arrived on board with one or no signatures on their certificates.

On arrival in one port the port medical officer announced that unless everyone on the ship had two signatures on his certificate no one would be allowed off the ship. He demanded to inspect all the certificates. The cruises are run on a very tight schedule and this operation would have taken at least two hours. The chief purser came to the rescue by suggesting glibly to the port doctor that it would be quicker to take any five passports from the rack and to inspect the enclosed immunization certificates. He assured him that he and the ships' doctor had inspected all the certificates and that they were all in order. A significant glance from the purser silenced me and, with bated breath, we watched the port doctor select five certificates—all of which bore two signatures. At the expense of my medical integrity all the passengers disembarked in time to complete their excursion ashore.

Conclusions

Twelve months' experience has shown me that medical practice in a ship is in many ways different from that on land. Shipboard medicine is very compact and its dramatic qualities are intensified. In the small, overcrowded, and inadequately-equipped cruising vessels described here the incidence of illness is relatively high, for reasons which I have indicated. The passengers are out of their normal environment—often for the first time in their lives—and are easily frightened. Any illness, no matter how minor, is interpreted as a threat to their holiday, with the possibility of their being sent home or, much worse, being admitted to hospital in a strange country. This is quite understandable, of course, but it causes some patients to delay reporting early symptoms, which often prolongs the course of their illness. This attitude affects some passengers in the opposite way in that they become over-excited and demand immediate attention, even with second opinions, before a proper assessment of the condition can be made.

All this often makes it very difficult to reach a decision which will be in the best interests not only of the patient but also of his fellow passengers. On a tight-scheduled cruise a diversion of the ship for a medical emergency can spoil the holiday for as many as 600 people. The difficulty may be unnecessarily intensified if passengers with pre-existing illnesses are unable to provide help in the form of a covering letter from their medical practitioner at home.

I wish to thank Miss J. Short and Miss D. Strain for their invaluable help in the preparation of this article.

Scientific Basis of Clinical Practice

Vitamin B_{12} and Folate Metabolism

N. K. SHINTON

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The two groups of substances which have been termed vitamin B_{12} and folate have in common their action as coenzymes in DNA synthesis and hence their use as therapeutic agents in the treatment of megaloblastic anaemia. Chemically they are unrelated and have entirely different forms of metabolism.

Vitamin B_{12}

CHEMISTRY

Vitamin B_{12} was isolated in 1948 as a haemopoietic factor of liver extract.[1 2] The molecular structure consists of a nucleotide, 5,6-dimethylbenziminazole linked at right angles to a four pyrrol ring, similar to a porphyrin with a cobalt atom attached—a corrin group (Fig. 1).[3 4] A number of similar compounds, termed cobalamins, have been found in nature, the difference between them being the ligand, or transfer of electrons, attached to the cobalt atom.

Cobalamin with a CN ligand (cyanocobalamin) was the first isolated. The other cobalamins include hydroxocobalamin (OH ligand), methylcobalamin (CH_3 ligand), and 5'-deoxyadenosyl cobalamin. They are all interconvertible. Cyanocobalamin readily changes to hydroxocobalamin on exposure to light, and this is reversible in the presence of cyanide. All cobalamins are

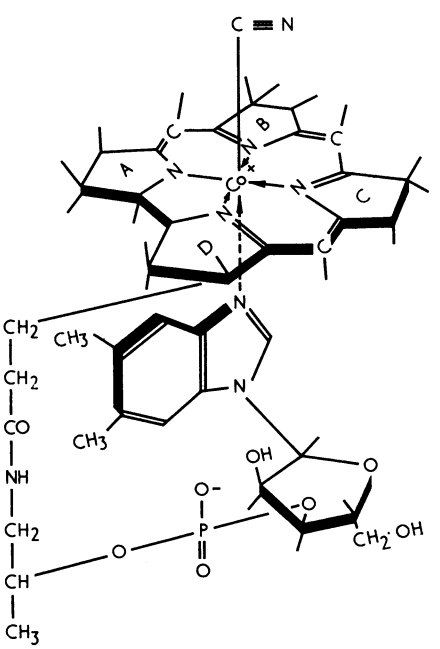

FIG. 1—Molecular structure of vitamin B_{12} (from *The Megaloblastic Anaemias*, by I. Chanarin.[6] Reproduced by permission).

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crystalline hygroscopic powders readily soluble in water, heat stable, and have molecular weights around 1,400.

SOURCE

The only source of cobalamins in nature is from synthesis by micro-organisms in soil, water, and animal intestine. The cobalamin content of a non-vegetarian diet varies from 1 to 85 μg daily, depending on economic means and social habits. Rich sources of cobalamin are the liver, kidney, and heart of sheep and cows. Moderate amounts are present in seafood and muscle meat, but only small amounts in dairy foods. Strict vegetarians may obtain enough cobalamin in the form of 5'-deoxyadenosyl cobalamin which is synthesized by micro-organisms in legume nodules of root vegetables and is present in tap water. Bacterial synthesis of cobalamin occurs in the human colon but it is not absorbed. About 5 μg is excreted daily in the faeces.

Food cobalamin is joined by peptide bonds to protein, but is readily dissociated by cooking, digestion in the stomach at acid pH, or by intestinal enzymes.

ABSORPTION

Active (Physiological) Mechanism

Free cobalamin is rapidly bound by enclosures to intrinsic factor (IF), a mucopolysaccharide secreted by the parietal cells of the gastric mucosa, in the proportion 2 mol cobalamin to 1 mol dimer IF.[5] This B_{12}-IF complex is resistant to intestinal digestion. Vitamin B_{12} also binds to the proteins of gastric juice, bile, and saliva. This binding can be distinguished by immunological means, since an antibody to intrinsic factor has the same binding site on the molecule as vitamin B_{12}. Intrinsic factor concentration is taken as the difference between radioactive B_{12} bound by gastric juice to which normal serum has been added and that bound to gastric juice to which serum containing intrinsic factor antibody has been added. One unit of intrinsic factor is that which binds 1 μg ^{58}Co-B_{12} per ml of gastric juice. Assays depend on stimulation of intrinsic factor secretion for one hour after an injection of pentagastrin. The range is 400-25,000 units an hour in subjects unsuspected of gastric abnormality.

The current hypothesis[6] of intrinsic factor action is that it has two receptor sites, one for vitamin B_{12} and the other for ileal intestinal microvilli which specifically requires a neutral pH and the presence of free calcium. The latter receptor site is readily saturated, so limiting the absorption of vitamin B_{12} to 1·5 μg from any single dose. Intrinsic factor has not been identified in plasma, so it is presumed that an intestinal releasing factor splits it off and the vitamin B_{12} passes into the mucosal cell. Other theories are that intrinsic factor acts as protector against bacterial decomposition of vitamin B_{12}-peptide, in which form it is absorbed. The rate of absorption is increased by carbachol, acting as a motility stimulant, and by food, particularly liver. There is no evidence that sorbitol increases absorption. Passage into the plasma takes two to three hours, and peak plasma levels are attained after 8-12 hours.[7] Cobalamin is transferred across the intestinal mucosa by complex processes.[8]

Passive Mechanism

About 1% of large concentrations of cobalamin throughout the whole length of the gastrointestinal tract are passively absorbed.[9] Such large molecules are unlikely to enter the water-filled pores of the lipid membrane surrounding the intestinal cell, so the absorption is either by discontinuity or facilitated diffusion. Similarly vitamin B_{12} is absorbed through the mucous membrane of the respiratory tract.[10]

TRANSPORT

Vitamin B_{12} is present in plasma as methylcobalamin, 5'-deoxyadenosylcobalamin, or hydroxocobalamin[11] bound to proteins, of which there are at least two—transcorrin I and transcorrin II.

Transcorrin I is a strong binder with α mobility. It is synthesized by granulocytes, and this is increased in myeloproliferative states. Hydroxocobalamin is bound more readily than cyanocobalamin.

Transcorrin II is a weak binder with β mobility. Probably the 1-10% of vitamin B_{12} carried on this protein is attached after initial absorption and is readily released for urinary excretion[12].

The plasma level of vitamin B_{12} in healthy subjects ranges from 140 to 750 pg/ml. There are variations of 80 pg/ml daily, 90 pg/ml weekly, and 160 pg/ml monthly. There is no difference between the sexes but the level falls after the seventh decade.[13] The plasma level represents only 0·1% of the total body content.

EXCRETION

The main excretion route of vitamin B_{12} is through the bile, and about 40 μg passes into the jejunum each day. An enterohepatic circulation results in most of this being reabsorbed in the ileum by the intrinsic factor mechanism. Small amounts of vitamin B_{12} also enter the intestine from the gastric, pancreatic, and intestinal secretions. Unabsorbed vitamin B_{12} passes into the faeces and together with that derived from bacterial synthesis in the colon amounts to 3-6 μg daily, depending on the size of the body stores.

Urinary excretion by glomerular filtration of vitamin B_{12} unbound to protein varies from 0-0·25 μg a day, the total loss from the body being from 2-5 μg daily. Owing to its greater binding, less hydroxocobalamin than cyanocobalamin is excreted after injection of the same dose of each. It has been postulated that conversion of hydroxocobalamin to cyanocobalamin by cyanide in the plasma acts as a route for the detoxification of smaller amounts of cyanide such as may arise from tobacco smoking.[14]

TISSUE COBALAMIN

Cobalamin bound to plasma protein passes to the tissues where diffuse protein binding occurs. Any excess enters the liver probably as 5'-deoxoadenosyl B_{12}. The total body cobalamin in normal adults is 2-5 mg, of which about 1·5 mg is in the liver.

ACTION

The cobalamins have an enzymatic action in many metabolic reactions of micro-organisms and of man. They include, firstly,

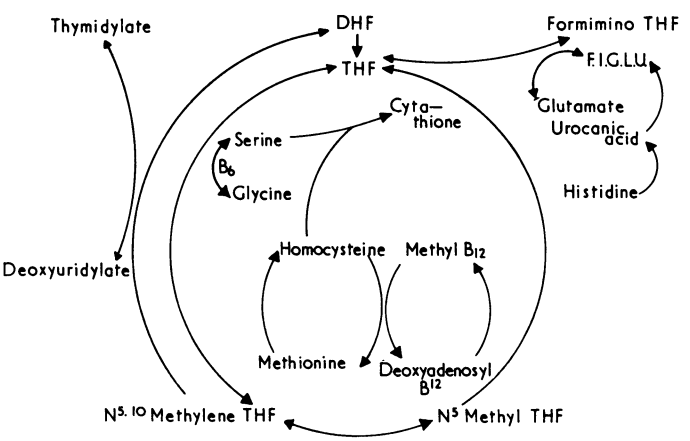

FIG. 2—B_{12}—Folate interrelationship.

isomerization of homocysteine to methionine with a single carbon unit transfer by methylcobalamin from tetrahydromethylfolate (Fig. 2); and, secondly, isomerization of methylmalonate to succinate by 5'-deoxyadenosylcobalamin. This may be the essential reaction for the production of lipoprotein in myelin sheaths.

Folates

CHEMISTRY

The name folate is derived from the Latin *folium* (leaf), and was given by H. K. Mitchell in 1941[15] to a factor in spinach leaves which was required for the growth of *Streptococcus lactis*. Some years later folates were recognized as another haemopoietic factor of liver extract. The parent compound is pteroic acid (Fig. 3), and its salts and radicles are pteroates and pteroyl.

Reduction Compounds.—"Dihydro" or "tetrahydro" compounds are formed with hydrogen atoms at positions 7, 8 or 5, 6, 7, 8 (Fig. 4). In-vivo reduction is brought about by ascorbate or folate reductases present in serum and intestinal juices. Folate antagonists (2, 4-diaminopyriminidine) act by competitive blocking of dihydrofolate reductase.

Polyglutamates.—These are conjugates of glutamic acid of up to 7 molecules attached through the γ-carboxyl group. They are broken down by enzymes (α-carboxypeptidases) present in serum and intestinal juices.

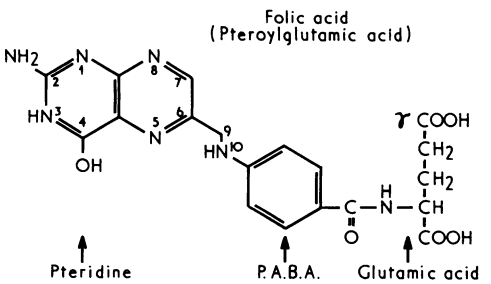

FIG. 3—Molecular structure of folic acid.

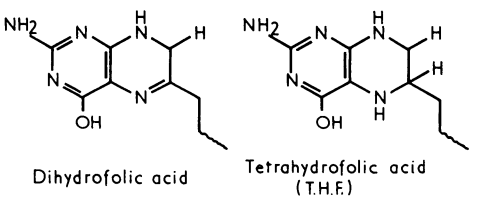

FIG. 4—Reduction compounds of folate.

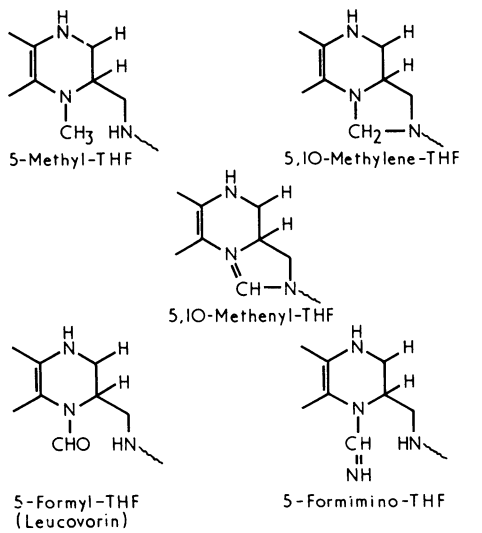

FIG. 5—Substitution compound of folate.

Substitution Compounds—Substitution occurs at N^5, N^{10} positions (Fig. 5).

SOURCE

Folates are present in a wide variety of plant and animal tissue, mainly as polyglutamates in reduced methyl or formyl forms.[16] Pteroylmonoglutamic acid is a minor component of the dietary folates. The richest sources are yeast, liver, and green vegetables, with moderate amounts in dairy foods, meat, and fish. There is little present in fruit. Some are unstable on exposure to air and sensitive to ultraviolet light and they steadily decline in storage. Cooking, particularly boiling, or heat preservation in canning destroys 50-90% of folates present.[17]

ABSORPTION

The availability of folates for absorption is uncertain owing to changes effected by bacterial enzymes in the intestinal tract. The average daily diet contains 160 μg before treatment with conjugase enzymes and 670 μg after treatment. The dietary content will also vary greatly with economic status and social habits.

Absorption probably occurs along the whole length of the small intestinal mucosa.[5] Polyglutamates probably enter the villous epithelial cells where the peptide chain is removed by α-carboxyl peptidases[18] and monoglutamates at the dihydro state of reduction are further reduced to tetrahydrofolates by folate reductases. All reduced folates are further converted to methyltetrahydrofolate, which is the form in which they enter the portal circulation. Small doses of pteroyl glutamic acid are methylated, but large doses are absorbed unchanged.[19] There is a rise in the portal vein concentration after 5-20 minutes, but peak concentration in the systemic circulation is delayed for 1-2 hours. This is owing to the exchange with tissue folate in the liver.

TRANSPORT

Folates are carried in plasma bound to protein in the methyltetrahydro form. Plasma levels vary from 3 to 21 ng/ml in apparently normal people. Levels do not differ with sex or age but there is a wide diurnal variation associated with meals. Similarly, with a low dietary intake of folate plasma levels fall within a few days owing to the cessation of the exchange mechanism with tissue folate in the liver. The transfer from plasma to tissue is rapid and continuous. The folate level of cord plasma is three times the maternal plasma level, but there is a rapid decline during neonatal life.

EXCRETION

Folate in faeces represents the unabsorbed 20% of the dietary folate, the daily 60-90 μg of folate in the bile which is not reabsorbed, and the bulk from bacterial synthesis in the colon which is unavailable for absorption. There is a small daily excretion in urine of 2-5 μg which is much increased after an oral dose when the tissues are saturated. Some folate is lost in sweat and saliva.

TISSUE FOLATE

The total content of folate in the body is about 70 mg, of which about a third is in the liver (5-15 μg/g).

Folate is incorporated in red cells during erythropoiesis, and there is only a slight fall during their life span. The folate is largely in polyglutamate form but is rapidly broken down in

haemolysates at acid pH by plasma conjugases. Red cell folate is a useful indicator of body folate status. The average level is 300 ng/ml whole blood corrected to PCV of 45% with a range of 160-640 ng/ml. The levels are higher in neonates and they fall during the first year of life. Premature infants have still higher levels, but this rate of fall is more rapid, particularly at 4-8 weeks.

Liver folate falls to around 1·5 μg/g in about 130 days on a folate-deficient diet with megaloblastic changes. To prevent such changes a diet containing up to 200 μg daily is necessary.[6] During pregnancy an additional 100 μg daily is required.[20]

ACTION

Folates in the tetrahydro form act as coenzymes in all mammalian metabolic systems in which there is a transfer of a one-carbon unit[21] (Fig. 2). These are (1) the formylation of glycinamide ribonucleotide and 5-amino-4-imidazole carboxamide ribonucleotide in early purine synthesis; (2) the methylation of deoxymandylic acid to thymidylic acid in pyrimidine nucleotide biosynthesis; (3) amino-acid conversions; and (4) the generation of formate and its utilization.

The amino-acid conversions are (1) serine to glycine (this also requires pyridoxine); (2) histidine to glutamic acid through formiminoglutamic acid (FIGLU); and (3) homocysteine to methionine, which also requires cobalamin as a coenzyme. Here there is a transfer of a methyl group from N^5, methyltetrahydrofolic acid to cobalamin to form methylcobalamin and a subsequent transfer of the methyl group to homocysteine to form methionine.

Disturbances of Vitamin B_{12} and Folate Metabolism

All disorders of vitamin B_{12} and folate metabolism arise from deficiency at the tissue level. Neither group of substances has a known toxic effect.

A folate-free diet in man[22] leads to a fall in serum folate after 1-2 weeks followed by a progressive fall in red cell folate over the next 17 weeks. From the tenth week there is an increase in urinary FIGLU excretion. Owing to its slower rate of utilization a fall in the serum level of vitamin B_{12} in patients with a known deficiency and receiving B_{12} injections does not occur for some 18-24 months after the cessation of parenteral administration. A deficiency does not become evident until the tissue stores are below 0·1 mg and the plasma level below 100 pg/ml.

Folate deficiency in the tissues may arise from the increased utilization, such as by the fetus in pregnancy or by the hyperplastic bone marrow in haemolytic anaemias and myeloproliferative disorders. It may also arise as a result of the inhibition of dihydrofolate reductase by such drugs as methotrexate. Anticonvulsant drugs also disturb cell utilization of folate by an undetermined mechanism. This may be either by interference in a metabolic pathway, such as thymidylate synthesis, or by inhibition of folate absorption in the intestinal tract.

MEGALOBLASTOSIS

Deficiency of either folate or vitamin B_{12} results in a disturbance of DNA synthesis which leads to cellular changes in the rapidly dividing tissue of the haemopoietic system and gastrointestinal mucosa. Hypersegmentation of the polymorph nuclei occurs from the seventh week on a folate-free diet with macrocytic anaemia after the eighteenth week.

Florid megaloblastic anaemia with gross changes in the bone marrow is associated with a shortened life-span of the cells produced and leads to a haemolytic anaemia with jaundice, leucopenia, and thrombocytopenia with purpura. The gastrointestinal disturbances include glossitis, stomatitis, and intestinal malabsorption sometimes accompanied by diarrhoea. Other organs affected are the cervical and vaginal squamous epithelium and the ovary and testes, causing infertility.

OTHER EFFECTS OF DEFICIENCY

Folate deficiency may be associated with a variety of skin disorders (psoriasis, dermatitis herpetiformis, rosacea, eczema, exfoliative dermatitis); obstetric disorders (toxaemia of pregnancy, accidental haemorrhage, abortion, congenital anomalies of the fetus); neuropathy; and psychiatric disturbances but it has not been proved to be the cause of these disorders. Possibly low levels of serum and red cell folate are not uncommon but do not necessarily lead to pathological changes.

Disturbance of the metabolism of myelin sheaths due to vitamin B_{12} deficiency leads to peripheral nerve lesions, optic atrophy, degeneration of the posterior and lateral columns of the spinal cord with both sensory and motor effects, and psychoses. Deficiency of hydroxocobalamin in the plasma may be the cause of toxic amblyopia associated with tobacco smoking, the failure to remove cyanide causing retrobulbar neuritis.[23]

This article is based on a lecture given in the Birmingham course under the title "The Scientific Basis of Clinical Practice" (see B.M.J. 27 November 1971, p. 510).

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