FOLATE ABSORPTION

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Abstract

Folate is the generic term given to numerous compounds of pteroic acid with glutamic acid. Knowledge of absorption is limited because of the complexities introduced by the variety of compounds and because of the inadequacy of investigational methods. Two assay methods are in use, namely microbiological and radioactive. Techniques used to study absorption include measurement of urinary excretion, serum concentration, faecal excretion, intestinal perfusion, and haematological response. It is probably necessary to test absorption of both pteroylmonoglutamic acid and one or more polyglutamates, and such tests would be facilitated by availability of synthesized compounds labelled with radioactive tracers at specifically selected sites.

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1. INTRODUCTION

1.1 Functions

Folate is the generic term given to compounds of pteroic acid with glutamic acid. In the body there are a number of different forms of folate which play important roles in intermediary metabolism. These functions have been reviewed by Stokstad and Koch[1] and Blakley[2]. From the clinical standpoint the most important of these functions is in deoxyribonucleic acid synthesis. When this is interfered with, cellular maturation throughout the body becomes abnormal and, in particular, megaloblastic anaemia develops.

1.2 Occurrence

During intrauterine life foetal folate stores are built up, mainly during the last trimester of pregnancy, by transfer from the mother. In post-natal life the individual is dependant entirely on dietary folate to maintain the body stores. The only exception to this in man occurs when, in certain conditions, there is bacterial overgrowth in the upper small intestine and some of the bacteria produce folate which may then be available to the host[3]. Folates are widely present in foods such as meat, dairy products, vegetables, fruits and cereals, and exist largely in the form of polyglutamates[4]. Most food folate is heat labile and losses on cooking may be considerable[5].

1.3 Mechanism of absorption

In plasma, folate is present mainly in the monoglutamate form[6], suggesting that during digestion or absorption the polyglutamates in the food are converted to monoglutamates. Butterworth et al.[7] studied the absorption of ^{14}C-labelled pteroylheptaglutamate, synthesised by the solid-phase method. When the label was in the pteroate moiety or in the first glutamic acid, the plasma folate was found to be radioactive, but when the label was in the second or subsequent glutamic acids, plasma folate was unlabelled and the ^{14}C appeared in the expired CO_2. In dogs with isolated intestinal loops, Baugh et al.[8], using ^{14}C-labelled folates, found that the major form of folate entering the portal blood was always the monoglutamate. When di- or tri-glutamate were placed in the intestinal lumen some diglutamate could be detected in the mesenteric blood, but this did not occur when higher polyglutamates were used. The rate at which folate appeared in the portal blood was inversly related to the polyglutamate chain length. For example, approximately twice as much folate appeared in the portal blood from ^{3}H-labelled monoglutamate as from an equimolar amount of ^{14}C-labelled pentaglutamate when these two forms were placed together in the isolated loop.

Deconjugation of dietary folate may theoretically occur in the intestinal lumen, at the brush border, within the mucosal cell or in the plasma. Polyglutamate forms of folate placed in the fluid bathing the mucosal surface of an everted sac of rat jejunum are converted into the monoglutamate form[9], but it is not clear whether this deconjugation occurs in the fluid or in the mucosal cells with subsequent egress of monoglutamate. Gammaglutamyl peptidase (conjugase) has been demonstrated in the succus entericus of man[10,11], but it is not known how much of this conjugase is derived from pancreatic secretions and how much from shed mucosal cells and other sources. However, the optimal pH of this enzyme is 4.5, and at the normal intraluminal pH its activity is greatly reduced. Further, in patients with achlorhydria, whose intraluminal pH is even higher, folate polyglutamates are still normally absorbed[12]. It is therefore doubtful if this luminal conjugase plays a significant role in folate digestion.

Although, by analogy with sugars, it is tempting to postulate a role for the brush border in polyglutamate hydrolysis, there is little if any enzyme demonstrable in the brush border, the majority being associated with lysosomal fractions[13]. This suggests that the site of maximum deconjugation is in fact

inside the mucosal cell. It has also been demonstrated that there is a plasma conjugase which can split polyglutamate forms to the monoglutamate[14]. The physiological significance of this plasma enzyme is not clear, but unless steps are taken to inhibit its action[8], failure to demonstrate polyglutamate forms in the plasma does not exclude the possibility that at least some polyglutamates may be absorbed as such and converted to monoglutamates by the action of the plasma conjugase.

In man, ingested pteroylmonoglutamic acid (PGA) passes into the portal blood stream as such[15]. In dogs pteroylmonoglutamate also appears in the portal blood, even when originally derived from ingested pteroylpolyglutamates[8]. However, in man, injested dihydro- and tetrahydro-pteroylmonoglutamic acid appears in the blood as methyltetrahydrofolate[15,16], suggesting that reduced folates are methylated during their transit through the mucosal cell. Our understanding of folate absorption is shown in Fig.1.

There is some evidence that PGA can be absorbed against a concentration gradient[17,18]; however, these experiments do not necessarily imply that folate absorption is an active energy-requiring process[19]. Hepner et al.[20], employing an intestinal perfusion technique, found that there appeared to be a maximum rate at which PGA could be transported, and suggested that their data favoured a process of active transport for folate absorption. However, these data have been shown to fit better to a weighted straight line than to a Michaelis-Menten curve[21], so this also cannot be taken as indicative of an energy-requiring process.

In our laboratory we studied the absorption of tritiated PGA in two normal subjects by the intestinal perfusion technique using concentrations ranging from 10 to 1600 ng/ml in the perfusate and could detect no evidence of saturation phenomena. In both subjects the percentage absorption over a 30 cm segment of intestine was similar at all concentrations.

The most important evidence that a specific transport mechanism is required for PGA absorption comes from the study of subjects with isolated defects of folate absorption[22,23] who cannot absorb any form of folate in physiological amounts. This can only be explained on the basis of a biochemical defect in a specific transport mechanism.

Clearly, considerably more work is needed before the biochemistry of folate absorption is fully elucidated.

1.4 Site of absorption

The optimal site of folate absorption appears to be the upper small intestine. In perfusion studies in the rat, Burgen and Goldberg[17] found a lower absorption of PGA from the ileum than from the jejunum. They also showed that the difference in absorptive capacity between jejunum and ileum was increased when the dose of folate was given with saline instead of water. Hepner[18] instilled PGA into the rat small intestine at different levels and found a significantly lower absorption in the ileum than in the jejunum when a dose of 1.5 µg was used, but this difference disappeared with higher doses. Izak at al.[23] short-circuited different parts of the rat intestine and found that nearly 5 times as much PGA was absorbed by rats with ileal exclusion as compared with those with jejunum exclusion.

In man, Cox et al.[25] found PGA absorption normal in subjects with ileal disease or resection. Hepner et al[20], using a double-lumen perfusion technique, showed that the proximal jejunum absorbed PGA best; absorption was reduced in the lower jejunum and almost absent in the ileum. Baker at al.[25] studied folate absorption in controls and two patients with intestinal resection, by following the rise in serum levels after oral administration of pteroylmono- di- and tri-glutamates. They found that the patient who had had a jejunal resection could absorb pteroylmonoglutamate but not the di- or triglutamates. However, these tests were done with large doses (5 mg PGA) and therefore may not represent the physiological situation.

1.5 Enterohepatic circulation

Folate, once absorbed, passes in the plasma to the liver where some is rapidly taken up and excreted in the bile at concentrations considerably higher than those in the plasma[27]. Presumably this folate is available for reabsorption and thus enters an enterohepatic circulation. The exact proportions of folate that are recycled and lost, respectively, are not known.

2. TESTS OF PTEROYLMONOGLUTAMIC ACID ABSORPTION

2.1 Methodology

Two main assay methods have been employed for studying absorption and excretion of folate, namely microbiological and radioactive. Microbiological assays have employed the organisms S.faecalis or L.casei, which require folate for their growth. The compounds these organisms can utilize are shown in Table 1. Radioactive compounds have usually been made by labelling with tritium.

Recently ^{14}C-labelled material has been prepared, in small amounts, by solid-phase synthesis with the ^{14}C label at predetermined positions[28], enabling more detailed study of the fate of the folate molecule to be undertaken.

In addition a few investigators have utilised the haematological response of subjects with folate-deficiency anaemia as an index of folate absorption[29].

A large variety of tests have been employed by different investigators based on one or more of these methods. Broadly they may be divided into five groups, namely urinary excretion, serum concentration, faecal balance, intestinal perfusion, and haematological response.

2.2 Urinary excretion test - microbiological

There is normally a small amount of folate excreted in the urine even when intake is restricted[30]. When intake is increased above a certain amount urinary excretion also increases. Thus with a parenteral dose of 300 µg there was a small increase in urinary excretion, which rose further with higher doses[31]. Various authors have used this urinary excretion as an index of intestinal absorption. A variety of test doses have been employed ranging from 500 µg[32] to 5 mg[33].

The amount of folate excreted in the urine following a given oral dose will vary depending on the degree of saturation of the tissues with folate. In folate deficiency more is retained in the tissues, and blood levels and urinary excretion will be reduced as compared with normal subjects. In order to overcome this, it is desirable to treat the individual with parenteral folate for several days, stopping 48-36 hours before performing the test[32], to "presaturate" the tissues. The urinary excretion of folate will also be affected by renal function. To allow for this Girdwood[34] introduced a "differential" excretion test, comparing the 24-hour urinary excretion of folate after an oral test dose of 5 mg of PGA with that following the same dose given by injection. The ratio

$$\frac{\text{excretion after oral dose}}{\text{excretion after parenteral dose}} \times 100$$

was termed the "excretion index"[35]. In this test normal subjects excrete more than 1.5 mg of folate in 24 hours and the excretion index is over 70.

The disadvantages of these urinary excretion tests are the necessity of presaturating the patients, and the large doses, either of which may produce haematological responses and improvement in jejunal function before investigatory studies are completed. Moreover, the 5 mg dose is an unphysiological one and the way this is handled may not mirror the physiological situation.

2.3 Urinary excretion test - radioactive

When a physiological dose of labelled PGA (200 μg) is given by mouth, a small amount of the label is excreted in the urine in the subsequent 24 hrs. However, using this dose, there was no detectable difference in excretion between control subjects and those with malabsorption[36]. When a larger dose (40 μg/kg) was given there was a greater separation between normal subjects and patients with malabsorption. Even better separation between the two groups was achieved when a flushing dose of 15 mg of unlabelled PGA was given immediately before or after ingestion of the labelled folate. Finally, in order to overcome the effects of tissue folate depletion, Anderson et al.[36] advised presaturation with non-radioactive folate. Klipstein[37] employed this test in a larger group of controls. The subjects were presaturated with injections of 15 mg/day for 3 days, then 36 hours after the last injection, a dose of 40 μg/kg of tritiated PGA was given, followed 3 hours later by an injection of 15 mg PGA as a flushing dose. Normal subjects excreted 26-60 % of the test dose in 24 hrs - figures which are similar to the smaller series of Anderson et al.[36]. Others have also employed this test with essentially similar results[38-43].

2.4 Serum test - microbiological

Small (e.g. physiological) doses of folate do not always cause a significant rise in blood folate concentration as measured by microbiological assay[30], but large doses (e.g. 5 mg) cause a rise that is readily measurable. Denko[33], after giving 5 mg of folate, found a peak rise of about 100 ng/ml occurring one or two hours after the oral dose. Butterworth et al.[44] and Cooke et al.[45] used this dose and found that the results in control subjects were clearly separable from those in patients with intestinal disease. Other investigators have used different doses; thus Spray and Witts[46] and Clark[32] followed serum levels after a 1 mg dose and Chanarin et al.[47] employed a 3 mg dose. In the studies of Clark[32] and Chanarin et al.[47], the subjects were presaturated to prevent differences in the rate of clearance from the blood stream due to tissue unsaturation.

Further refinements of this test were introduced by Chanarin and Bennett[48]. In order to allow for differences in the size of the test subjects they used a dose of PGA of 40 μg/kg body weight. The subjects were presaturated by giving 15 mg of PGA for one or more days before the test, the last injection being 36 hr before the oral dose. These authors also measured the blood levels with

S.faecalis, which responds to PGA, rather than with L.casei, which will also respond to methyl folates and so will measure both absorbed PGA and methyl folates displaced from the tissues. By this technique peak serum levels in normal subjects were found to be in the range 51-142 ng/ml with a mean of 91 ng/ml, and there was good separation between normal subjects and patients with intestinal disease.

2.5 Serum test - radioactive

The rise in serum folate can be measured by determining the rise in blood radioactivity following an oral dose of labelled folate. As with the microbiological serum test it is necessary to presaturate the subject with PGA before giving the oral test dose. Halsted et al.[38] used this test employing a dose of 15 μg/kg body weight. They found a peak total plasma radioactivity of 2.8 (± 1.3) % of the administered dose (total plasma volume was estimated at 45 ml/kg body weight) in normal subjects. In view of the popularity of the serum microbiological test it is surprising that the radioactive serum test, which is considerably easier to carry out, has not become more popular with investigators.

2.6 Faecal balance - radioactive

Because of the production of folate by intestinal bacteria in the large and small intestine[3,49], meaningful balance studies can be carried out only with labelled folate.

The method is based on the assumption that when a known amount of labelled folate (D) is given by mouth and the amount subsequently excreted in the stools (S) is measured, then the amount absorbed (A) is given by the equation

$$A = D - S \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The advantages of this technique are that small doses, within the physiological range, can be employed and saturating or flushing doses of folate are not required. This means that even repeated tests can be carried out on the same individual without having to alter existing conditions by treating with folic acid. The disadvantages of this technique are the difficulties of obtaining full collection of all stools and the technical problems of processing the stools. The former can be largely overcome by performing the test in a metabolic ward. Several methods for processing of stools have been introduced. Anderson et al.[36] oxidised the faeces with nitric and perchloric acids and counted the tritium as tritiated water. Radhakrishnan and Baker[50] and Kremenchuzky et al.[41] decolourised an aliquot of faeces with sodium peroxide

and hydrogen peroxide and added 0.5 - 0.75 g of processed stool to a dioxane-based scintillation mixture containing "Cabosil". This gave counting efficiencies of 15 % to 20 %, and experiments with tritiated folate added directly to 24 hour stool collections showed a mean recovery of 95 %.

Jeejeebhoy et al.[51] used a method of wet oxidation of the stool with potassium permanganate and hydrogen peroxide, which gave a mean recovery of 98 % of added tritium. These workers also used a non-absorbable marker (^{51}Cr, as chromic oxide) to obviate the necessity for obtaining complete stool collections. Although theoretically chromic oxide cannot be considered a good marker for the water-soluble folic acid, a good correlation was obtained between the amount absorbed as calculated from the total stool collection and the amount absorbed as calculated from the first 24-hour stool collection corrected for inadequate recovery by use of the marker.

The amount used in the test dose has varied from 100 µg to 320 µg of PGA and the amount of radioactivity from 20-80 µCi. Normal subjects have been found to absorb more than 40 %[36] to more than 78 %[41] with a 200 µg dose, or more than 60 %[51,52] with a 100 and 320 µg dose, respectively.

It should be noted that this faecal balance test is dependant on the validity of equation (1). Clearly this applies only if the enterohepatic circulation of folate[27] makes an insignificant contribution to the faecal losses. Any radioactivity absorbed and re-excreted through the bile, which is not again re-absorbed, will be excreted in the faeces, and will falsely depress the total apparent absorption from the intestine.

In carrying out these faecal balance studies we observed that if the stool radioactivity were followed for a prolonged period of time, there was frequently a second peak of excretion of the label, usually on about the 5th day (Fig.2). The significance of this is not clear. It cannot be accounted for by the enterohepatic circulation and it is rather too late to represent material which perhaps entered the mucosal cells and was subsequently shed into the lumen. However, since it occurs after other non-absorbed substances have been excreted this was ignored in calculating net absorption.

2.7 Intestinal perfusion

This method is based on the principal of measuring the amount of folate absorbed, per unit time, over a given length of intestine, by reference to a non-absorbable marker infused along with the folate.

This test has the advantage that it employs a physiological dose of folate and that the rate of uptake by the intestine is measured directly. However, it has the disadvantage that it tests the absorptive capacity of only a small segment of the intestine and does not therefore measure the total absorptive capacity of the individual, which may be clinically more important.

The only studies employing this technique in groups of subjects are those of Halsted et al.[53] and Gerson and Cohen[53a]. The former employed a solution of tritiated folic acid (25 ng/ml, 0.4 μCi/μg) at pH 7.2 containing 16.7 millimoles of glucose per litre made isotonic with sodium chloride and containing 10 g of PEG per litre as a marker. Infusion was at the rate of 9.7 ml/min through a tube which allowed a 15 cm mixing segment and a 30 cm segment for studying absorption. The mean uptake from the jejunum on 14 tests in 9 control subjects was 36.5 %. Gerson and Cohen also employed tritiated PGA in an isotonic glucose solution and found absorption in normals in the range 26-48 %.

2.8 Haematological responses

This method depends on observing the haematological effect of a small daily oral dose of PGA given to patients with megaloblastic anaemia due to folic-acid deficiency. The method is applicable only when there is at least a moderate degree of anaemia and moreover it is at best only crudely quantitative. The latter objection depends on the fact that when a person with folate-deficiency megaloblastic anaemia is treated with a suboptimal dose of folate a reticulocyte response occurs. If a second dose of folate, larger than the first, is then substituted, a second reticulocyte response will occur, but if it is the same as or smaller than the first a second reticulocyte response will not occur.

This test is clearly inapplicable to normal subjects but has been used to demonstrate absorption of physiological amounts of PGA in certain disease states[29,52].

3. TESTS OF POLYGLUTAMATE ABSORPTION

3.1 Microbiological

Until very recently synthetic polyglutamates were unavailable, so that most studies of polyglutamate absorption have been done using crude forms of food folate and employing microbiological methods to detect changes in serum or urine folate concentration.

Swendseid et al.[54] fed yeast heptaglutamate, from which the conjugase inhibitor had been removed, to normal subjects at a dose level equivalent to 4 mg of PGA, and measured the folate excretion in the urine. This excretion was compared with that following a similar dose of PGA. They found that the heptaglutamate appeared to be absorbed as well as PGA. Markkanen[55] fed raw ox liver, 1 g/kg body weight, to subjects who had previously been saturated with folic acid, and studied the rise of L.casei-active material in the plasma. In normal subjects there was a peak at $\frac{1}{2}$ - 2 hr, which was about 3 times the level during fasting.

Hoffbrand et al.[56] prepared partially purified polyglutamates from yeast and fed doses which gave 200 µg of folate, as measured by L.casei after treatment with folate conjugase. Patients were first saturated with 15 mg of PGA given intramuscularly for at least 3 days, the last injection being 48 hr before the test dose. The rise in serum-folate L.casei-activity following the dose of polyglutamate was compared with the rise after a dose of 200 µg of PGA given the following day. In normal subjects the mean rise of serum folate was similar with both tests (11.0 ng/ml with polyglutamates and 11.8 ng/ml with PGA), but in subjects with malabsorption there was a significantly lower rise in serum levels when the patients were given polyglutamates.

Perry and Chanarin[12] and Jeejeebhoy et al.[57] carried out a similar type of test in presaturated subjects. However, they fed higher amounts of folate, 20 µg/kg and 40 µg/kg respectively, of PGA or the equivalent as yeast polyglutamate. In each case the rise in serum level was considerably lower when the polyglutamate was fed than when PGA was fed. In the study of Perry and Chanarin this was also reflected in the urinary excretion.

3.2 Radioactive

Rosenberg and Goodwin[58] compared the absorption of tritiated PGA with that of tritiated heptaglutamate fed to 6 normal subjects. After a dose equivalent to 250 µg of PGA the urinary excretion of the label ranged from 50-91 % with each substance. These authors therefore concluded that in normal subjects these substances are absorbed with similar efficiency, thus confirming the previous studies of Hoffbrand et al.[56] based on microbiological assays.

Butterworth et al.[7] administered ^{14}C-labelled folates to five subjects with different haematological conditions. Unfortunately, they employed large doses of folate (10 µM - the equivalent of 4.4 mg of PGA) and each person had only one test. It is therefore not possible to draw any quantitative conclusions from

the study, except perhaps to say that the faecal losses tended to be greater with increasing numbers of glutamyl moieties.

The conflicting results obtained with both microbiological and radioactive techniques when PGA and polyglutamate absorption are compared (Table 2) cannot be explained on the basis of the available data. It is, however, worthy to note that those who have employed doses in the microgram range have found no difference between PGA and polyglutamate absorption, whereas with the exception of Swendseid et al.[54], those who have used doses in the milligram range have found that PGA is better absorbed. This suggests that the normal person may be able to absorb small doses of polyglutamate as well as he can PGA, but that this is not the case with larger doses.

4. CONDITIONS AFFECTING FOLATE ABSORPTION

4.1 Congenital folate malabsorption

Three patients have been described in the literature with an apparently specific and isolated defect of folate absorption[22,23]. The patient of Lanzkowsky[23] could apparently absorb enough folate to maintain health when very large doses (40 mg) of PGA were given orally, but could not absorb physiological doses or even pharmacological doses (3 mg) of pteroylmonoglutamic acid or other related folates. When the patient was in haematological relapse there was no response to 250 µg PGA by mouth, but a prompt response occurred to the same dose given parenterally, clearly indicating a defect at some stage of absorption.

4.2 Intestinal resection

Even massive small-bowel resection may not necessarily be associated with folate deficiency. Folic-acid absorption as measured by the microbiological serum test was normal in a patient with only four feet of jejunum remaining and in another subject who had a resection of 8 feet of jejunum[59]. Three patients with large segments of jejunum removed, who were studied by Klipstein[60], were found to have defective absorption of PGA when measured by the microbiological urinary-excretion test. One of these patients had a folate-deficiency megaloblastic anaemia.

4.3 Gastrectomy

Folate deficiency may sometimes occur after gastrectomy[61-64]. Doig and Girdwood[35] found folate absorption, measured by the microbiological urinary-excretion test, to be normal in patients after partial gastrectomy. Hoffbrand[65] and Chanarin[66], using the microbiological serum test, found reduced absorption

of PGA in 6 out of 22 and 6 out of 31 patients, respectively, following gastrectomy.

Markkanen[55] found depressed serum concentration of folate in 10 % of gastrectomised subjects following the administration of ground liver as the source of folate polyglutamates. It therefore appears that PGA and pteroyl polyglutamate malabsorption may occur in a small percent of cases after gastrectomy. The aetiology of this folate malabsorption is not clear. It is presumably related to the generalised disorder of small intestinal function which frequently occurs following gastrectomy and in many patients is accompanied by steatorrhoea, biopsy changes, and other absorptive defects.

4.4 Coeliac disease

Folate deficiency is a common complication in patients with coeliac disease[45]. Employing the microbiological urinary-excretion tests, 86 % or more of subjects with active coeliac disease have been found to have PGA malabsorption[25,35,45]. With the microbiological serum technique, 88 % or more have been found to have an abnormal absorption[45,47,48,67].

Studies with tritiated PGA have been carried out by several groups of investigators. Anderson et al.[36] used the faecal-balance and urinary-excretion technique and found that, as a group, subjects with coeliac disease, after a test dose of 200 µg, excreted more label in their stools than did normal subjects, although there was a considerable overlap. The differences between patients and controls were more marked when the dose of PGA was increased to 40 µg/kg. This difference in absorption was also mirrored in the urinary excretion of folate, with or without a flushing dose of unlabelled folate. Kinnear et al.[39], using a flushing dose of 30 mg of unlabelled PGA and an oral dose of 15 µg/kg of tritiated PGA, and Klipstein[37], using an oral dose of 40 µg/kg of tritiated PGA, measured urinary excretion and found a marked difference in excretion between controls and subjects with coeliac disease. Gerson and Cohen[53a] report briefly on an intestinal perfusion study of folate absorption in what is presumably adult coeliac disease where absorption was reduced to 0-15 % (normal 26-48 %).

The absorption of polyglutamates in coeliac disease was investigated by Hoffbrand et al.[68], who used the microbiological serum method with an oral dose of 0.45 µM yeast polyglutamate, or PGA, fed to previously saturated subjects. Patients with coeliac disease showed evidence of malabsorption of both PGA and polyglutamate.

4.5 Tropical sprue

There is a high prevalence of folate deficiency in subjects with tropical sprue[52,69,70]; however, tests of folate absorption have given conflicting results. Butterworth et al.[44], using a 5 mg dose and employing the microbiological serum and urinary-excretion tests, demonstrated decreased urinary excretion and lower serum levels in all of 10 patients with sprue as compared with controls. With the microbiological serum test, using a dose of 40 µg/kg of PGA and prior saturation, absorption was judged abnormal in 12 out of 19 patients from Puerto Rico and 7 out of 18 from Haiti[69]. In Bombay, 76 % of 50 patients studied by means of the same test had abnormal absorption[71], but in a subsequent report from the same laboratory 12 out of 12 patients had peak serum concentrations that were not significantly different from those of controls[57].

With physiological doses of labelled PGA an absorptive defect has been demonstrated less frequently. Using faecal balance techniques and a 320 µg dose of tritiated PGA, Jeejeebhoy et al.[51] demonstrated malabsorption in 8 out of 17 patients. With a 200 µg dose Baker and Mathan[52] could demonstrate malabsorption in only 9 of 69 patients with tropical sprue. The normality of PGA absorption in some of these subjects was further documented by demonstrating a haematological response to an oral dose of 100 µg of PGA[52]. Using a 25 µg dose Klipstein[72] found normal absorption in all of three patients.

The fact that apparently normal absorption of physiological doses of PGA can be demonstrated even in patients with sprue, who have folate-deficiency megaloblastic anaemia, suggests that there may be interference with polyglutamate absorption in spite of normal PGA absorption. Haematologic studies in which food folate was fed have in fact shown in some cases that food folate was less well absorbed than PGA[73]. Jeejeebhoy et al.[57], using the microbiological serum test and a PGA dose of 40 µg/kg, or the equivalent as yeast polyglutamate, found that 12 patients with sprue had normal PGA absorption but defective absorption of polyglutamates. Hoffbrand et al.[56], using the microbiological serum test with a 200 µg dose of PGA and preloading of the subjects, found that four of eight subjects with tropical sprue had a subnormal rise in serum level. Following yeast polyglutamate, at an equivalent dosage, all had a rise less than normal, indicating a greater defect of absorption of the polyglutamate forms. Bernstein et al.[74], using an oral test dose of polyglutamate equivalent to 500 µg PGA, showed a decreased rise in serum concentration as compared with controls in 5 out of the 6 patients with tropical sprue.

The pathogenesis of the folate absorptive defect in coeliac disease and tropical sprue is not clear. Presumably if folate is absorbed by a specific mechanism there must be interference with this mechanism at one or more points. The fact that, at least in tropical sprue, polyglutamate absorption may be more affected than monoglutamate suggests that there may be interference with the mechanisms of deconjugation. However, conjugase levels in mucosal biopsies from patients with both tropical sprue and coeliac disease have been found to be no different from those of normal subjects[56,68]. It has been claimed that the defect of PGA absorption in both these diseases can be overcome by feeding an extract of calf jejunum along with the test dose[75]. However, the way this works is not clear and this effect has yet to be confirmed.

4.6 Drugs and alcohol

Patients receiving anticonvulsant drugs such as phenobarbitone, primidone and diphenylhydantoin are prone to develop a folate responsive megaloblastic anaemia[76]. It has been suggested that these drugs interfere with PGA absorption. Maynell[77], using a 5 mg dose with serum and urinary microbiological tests, Dahlke and Mertens-Roesler[78] using a 600 µg dose in the microbiological serum test and Gerson et al.[79] using intestinal perfusion, have all found evidence of PGA malabsorption induced by diphenylhydantoin. However, other investigators have failed to confirm this effect[12,80,81], and at least one patient has been described whose megaloblastic anaemia was cured by an oral supplement of 25 µg of PGA even though dilantin and phenobarbitone administration were continued[82]. Polyglutamate absorption has been found to be depressed by Hoffbrand and Necheles[80] and by Rosenberg et al.[81] who suggest that the drug may inhibit folate conjugase. However, others have not been able to confirm this effect[12,74,83].

Patients with chronic alcoholism may develop folate-deficiency megaloblastic anaemia[84]. In an attempt to study the effect of alcohol on PGA absorption Halsted et al.[38,53] employed the radioactive serum and urine tests, using a dose of 15 µg/kg of tritiated PGA and a flushing dose of 30 mg of unlabelled PGA. Significantly lower serum levels were found in 23 patients admitted after at least a "3 week debauch", but urinary excretion was within normal limits. In acute experiments in five normal subjects, the rise in serum radioactivity after alcohol (4 - 5 ozs whisky in one hour) was abnormal only in one. The authors concluded that the malabsorption seen in chronic alcoholics was probably related more to associated malnutrition than to the direct ingestion of alcohol.

5. CONCLUSIONS

Our present knowledge of the whole field of folate absorption and malabsorption is beset with inadequate knowledge and apparent contradictions and inconsistencies. These arise largely because of the complexity of the situation and the inadequacy of investigational methods. The recently acquired ability to synthesize specifically labelled polyglutamates offers a useful tool to elucidate more fully the steps of normal absorption and the disorders of this process in various clinical states.

The ideal test of folate absorption has yet to be devised. From available evidence it is probably necessary to test the absorption of both PGA and at least one polyglutamate form. The test should not alter the metabolic status of the patient and therefore should not require prior saturation with PGA nor involve a "flushing" dose of unlabelled folate. For testing overall absorption and detecting physiologically significent degrees of malabsorption, faecal balance studies are probably best. The best dose of PGA for such a test has yet to be determined and much more study of this test with specifically labelled polyglutamates is required. Faecal-balance tests are influenced by the whole intestine and may not reflect minor damage to one part of the intestine. In such cases intestinal perfusion studies may be expected to give more useful information.

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TABLE 1. GROWTH RESPONSE OF L.CASEI AND S.FAECALIS TO DIFFERENT FORMS OF FOLATE

	L.casei	S.faecalis
Pteroylmonoglutamic acid	+	+
N^5 methyltetrahydrofolate	+	-
Other reduced pteroylmonoglutamates	+	+
Pteroyldiglutamic acid	+	+
Pteroyltriglutamic acid	+	-
Higher polyglutamates	-	-

TABLE 2. THE ABSORPTION OF POLYGLUTAMATE AS COMPARED WITH THAT OF PGA BY DIFFERENT AUTHORS

Authors	Method	Dose	Conclusion
Hoffbrand et al.[56]	Serum	200 µg	Same
Rosenberg and Goodwin[58]	Urine	250 µg	Same
Swendseid et al.[54]	Urine	5 mg	Same
Jeejeebhoy et al.[57]	Serum	40 µg/kg	Lower
Perry and Chanarin[12]	Serum Urine	20 µg/kg	Lower
Butterworth et al.[7]	Faecal	4.4 mg	Lower

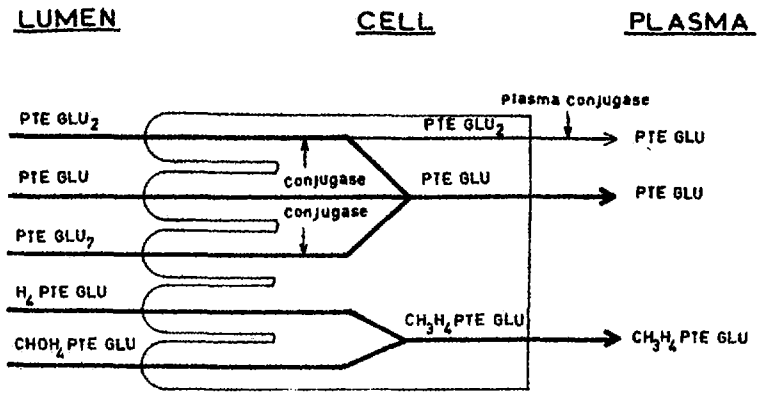

Fig. 1 Schematic representation of folate absorption

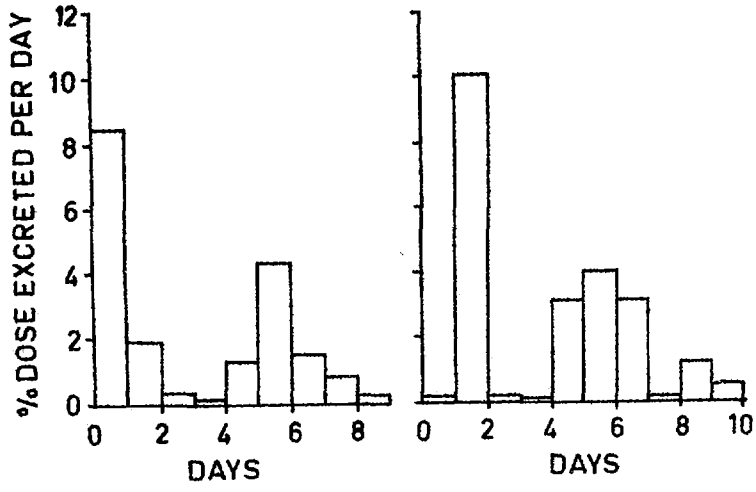

Fig. 2 Daily percent faecal excretion of label in two subjects following an oral dose of 200 µg of tritiated pteroylmonoglutamic acid