tissues, such as muscle, does not bring about the clinical signs of folic acid deficiency. Thus many elderly patients on poor diets, but without haematological abnormality, will excrete low quantities of folic acid following similar test doses. It seems probable that the poor dietary intake of many patients with pernicious anaemia similarly leads to tissue depletion of folic acid.

4. Clearance of Intravenously Administered Folic Acid

Chanarin, Mollin and Anderson (1958a) observed an increased rate of clearance from the plasma of folic acid (15 µg. per kg.) administered intravenously in patients with folic acid deficiency. However, similar rates of clearance were observed in patients with deficiency of vitamin B₁₂ (varying with the degree of anaemia), haemolytic anaemia and in some patients with iron deficiency. Increased rates of clearance were also recorded in a proportion of normal pregnancies. The increased rate of clearance in such disorders could be due to tissue depletion, to increased demand owing to the abnormal or increased haematopoiesis or to excessive utilization associated with the disturbed metabolism of folic acid deficiency.

5. Urinary Excretion of Formiminoglutamic Acid (FIGLU)

Formiminoglutamic acid (FIGLU) is excreted in the urine in increased amounts in folic acid

deficiency (Broquist, 1956). Tetrahydropteroyl-glutamic acid is required for the conversion of FIGLU to glutamic acid (Miller and Waelsch, 1957). Methods for estimating FIGLU in the urine are not sufficiently sensitive to differentiate between the patients with pernicious anaemia and those with folic acid deficiency. Since FIGLU is a breakdown product of histidine, preliminary loading with this amino acid permits the differentiation between the urinary excretion of FIGLU by folic acid-deficient and replete patients (Luhby, Cooperman and Teller, 1959). Though these findings have been confirmed by a number of workers, exceptions have already been noted (Herbert, 1959). Increased excretion of FIGLU has been noted in vitamin B₁₂-deficient rats despite adequate intake of folic acid. Increased amounts have also been found in the urine of patients with apparently uncomplicated pernicious anaemia (Marshall and Jandl, 1960).

6. Aminoaciduria

An abnormal pattern of urinary excretion of amino acids has been reported in patients with megaloblastic anaemia with the suggestion that it might be of importance in differential diagnosis. While confirming the frequent occurrence of such patterns, we had similar results in hypochromic anaemia due to iron deficiency and inconsistent differences between the megaloblastic anaemias due to folic acid deficiency and those due to deficiency of vitamin B₁₂ (Fowler, Cox, Cooke and Meynell, 1960).

7. Folic Acid Excretion Test

This test is based on the principle that comparison between the amounts of folic acid excreted in the urine after an intramuscular dose and those after an oral dose of folic acid should bear a close relationship to the ability to absorb folic acid from the intestine. In practice a normal subject following 5 mg. folic acid, either orally or intramuscularly, excretes approximately similar amounts. The amount excreted after an oral dose, expressed as a percentage of that excreted after an intramuscular dose given 48 hours previously, is referred to as the excretion index. In normal subjects this is around 100% and varies from 70 to 120%. Over 90% of patients with adult coeliac disease or idiopathic steatorrhoea have low indices. Patients with regional enteritis affecting the jejunum also have low indices, a finding which has led to the suggestion that folic acid is absorbed through the jejunum. The test is normal in pernicious anaemia and in the majority of patients with megaloblastic anaemia of pregnancy. It is also normal in patients with steatorrhoea due to pancreatic disorders, gastric operations and regional

enteritis when the upper small intestine is normal (Cox *et al.*, 1958a).

8. Oral Folic Acid Tolerance Curves

All tolerance curves are justly criticized because they are not only influenced by the amount of absorption, but also, and probably to a greater extent, by the rate of absorption and the rate of clearance from the blood stream. Chanarin, Anderson and Mollin (1958b) have found the plasma folic acid levels following 3 mg. folic acid orally a satisfactory means of determining defective absorption of folic acid in patients with idiopathic steatorrhœa. They did, however, find similar curves in pregnant patients, both in late normal pregnancy as well as those with megaloblastic anæmia. They suggest that this may be of ætiological significance in the production of megaloblastic anæmia of pregnancy (Chanarin, MacGibbon, O'Sullivan and Mollin, 1959).

9. Tritium-labelled Folic Acid

Anderson, Belcher, Chanarin and Mollin (1960) have used tritium-labelled folic acid in the investigation of folic acid deficiency. Following oral administration of 0.2 mg. or 40 µg./kg. ^3H folic acid, the fæcal excretion was usually less than 25% in normal patients and greater than 50% in patients with idiopathic steatorrhœa. The urinary excretion of radioactive material, especially when enhanced by a preliminary dose of 15 mg. folic acid intramuscularly, was also considered reliable in demonstrating 'malabsorption'. It could be done more rapidly and less laboriously than fæcal estimations. They did point out that the fæcal excretion was quantitative and the difference between the amounts excreted in the fæces and those in the urine were still considerable. Endogeneous factors could cause significant errors in interpretation when based on urinary excretion only.

The Ætiology of Folic Acid Deficiency

Deficient diet, impaired absorption, increased demand and defective utilization cover in general terms the possible factors. A combination of any of these is not uncommon. For example, green vegetables, a major dietary source of pteroylglutamates, may exacerbate alimentary symptoms and be avoided by patients with cœliac disease and idiopathic steatorrhœa; in pregnancy, when an increased demand is to be expected, disturbance of dietary habits or nausea and vomiting can reduce the intake of folic acid. Defective utilization of folic acid may not only be encountered during anti-folic therapy, but also in pregnancy and in cœliac disease. Other deficiencies, such as iron and ascorbic acid, may aggravate the disturbance (Cox *et al.*, 1960).

It is evident that folic acid deficiency must be considered from two points of view. The first is that the tissues may contain pteroylglutamates reduced in quantity to such a degree that a disturbed metabolism results. An alternative possibility exists when the tissue content of pteroylglutamates is normal, but its metabolic activity is in some way disturbed or blocked so that the normal requirement is increased. Treatment with anti-folic compounds, and possibly the anti-convulsant drugs mentioned, might be examples of this second possibility. These two types of deficiency may account for the contradictory findings reported in other conditions associated with folic acid deficiency.

It seems logical that patients presenting with the severe anæmia and obvious clinical manifestations of folic acid deficiency, particularly in adult cœliac disease and idiopathic steatorrhœa, would show greater degrees of malabsorption of folic acid than those not so severely affected. However, as judged from the folic acid excretion test and the oral tolerance curves following the administration of 5 mg. folic acid by mouth, the converse appears to be true. Using tritium-labelled folic acid, Anderson, Belcher, Chanarin and Mollin (1960) have made similar observations, i.e. that the patients with severe megaloblastic anæmia absorbed folic acid rather better. Fone, Cooke, Meynell, Brewer, Harris and Cox (1960) found that the majority of patients with a severe megaloblastic anæmia and steatorrhœa did not have the complete flattening of the jejunal villi seen in cœliac disease. In view of the shorter clinical history and other small differences, it might be postulated that in some of these patients there was a fundamental ætiological distinction and that malabsorption is not the complete explanation. It is also of great interest that patients in this group have complete clinical and hæmatological remission lasting many months and up to more than five years following the administration of only 5 mg. folic acid, the performance of a folic acid excretion test or to the single injection of 5 mg. citrovorum factor.

Megaloblastic anæmia of pregnancy poses somewhat similar questions. The generally accepted view that megaloblastic anæmia of pregnancy in temperate zones is the result of a pteroylglutamate deficiency in the strict sense is open to doubt. Girdwood (1956) found the folic acid excretion test in many of these patients was normal; in approximately half, however, there was a reduced urinary excretion of folic acid following the intramuscular test dose. Cox *et al.* (1960) encountered one patient with megaloblastic anæmia of pregnancy in whom the concentrations of folic acid activity, ascorbic acid and vitamin B_{12} were normal.

in both the blood and marrow cells. Thompson and Ungley (1951) found that some anæmic patients with no alimentary dysfunction were taking better diets than other pregnant women who had no anæmia or disorder of folic acid metabolism. Again a single injection of 5 mg. folic acid may be sufficient to bring about complete hæmatological remission.

The effect of folic acid antagonists in causing megaloblastic reversion of the bone marrow was first noted by Stickney in 1952. Following this lead, it seemed possible that anticonvulsant drugs might act similarly. Badenoch (1954), closely followed by Hawkins and Meynell (1954), were the first to record this phenomenon. Subsequently, many similar examples have been recorded. Hawkins and Meynell (1958) reported detailed investigations on eight patients. In all serum levels of vitamin B₁₂ were normal, as were the folic acid excretion tests. The bone marrow and peripheral blood pictures were indistinguishable from those found in pernicious anæmia. Despite normal serum levels, some of the patients improved with vitamin B₁₂. All, however, attained hæmatological remission with folic acid; a daily dose of 5 to 10 mg. given with the anticonvulsant drug provides complete protection. Girdwood and Lenman (1956) pointed out the structural similarities between folic acid, primidone, phenobarbitone and phenytoin. The precise action of the anticonvulsant drugs is not yet fully understood, but it has been suggested that they act as enzyme inhibitors.

The Pteroylglutamates and Pernicious Anæmia

Megaloblastic anæmias are due to deficiency either of cyanocobalamin or of folic acid. Whilst those due to folic acid deficiency respond to small doses of folic acid, those due to vitamin B₁₂ deficiency do manifest hæmatological response to larger doses of folic acid. It has long been known that patients with pernicious anæmia will respond to folic acid in relatively large doses, though this therapy does not prevent neurological complications. Evidence of a tissue depletion of folic acid has been shown in pernicious anæmia. Low F.A.A., as assayed with *S. faecalis*, occurs in approximately 50% (Nieweg, Faber, DeVries and Kroese, 1954; Cox *et al.*, 1960). The urinary excretion following 5 mg. folic acid is reduced in a similar number (Cox *et al.*, 1958). The plasma

clearance of intravenously administered folic acid is increased proportionate to the degree of anæmia (Chanarin *et al.*, 1958a). On the other hand, folic acid is rarely required to supplement vitamin B₁₂ therapy in the treatment of pernicious anæmia (Ungley, 1955). Following B₁₂ therapy, a retention of folic acid by the body takes place, usually after the maximum reticulocytosis, and is associated with a rise in F.A.A. of the blood. The theory put forward by Vilter and his colleagues (1950) to explain the effect of folic acid in vitamin B₁₂ deficient states as a 'mass action' effect seems possible, though not very enlightening. At this stage it must be admitted that the interrelationships of folic acid and vitamin B₁₂ are poorly understood.

Requirements of the Pteroylglutamates

No mention has yet been made of the normal daily requirements of folic acid in man, for data have been inadequate. Jandl and Lear (1956), basing their calculations on the daily urinary excretion, suggested that the requirements do not exceed 0.2 to 0.3 mg. per day. Marshall and Jandl (1960) used dosages of folic acid of this order (0.4 mg.) intramuscularly daily to distinguish between megaloblastic anæmias due to folic deficiency and those due to cyanocobalamin deficiency. Such small 'physiological' doses brought about hæmatological remission in 'folic acid deficiency', but had little or no effect in deficiency due to vitamin B₁₂. All that can be stated is that the requirements for folic acid in the normal are considerably less than the doses, 5 to 15 mg. daily, usually given in clinical practice.

Conclusion

Though much has been written on this subject, our understanding of the metabolic activity of the pteroylglutamates is far from complete (Hutner *et al.*, 1959). The only point of general agreement is that the active members are concerned with the transfer and utilization of single carbon moieties. It can be appreciated that there are many problems posed by what constitutes 'folic acid deficiency'. Difficulty arises from the lack of knowledge of the naturally occurring substances with 'folic acid' activity. Any attempt to identify what happens when folic acid, a synthetic substance, is administered with what has occurred in a particular disease is limited. Malutilization as well as deficiency must be considered.

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