derive their vitamin B_{12} by absorption of microbially synthesized material from the gut. Carnivorous animals may derive theirs partly in this way and partly from their food.

Vitamin B_{12} has been found in significant amounts in nature only in fermented materials like faeces and in animal products rich in protein. Liver and kidney appear to be the only relatively rich sources and contain around 0·5 p.p.m. Other meats, egg yolk, cheese and casein contain only a few parts per 100 million.

For literature references, see reviews on vitamin B_{12} (Smith, 1950–1; Ungley, 1951–2).

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The Pathogenesis of Megaloblastic Anaemias and the Value of Vitamin B_{12}

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Origins of megaloblastic anaemias

After the discoveries of Minot & Murphy (1926) and Castle (1929) earlier toxic theories were discarded and all megaloblastic anaemias were attributed to either simple or 'conditioned' deficiencies of an active principle present in food and stored in the liver. The subsequent isolation of vitamin B_{12}, folic acid and the citrovorum factor further strengthened current beliefs in the purely nutritional origin of this group of anaemias, although dissenting voices had been raised by Dock (1938) and Bomford (1946). In this paper nutritional and toxic theories are welded into a single working hypothesis.

The main dietary sources of vitamin B_{12} are of animal origin: organ meats, muscle meats, fish, milk and eggs. The folic-acid group of substances (including citrovorum factor) comes not only from animal sources but from fruits and green leaves. Yeast is a source of the folic-acid group and of the hypothetical Wills's factor, if this is a separate entity. Moreover, although lacking vitamin B_{12}, some yeast extracts have the properties of an extrinsic factor of Castle in that their haemopoietic effects in pernicious anaemia are potentiated by the simultaneous oral adminstration of a source of intrinsic factor (Strauss & Castle, 1932; Ungley & Moffett, 1936). A deficient intake

of these haemopoietic factors or of their precursors is common in the megaloblastic anaemias of tropical and subtropical regions, and not unknown in temperate zones. The low intake may be due to poverty or ignorance, to peculiar food habits based on fads or fancies, religious beliefs, prescribed diets or real or imagined association of symptoms with the eating of certain foods.

Absorption may be impaired by abnormalities in the alimentary tract. Gastric atrophy causes permanent lack of Castle's intrinsic factor in Addisonian pernicious anaemia. The secretion of this gastric factor may be temporarily reduced, e.g. during pregnancy (Strauss & Castle, 1933). The part played by structural and biochemical abnormalities in the intestine and by micro-organisms and helminths is discussed later.

Utilization may be interfered with by infection or by damage to vital organs such as the liver. Extreme deficiency of one haemopoietic factor may interfere with the utilization of another (see p. 303). Demands for haemopoietic factors may be increased by pregnancy, hyperthyroidism or infection and perhaps by prolonged excessive haemolysis, as in acholuric jaundice or malaria. Sometimes there is a vicious cycle, deficiency leading to anorexia, vomiting, diarrhoea or other alimentary disturbances, which in turn accelerate depletion of the nutrient.

Several facts, some clinical or biochemical, others deriving from experiments with animals or marrow culture, are difficult to reconcile with these simple nutritional concepts. For example, certain manifestations of megaloblastic anaemia are more readily explicable on a toxic than on a purely nutritional basis (Ungley, 1950*b*, 1951–2). One recalls the pyrexia that subsides dramatically after therapy, even before the red-cell count has risen appreciably, the patchy and sometimes perivascular distribution of degeneration in the nervous system, and the vivid red patches of glossitis. Sometimes haemolysis is excessive and even red blood cells transfused from normal donors are rapidly eliminated. In five out of six such cases the cessation of excessive haemolysis and the change to a normal rate of elimination occurred within 2 weeks of the administration of vitamin B_{12} or folic acid (Ungley & Walker, unpublished).

Serum from patients with pernicious anaemia in relapse inhibits the maturation of megaloblasts in marrow culture (Rusznyák, Löwinger & Lajtha, 1948; Lajtha, 1950). In vitro the inhibitor can be overcome by adding enough folic acid, citrovorum factor, normal serum, or vitamin B_{12} with gastric juice. During remission the serum ceases to inhibit the maturation of megaloblasts in marrow culture. All these findings have been confirmed in my laboratory either by Thompson (1950) or by Cox (to be published). The nature and origin of the inhibitor are unknown. Its presence in cerebrospinal fluid suggests that it may be ultrafiltrable. It was not destroyed by heating for from 1 to 2 h at 56° (Lajtha, 1950).

The urinary excretion of certain phenols is excessive in relapse and falls to normal after therapy with liver extract (Swendseid, Wandruff & Bethell, 1947) or vitamin B_{12} (Abbott & James, 1950). Another possible toxic agent is indol, which produced haemolysis and anaemia in dogs on a diet deficient in the vitamin B complex, but not in dogs on a normal diet (Rhoads, Barker & Miller, 1938).

In animals neither deprivation of vitamin B_{12} nor complete gastrectomy leads to megaloblastic anaemia. Yet megaloblastic anaemia can be produced both in man and

in animals by operations or diseases that lead to stenosis, blind loops or anastomoses in the gastrointestinal tract. All these conditions encourage the growth in the small intestine of micro-organisms which normally flourish in the colon.

Megaloblastic anaemia associated with intestinal strictures and blind loops

The essential feature of such anaemia is an area of stagnant and infected small intestine. When Watson, Cameron & Witts (1948) made culs-de-sac in the intestines of rats, the onset of anaemia was preceded by a long latent period. Schofield, Cox and I are repeating this work using larger animals so as to permit sampling of the blind sac. Our hypothesis is this. A toxic factor or inhibitor is formed by bacterial action in the stagnant area. In the initial stages this inhibitor is detoxicated by postulated enzymes of which folic acid or vitamin B_{12} or both form a part (see below). The increased call for detoxication leads to an increased demand for one or both vitamins, stores of which are gradually depleted. When depletion reaches a certain level, detoxication fails and the animal suddenly becomes anaemic and ill. The experimental anaemia can be prevented or alleviated by the administration of antibiotics (Toon & Wangensteen, 1950; Witts, 1951; Watson & Witts, 1952). In the rat, folic acid was effective, but not liver extract or vitamin B_{12}. In man, subacute combined degeneration may develop and responses to vitamin B_{12} have been observed. Two examples may be cited from a larger series (Thompson & Ungley, to be published).

In Mr C., with intestinal stenosis, the anaemia was initially hypochromic and responded to iron. Later the anaemia became macrocytic and responded to liver extract (see Fig. 7 in Ungley, 1938). In the latest relapse 80 μg vitamin B_{12} produced an effect equivalent to the response expected from about 20 μg vitamin B_{12} in Addisonian pernicious anaemia. At autopsy there was a stagnant and infected area of small intestine.

In Mr H., who had had multiple operations on the intestine for Crohn's disease, oral administration was ineffective at first, but after parenteral therapy had restored normal blood values, daily doses of 100 μg vitamin B_{12} orally were enough to maintain remission. In relapse the serum showed an inhibiting effect on the maturation of megaloblasts in marrow culture (Thompson & Ungley, to be published).

Addisonian pernicious anaemia

Lesion. The essential underlying lesion is gastric atrophy. The atrophy is confined to the body of the stomach (corpus, fundus). It involves not only the mucosa but the underlying muscle coats (Magnus & Ungley, 1938). I suggest that this peculiar lesion may be genetically determined; the individual is born not with gastric atrophy, but with a tendency for the stomach to atrophy later in life. Of those whose stomachs atrophy probably all have achlorhydria, but only some develop pernicious anaemia, and then probably after a long interval. Others develop a simple iron-deficiency anaemia, or never become anaemic at all. Similar remarks hold true for the subjects of total gastrectomy.

My working hypothesis is that when gastric atrophy leads to pernicious anaemia it does so by two pathways:

(*a*) Loss of Castle's intrinsic factor leads to defective absorption of vitamin B_{12} and so to depletion.

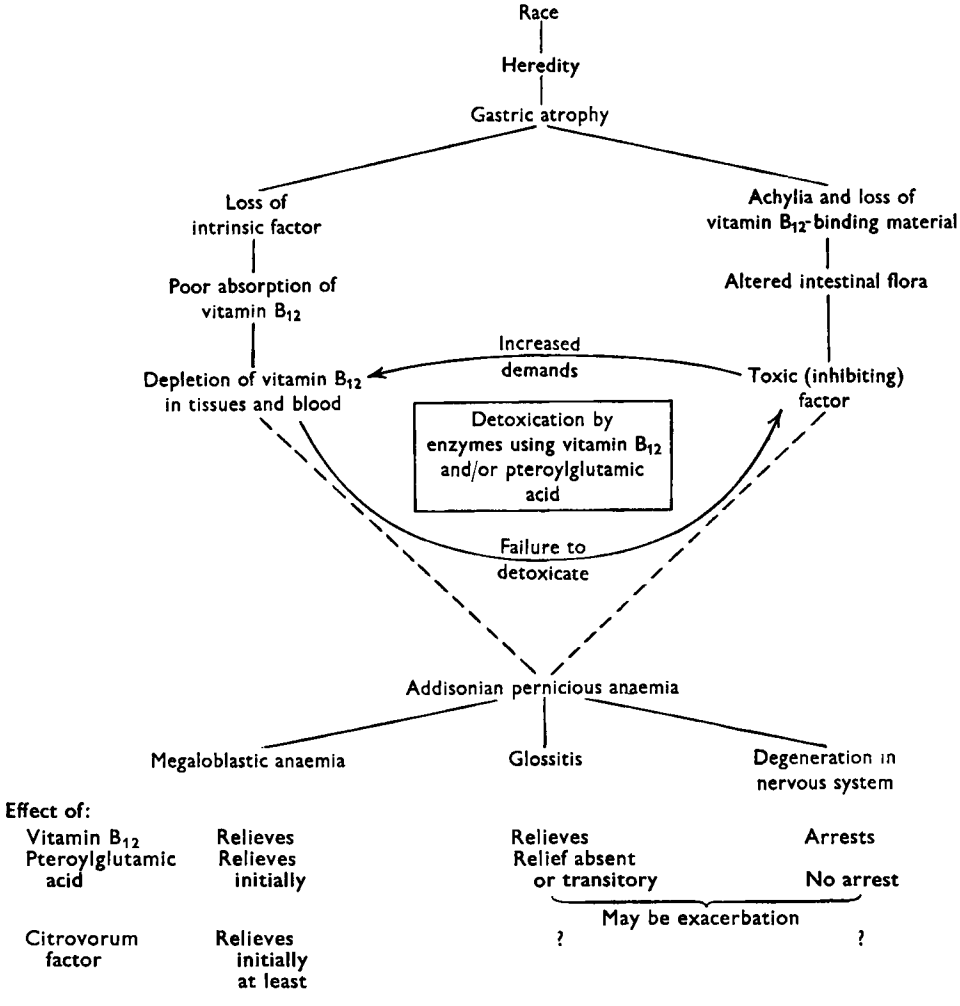

Fig. 1. Suggested pathogenesis of Addisonian pernicious anaemia (working hypothesis only).

(*b*) Loss of free hydrochloric acid reduces the antiseptic action of gastric juice. The contents of the upper intestine lack peptic predigestion. Moreover, the small amounts of gastric juice with which the food is admixed are deficient in substances that bind vitamin B_{12} and render it unavailable to certain bacteria. In this abnormal medium micro-organisms flourish and produce the postulated toxic or inhibiting factor (Fig. 1). In the diagram these two pathways are linked by enzyme systems in which vitamin B_{12} and the folic-acid group of vitamins play an important part.

These vitamins are known to be concerned in methylation and in nucleic-acid metabolism. Here we postulate another, but possibly related function, namely to

detoxicate or otherwise eliminate the inhibitor. One recalls the protective action of vitamin B_{12} against poisoning by pyridine (Dinning, Keith, Parsons & Day, 1950) and by carbon tetrachloride (Koch-Weser, Szanto, Farber & Popper, 1950). Day, Hall & Pease (1949) have demonstrated in vivo an effect on the methylation of guanidoacetic acid in rats. Vitamin B_{12} has also been shown to prevent uraemia (and high arginase activity in the liver) in newborn rats from mothers maintained on all-vegetable rations (Liener & Schultze, 1950).

Where then do folic acid and the citrovorum factor fit into this scheme? Why should the giving of folic acid relieve the anaemia, at least temporarily, and yet leave the tongue and the nervous system unprotected or more vulnerable? Conceivably, the giving of an excess of folic acid facilitates the action of the (postulated) vitamin B_{12}-containing enzyme, but in doing so it hastens the depletion of traces of vitamin B_{12} remaining in the body. When these are exhausted folic acid is without effect. Relapse follows, which may be neurological, lingual or haematological. The citrovorum factor is probably a stage in the normal metabolism of folic acid. Conversion of pteroylglutamic acid to citrovorum factor is aided by ascorbic acid, and also apparently by vitamin B_{12} (Dietrich, Monson & Elvehjem, 1951). In marrow culture—but not clinically, except in scorbutic monkeys—citrovorum factor is about 200 times more active than pteroylglutamic acid (Callender & Lajtha, 1951*a*, also personal communication).

In Addisonian pernicious anaemia the sole limiting factor seems to be vitamin B_{12}, although in non-Addisonian anaemia multiple deficiencies are probably not uncommon. In such cases a response to one haemopoietic factor may be inhibited by lack of another. Here are two possible examples (see Figs. 2 and 3). Both resembled Addisonian pernicious anaemia in having histamine-fast achlorhydria and a continued need for maintenance therapy, but one had syphilis and the other had steatorrhoea.

Mr C. (Fig. 2), aged 59 years, had anorexia, vomiting, weakness, dyspnoea, pallor, a smooth tongue and achlorhydria. Treatment for syphilis for 5 years resulted in serological negativity 8 months before admission. Stomach and duodenum were radiologically normal and diet was normal. Values for calcium, phosphorus, proteins, carotenoids and ascorbic acid in the blood spoke against a malabsorption syndrome. With serum bilirubin, 0·8 mg/100 ml., the direct reaction was negative. The marrow was megaloblastic. Red blood cells numbered 1,260,000/cu.mm, haemoglobin was 4·7 g/100 ml., packed cell volume 14 %, mean corpuscular volume 111·1 μ^3, mean corpuscular haemoglobin 37·7 $\mu\mu$g, mean corpuscular haemoglobin concentration 33·9 %, reticulocytes 0·6 %, white blood cells 2000/cu.mm. Transfusion was followed by a control period of 19 days, during which the highest reticulocyte level was 2·6 % and red-cell count fell to 1,300,000. The injection of Smith's slow-moving red component, a concentrate of vitamin B_{12b} (Smith, 1948; Fantes, Page, Parker & Smith, 1949), in a dose colorimetrically equivalent to 10 μg vitamin B_{12} was followed by an insignificant reticulocytosis of 2·8 % without improvement in the anaemia. After an injection of 10 μg ordinary vitamin B_{12} (cyanocobalamin) reticulocytes rose to 6 % on the 5th day, again without improvement in the anaemia. The marrow was still megaloblastic. In the next 6 days a total of 15 mg folic acid was administered orally.

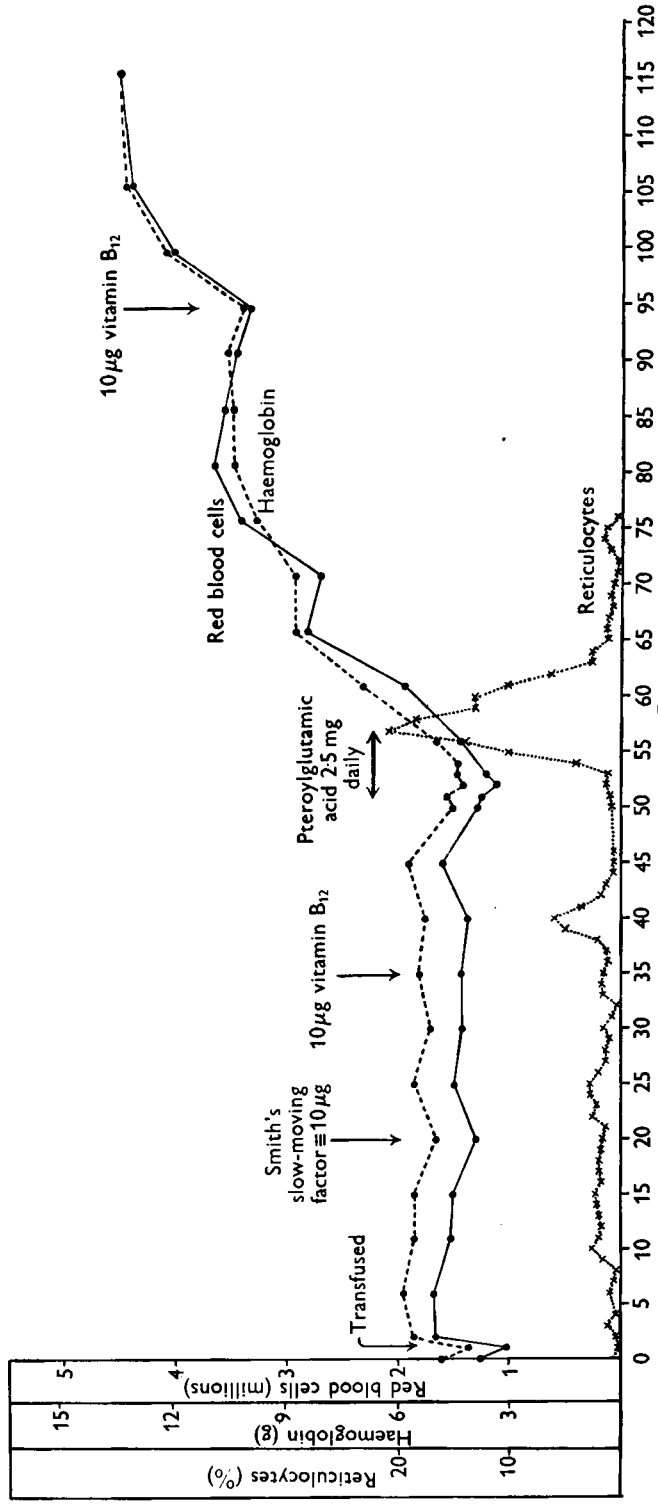

Fig. 2. Mr C., megaloblastic anaemia with syphilis. On the first two occasions 10 μg vitamin B_{12} or its equivalent had very little haemopoietic effect, although there was an excellent response to a total of 15 mg pteroylglutamic acid. When this response had ceased a similar dose of the same batch of vitamin B_{12} which had failed in the first instance now caused a satisfactory increase of red blood cells.

Reticulocytes reached a peak of 20·8 % on the 6th day, with rapid clinical and haematological improvement. On the 6th day the marrow was normoblastic.

	Days									
	0	5	10	15	20	25	30	35	40	44
Red blood cells (10^6/cu.mm)	1·24	1·42	1·93	2·80	2·67	3·39	3·62	3·53	3·43	3·30
Haemoglobin (g/100 ml.)	4·6	4·9	6·8	8·6	8·6	9·6	10·2	10·2	10·4	9·9
Packed cell volume (%)	14·5	17·0	—	32·0	30·0	35·0	37·0	—	—	—

After the effects of folic acid had waned, he received 10 μg of the same batch of vitamin B_{12} that had previously proved ineffective. This time the response was satisfactory.

	Days			
	0	5	11	21
Red blood cells (10^6/cu.mm)	3·30	3·98	4·35	4·45
Haemoglobin (g/100 ml.)	9·9	12·0	13·0	13·2

Normal blood values have since been maintained on vitamin B_{12} alone.

Although he had syphilis, this patient would probably have been regarded as suffering from true Addisonian pernicious anaemia but for the fact that he failed initially to respond to vitamin B_{12}.

The second possible example of dual deficiency (Mrs P., Fig. 3) is described under anaemias associated with idiopathic steatorrhoea (p. 308).

These cases recall the pigs described by Heinle, Welch & Pritchard (1948) which were so completely depleted of haemopoietic factors that folic acid failed unless liver extract was supplied; and liver extract failed unless small amounts of folic acid were supplied.

In some patients a small and delayed haemopoietic response has followed the administration of aureomycin (Lichtman, Ginsberg & Watson, 1950). Such responses —if they were indeed the effect of the antibiotics—may have arisen either from increased biosynthesis of citrovorum factor or vitamin B_{12} in the intestine, or from the elimination of bacterial sources of the (postulated) inhibitor.

It has long been a puzzle that some patients should get severe involvement of the central nervous system (or tongue) without anaemia. Girdwood (1951) suggests that such patients produce by intestinal biosynthesis enough folic acid to keep them free from anaemia, although they lack the vitamin B_{12} needed to protect the nervous system. Even if this hypothesis proves correct, we still need to determine why the nervous system remains undamaged in so many patients with severe and long-standing pernicious anaemia.

Depletion of vitamin B_{12} in pernicious anaemia is mainly due to loss of Castle's intrinsic factor. When doses of vitamin B_{12} as large as 3000 μg are given orally or *per rectum*, enough may be absorbed to produce a good haemopoietic response even though intrinsic factor is not supplied (Ungley, 1951). The absorption of small doses depends very much on the supply of Castle's intrinsic factor (see Ungley, 1950*a* part 2, Fig. 1). The mode of action of the latter is not known. Even when intestinal bacteria that absorb vitamin B_{12} are destroyed by antibiotics, intrinsic factor still

potentiates the effect of small amounts of the vitamin administered orally (see Ungley, 1950*a*, part 4, Fig. 4 and Ungley, 1951–2). Moreover, normal gastric juice which has been heated sufficiently to destroy its intrinsic-factor activity may still retain the capacity to bind vitamin B_{12} (Spray, 1952). We are still exploring the possibility that

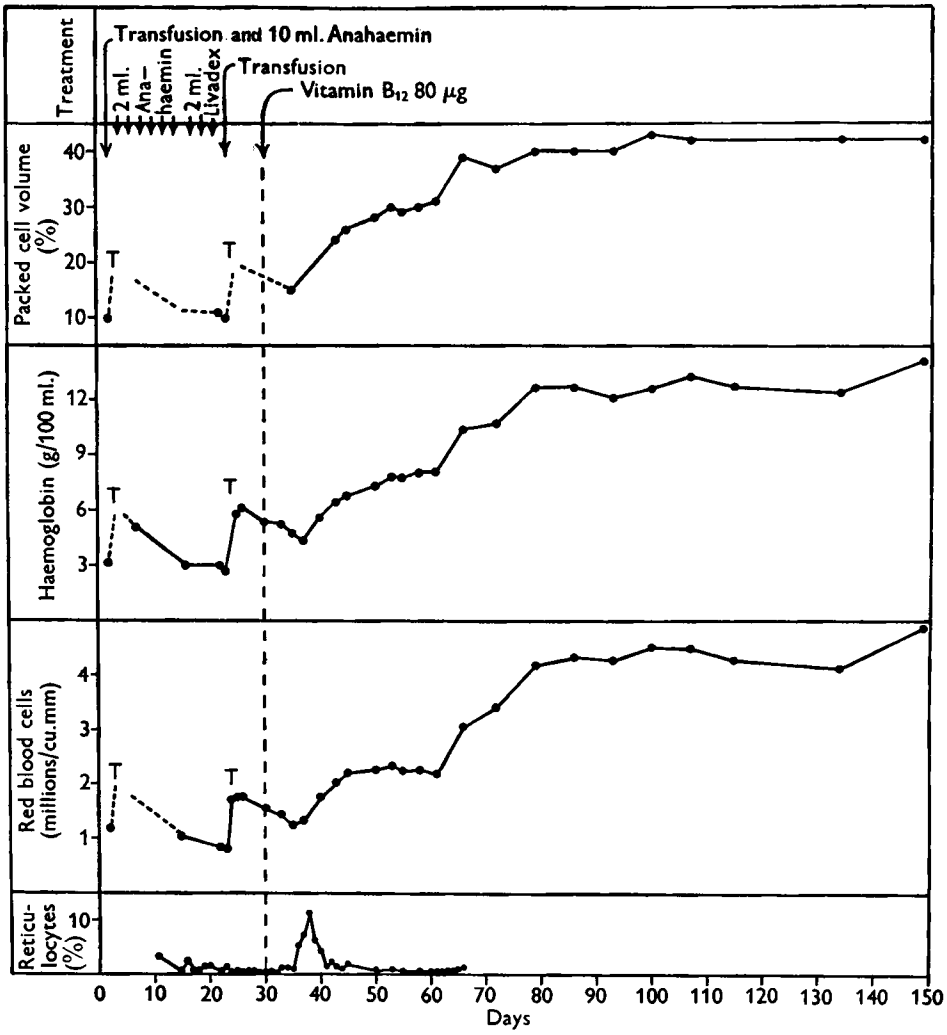

Fig. 3. Mrs P., megaloblastic anaemia with history of diarrhoea and poor diet. Vitamin B_{12} was ineffective at first, but effective later. In the interval she had received an injection of crude liver extract which had no haemopoietic effect, but may have supplied a missing nutrient needed for potentiating the action of vitamin B_{12} in this patient. T = transfusion.

interaction of vitamin B_{12} with intrinsic factor followed by enzymic splitting of the compound in the upper intestine leads to the release of a new compound which is more rapidly absorbed or utilized (Bethell, Swendseid, Miller & Cintron-Rivera, 1951; Ungley, 1951–2). It is unlikely, however, that the role of Castle's intrinsic factor is confined to absorption. Callender & Lajtha (1951*b*) have shown that, whereas neither free vitamin B_{12} nor unheated gastric juice is effective alone, the two together will

change megaloblasts to normoblasts in marrow culture. The vitamin B_{12} in normal serum is likewise bound to a thermolabile substance and has a similar effect in marrow culture. Heating the serum or the mixture of normal gastric juice and vitamin B_{12} frees the vitamin and renders the material inactive in marrow culture. This suggests that

intrinsic factor + extrinsic factor = haemopoietic factor

as in Castle's original hypothesis.

Effects of vitamin B_{12}. The effects of parenterally administered vitamin B_{12} on symptoms referable to the anaemia, alimentary tract and the nervous system could be explained as direct, due to restoration of normal tissue metabolism, or as indirect, due to elimination of a toxic factor or inhibitor. Perhaps both actions are involved, the balance between nutrient and inhibitor governing the occurrence of relapse or remission.

To ascertain the relation between dose and response following a single injection, Ungley (1949*a*) spaced doses logarithmically: Fig. 1 in Ungley (1949*a*) shows some typical responses. Ten μg was just enough to produce, on average, a 'maximal' reticulocytosis and a rise of red blood cells which was 'optimal' according to standards for injectable liver extract. Individual variations were wide—especially for doses below 10 μg—but for groups of ten or more cases the deviation between observed and expected response was small. At least for doses ranging from 5 to 160 μg there was a constant relation between the logarithm of the dose and the mean increase of red blood cells in 15 days (see Ungley, 1949*a*, Fig. 5). A similar relationship exists between the logarithm of the dose and the mean increase of packed cell volume in 15 days. When the dose is increased to 320 μg this relationship no longer holds, so that 160 μg is probably somewhere near the top of the dose-response curve (Ungley & Campbell, to be published).

Other effects are: White blood cells and platelets usually increase to normal levels. The marrow changes from megaloblastic to normoblastic within about 72 h after a single injection. The effect lasts for days or weeks according to the size of the dose (Mollin & Dacie, 1950). Appetite returns and gain in weight follows. Soreness of the tongue is relieved in 4 or 5 days. After 2 or 3 weeks the papillae have usually regenerated and the tongue has its normal coating of fur.

Gastrointestinal symptoms usually vanish, but gastric atrophy persists and so does the achylia. Bilirubinaemia and the elimination of excessive amounts of urobilinogen cease (Concha, Etcheverry & Guzman, 1949). Changes in excretion of phenols and the elimination of an inhibitor from serum were mentioned earlier.

Here is an interesting example:

A woman had a very sore tongue with vivid red patches. The vitamin B_{12} content of her serum (Dr G. I. M. Ross) was 40 $\mu\mu$g/ml.—a low level. She had achlorhydria but no anaemia. A few days after a single injection of vitamin B_{12} she ate in comfort for the first time for 2 years, and within 2 weeks the tongue was entirely normal in appearance.

Liver extract versus vitamin B_{12}. In our experience vitamin B_{12} is in no way inferior to liver extract for initial therapy and maintenance in pernicious anaemia.

Low prothrombin values persisting after therapy with vitamin B_{12} (Owren, 1950) have not been encountered in our series (unpublished observations with Dr F. K. Herbert). The persistent macrocytosis reported by Larsen (1950) was not seen in Price-Jones curves of six cases treated solely with vitamin B_{12} for 2 or 3 years (Ungley, 1951–2).

Girdwood & Carmichael (1950) assayed liver extract microbiologically and clinically and found that the whole of the clinical activity could be accounted for on the basis of vitamin B_{12} (see also Girdwood, 1951).

Maintenance dose of vitamin B_{12}. Now that vitamin B_{12} is relatively cheap, enough should be given to cover wide individual variations and to maintain a reserve. The less frequent the injection, the more material is required to maintain remission. Some patients need more than others. A safe compromise is to give 100 μg every 2 weeks to patients with subacute combined degeneration, and 100 μg a month to those without neurological involvement. A few patients may need more frequent injections, and extra doses should always be given if intercurrent illness or infection supervenes.

Vitamin B_{12} and neurological manifestations. Vitamin B_{12} is as effective as liver extracts, crude or refined, in the treatment of subacute combined degeneration of the cord (Ungley, 1949*b*, Figs. 18 and 12). Paraesthesias diminish in extent and in severity, and may disappear. Difficulty in walking and ataxic gait almost always improve. Even patients who are hardly able to move their legs in bed may recover ability to walk. Incoordination of the extremities and Rombergism lessen or disappear.

Mental changes often clear up rapidly coincident with the relief of the anaemia. Some, which may be due to 'subacute combined degeneration of the brain', improve more gradually.

Optic atrophy is a less common but equally serious complication of pernicious anaemia.

Mr E. had achlorhydria, mild spinal cord involvement, early marrow changes and very little anaemia, but was virtually blind from optic atrophy. After treatment with vitamin B_{12} for 2 months the improvement in vision is already remarkable. He can read small newspaper headlines and walk through busy streets alone. Central scotomata are decreasing in size and density (Ungley & Maw, to be published).

The megaloblastic anaemias associated with idiopathic steatorrhoea

These form a mixed group. Some respond to vitamin B_{12}, others do not. Here are three examples from a larger series to be published later. In Mr McK. the response to 80 μg was about equal to the response expected from 5 μg in pernicious anaemia. In a former relapse the response to folic acid had also been slow. Red-cell counts seldom exceeded 4 millions/cu.mm with any form of therapy.

In a patient, Mr W., with steatorrhoea associated with thyrotoxicosis, the anaemia entirely failed to respond to vitamin B_{12}, whereas folic acid was effective. In this instance a toxic or haemolytic factor seemed to play a part; excessive haemolysis and rapid elimination of transfused cells from normal donors ceased about 2 weeks after the administration of folic acid.

The third case is a possible example of dual deficiency already referred to on p. 305 (Fig. 3). Mrs P., aged 71 years had abdominal pain and flatulence intermittently for

2 years. In a week she might eat bacon or an egg once, cabbage once or twice, little meat or fish, but plenty of potatoes, bread, sugar and tea. An attack of diarrhoea 2 months before admission was treated for 8 days with phthalylsulphathiazole. As the diarrhoea diminished, anaemia developed suddenly—so suddenly that melaena was wrongly suspected. Her condition improved slightly with iron and then deteriorated rapidly. When admitted she was thin, pale and drowsy. Red blood cells numbered 1,140,000/cu.mm, haemoglobin 2·9 g/100 ml., mean corpuscular volume 88 μ^3, mean corpuscular haemoglobin concentration 29 %, white blood cells 3300/cu.mm. The marrow was megaloblastic. There was achlorhydria. After transfusion, 10 ml. of 'refined' liver extract (Anahaemin, British Drug Houses Ltd.) were injected, then 2 ml. on alternate days. There was no response, and 12 days later red blood cells numbered 1,050,000/cu.mm. Three injections of 2 ml. 'crude' liver extract (Livadex, British Drug Houses Ltd.) were then given on alternate days. The total dose of vitamin B_{12} in the refined and crude liver extracts, kindly assayed by Dr W. F. J. Cuthbertson of Glaxo Laboratories, was not less than 70 μg. Again there was no response and after 5 days red blood cells had fallen to 830,000/cu.mm, haemoglobin was 3 g/100 ml., M.C.V. 132·5 μ^3, M.C.H. 35·5 $\mu\mu$g, mean corpuscular haemoglobin concentration 26·9 %, reticulocytes 0·6 %, white blood cells 4700/cu.mm.

When seen by us at this stage the patient was emaciated and pale, but cheerful. The bowels had been constipated, and the appetite good. The tongue was dry and smooth but never sore. The abdomen was distended but lax; the liver was enlarged half-way to the umbilicus; spleen was not palpable. Co-ordination of the limbs was normal, vibration sense unreliable. Idiopathic steatorrhoea was suspected because of the history of diarrhoea and the failure to respond to liver extract. Later a 2-day test on a diet containing 50 g fat showed 36 % fat in the dried faeces. Analysis of blood showed total proteins 6·15 g, albumin 3·72 g, globulin 2·43 g, calcium 8·0 mg and phosphorus 3·5 mg/100 ml., alkaline phosphatase 7·9 units, ascorbic acid absent, carotenoids absent, prothrombin 64 % of normal average, methaemalbumin absent, bilirubin 0·8 mg/100 ml. serum. A glucose-tolerance curve was within normal limits—fasting 105, maximum at 1½ h 127 mg/100 ml. Radiography (performed when the patient was not having diarrhoea) showed apparent thickening of mucosal folds of the jejunum, but no segmentation of barium or delay in emptying the small intestine. After another transfusion and a satisfactory control period, during which reticulocytes did not exceed 1·4 %, 80 μg vitamin B_{12} were injected. Reticulocytes rose on the 6th day, reaching a peak of 11·2 % on the 8th day. The anaemia improved slowly, but normality was attained in 70 days without further therapy and without relaxing dietary restrictions.

	Days					
	0	5	10	15	20	25
Red blood cells (millions/cu.mm)	1·52	1·26	1·78	2·20	2·26	2·24
Haemoglobin (g/100 ml.)	5·3	4·6	5·6	6·8	7·3	7·7
Packed cell volume (%)	—	15·0	—	26·0	28·0	29·0

After about a year without therapy, and with the patient taking her ordinary diet, blood examination showed evidence of relapse. The decline in blood values was not arrested by the oral administration of vitamin B_{12} even when the dose was increased

to 200 μg daily. There was no diarrhoea, but occasionally she passed two or three soft stools a day. The injection of 160 μg of vitamin B_{12} was followed by a rapid clinical and haematological improvement, red cells increasing from 2·69 to 4·55 millions/cu.mm in 15 days. For the last 5 months she has remained well without further injections. A second relapse occurred after 11 months without further injections. Normal blood values were restored following the injection of 50 μg vitamin B_{12}.

Megaloblastic anaemias of infancy

Vitamin C deficiency may be partly responsible for some of these anaemias—they are said to be much less common in America since a prominent manufacturer added ascorbic acid to his baby foods. As already mentioned, ascorbic acid facilitates the conversion of pteroylglutamic acid to citrovorum factor. May produced megaloblastic anaemia in monkeys by a diet deficient in ascorbic acid and in folic acid. In these scorbutic monkeys as little as 7·5 μg of citrovorum factor changed the marrow to normoblastic, whereas several milligrams of pteroylglutamic acid were needed to produce the same effect (May, Nelson, Lowe & Salmon, 1950; May, 1951).

In infants, however, the effective dose of citrovorum factor is much the same as that of pteroylglutamic acid. Moreover, some of the infants show no signs of vitamin C deficiency and some (unlike monkeys) respond to vitamin B_{12}, so that 'megaloblastic anaemias of infancy' are probably a mixed group too.

Macrocytic anaemias associated with cirrhosis of the liver

These anaemias are usually macrocytic and normoblastic. In a few cases where the marrow was megaloblastic, there is said to have been severe nutritional deficiency. One such patient responded excellently to vitamin B_{12} (Movitt, 1950).

Megaloblastic anaemia after total gastrectomy

One presumes that the mechanism of origin of these cases is the same as for Addisonian pernicious anaemia, which is also in a sense an 'agastric' anaemia. One of my patients is responding to 1000 μg vitamin B_{12} administered orally. In a case described by Conway & Conway (1951), however, the response to vitamin B_{12} was poor and incomplete, whereas folic acid quickly restored normal blood values.

Megaloblastic anaemias of pregnancy and the puerperium

In this country such anaemias usually fail to respond to vitamin B_{12} (see Fig. 1 in Ungley & Thompson, 1950 and Fig. 1 in Thompson & Ungley, 1951) and Castle's theory therefore cannot be invoked to explain them.

In Mrs A. vitamin B_{12} was ineffective, but the anaemia responded to folic acid (Ungley & Thompson, 1950, case 1). In Mrs M. E. H. the peripheral blood was rather microcytic and hypochromic, but the marrow was megaloblastic. Vitamin B_{12} failed, but there was a good response to folic acid. Iron was ineffective while the marrow was megaloblastic, but effective after folic acid had been supplied (Thompson & Ungley, 1951, p. 201).

A report on combined therapy with vitamin B_{12} and ascorbic acid (Holly, 1951) awaits confirmation. The basis for the haemopoietic efficacy of yeast extracts is uncertain and the existence of a 'Wills's factor' distinct from folic acid and the citrovorum factor has yet to be excluded. The anaemias respond to folic acid, but this does not necessarily imply that they are due to a deficiency of folic acid. The fallacy of such reasoning became evident after painful experiences with pteroylglutamic acid in pernicious anaemia.

I doubt whether megaloblastic anaemia of pregnancy is a nutritional disorder in the ordinary sense. Several of our patients had excellent diets, a good appetite, no vomiting or other alimentary disturbances, and no achlorhydria. In others, the diet, although not good, was at least no worse than that of other women who did not develop anaemia. Only in eleven out of twenty-seven in whom dietary histories were available could it be said that a deficient diet preceded the symptoms of anaemia (Thompson & Ungley, 1951).

One patient had a transient attack of fatty diarrhoea with definitely impaired fat absorption; this attack occurred between two pregnancies. In both these pregnancies megaloblastic anaemia developed but without diarrhoea or clinical evidence of steatorrhoea. Indeed diarrhoea of any kind was most unusual in our series. Much more evidence would be needed to incriminate malabsorption as a cause of megaloblastic anaemia in pregnancy and the puerperium.

The only constant aetiological feature is the relationship to pregnancy or the puerperium. The incidence is relatively higher in twin pregnancies and in multiparas, but the anaemia seldom recurs in subsequent pregnancies. An interesting possibility is that the development of anaemia depends on quantitative or qualitative differences in the output of sex hormones. The relationship between sex hormones and the erythrocyte count is well known. Sex differences between blood counts of male and female chicks (Taber, Davis & Domm, 1942–3) and rats (Vollmer, Gordon, Levenstein & Charipper, 1941) can be reversed by the administration of the hormone—oestrogen or androgen—appropriate to the opposite sex. The rapidity of growth of the genital tract of the chick (Hertz, 1948*b*) and monkey (Hertz, 1948*a*) in response to oestrogen varies with the amount of folic acid available. A normal supply of folic acid is adequate for a full androgen response in the male rat but not for a full oestrogen response in the female rat (Overbeek & Tausk, 1950). In the rat optimum lactation depends on the availability of folic acid (Cerecedo & Vinson, 1944).

Conceivably in man, too, there is an increased demand for folic acid as the uterus increases in size during pregnancy under the influence of oestrogen, and in susceptible persons this might lead to a deficiency of folic acid. But why are so few women susceptible? Do they produce excessive amounts of oestrogen? There is almost no information on this point. Day *et al.* (1949) record tests in one patient 5 days before delivery which showed an excretion of 4 mg of 17-ketosteroids, as compared with a normal range of 5·0–17·0 mg., in 24 h, 140 rat units of oestrogen in contrast to the normal 1000 or more rat units for the 9th month of pregnancy, and 15,300 i.u. of chorionic gonadotropins per 24 h, which is a high normal value. In one of our patients the excretion of ketosteroids, pregnanediol and total

oestrogens at the 7th month of pregnancy was within normal limits (Thompson & Ungley, 1951).

The possibility remains that hormone output is qualitatively abnormal. Do these patients produce an abnormal steroid with properties antagonistic to folic acid? Chemically there is no similarity between steroid hormones and pteroylglutamic acid. It is of interest, however, that at least one sex hormone, dehydroisoandrosterone, has properties like folic acid for promoting the growth of certain micro-organisms (Gaines & Totter, 1950). The circulation of an abnormal steroid, if it exists, might explain the haemolytic manifestations seen in some cases of megaloblastic anaemia of pregnancy and the puerperium. In three patients red cells transfused from normal donors were rapidly destroyed until raw liver or folic acid was administered, when the rate of elimination became normal (Thompson & Ungley, 1951).

To summarize, then, there are three possible ways in which a deficiency of folic acid might arise in pregnancy and the puerperium:

(1) If the heavy but normal demands for folic acid at these times were not met from dietary sources because of dietary deficiency, vomiting or defective absorption. For reasons already given, this explanation is unlikely. It also fails to explain the rarity of recurrence in subsequent pregnancies.

(2) If the demand for folic acid were abnormally high, owing, say, to an excess of oestrogen. At present there is almost no evidence about demands for folic acid or about the level of oestrogen production in megaloblastic anaemia of pregnancy and the puerperium.

(3) If folic acid were rendered less available by the presence of an inhibitor or antagonist, arising perhaps from an abnormal variant of one of the steroid hormones. On such a hypothesis it would be easier to account for the haemolytic component in some cases of megaloblastic anaemia of pregnancy and the puerperium. Efforts to test these hypotheses continue.

Nutritional megaloblastic anaemias

These, attributed to dietary deficiency of either vitamin B_{12} or of the folic-acid group, are uncommon in temperate climates. Free hydrochloric acid is usually present in the gastric juice, as in the three cases mentioned below, or returns on treatment of the anaemia.

In two patients the anaemia responded to yeast extract or wheat germ. One was a widow aged 67 years, who had restricted her diet because of epileptic fits; and the other was a woman aged 39 with prolonged dietary deficiency and dysphagia of the Plummer-Vinson type (see cases 9 and 10, Figs. 2 and 3 in Ungley, 1933).

The third patient was a male epileptic aged 24, who had restricted his diet chiefly to tea and white bread and butter, consuming almost no meat, eggs, fresh green vegetables or fruit. The anaemia responded to parenteral liver therapy (see Fig. 1 in Ungley, 1938).

Tropical megaloblastic anaemias

In these, whether associated with pregnancy or not, dietary deficiency is the rule, and vitamin B_{12} is often effective (Patel, 1948; Patel & Kocher, 1950; Das Gupta (personal communication), 1951; Chaudhuri, 1951).

Megaloblastic fish-tapeworm anaemia

von Bonsdorff and his colleagues in Helsinki have done much to advance our knowledge in this field (von Bonsdorff, 1948; von Bonsdorff & Gordin, 1951, 1952; Björkenheim, 1951). Infestation with *Diphyllobothrium latum* is common in country districts in Finland, but very few of those infested develop megaloblastic anaemia. In those who do, the worm is situated high up in the small intestine. The clinical picture resembles Addisonian pernicious anaemia even to the incidence of neurological phenomena, but there is usually free HCl in the gastric juice. Symptoms can be relieved either by expelling the worm or by giving vitamin B_{12} parenterally with the worm still in situ.

Many attempts have been made to isolate a toxin from the worm. Now it appears that far from being toxic, the dried worm is curative, being a rich source of vitamin B_{12}! Presumably the living worm in the intestine takes up vitamin B_{12} of which the host is thus deprived. Dietary deficiency and depression of gastric secretion, if present, are of secondary importance. Whether or not an inhibitor is present in the serum of the patient has yet to be determined.

Conclusions

In the management of patients suspected of having megaloblastic anaemia it is essential to examine the marrow before treatment obscures the picture. The blood findings alone may be misleading, for not all macrocytic anaemias are megaloblastic and not all megaloblastic anaemias are macrocytic.

Controlled therapeutic tests are important for diagnosis and prognosis. If vitamin B_{12} fails, the effect of some form of folic acid, e.g. pteroylglutamic acid or citrovorum factor, should be observed. Even if vitamin B_{12} is effective, the anaemia may not be Addisonian. If free hydrochloric acid is found in the gastric juice, other tests such as fat balance or radiology of the intestine may be required. These may reveal some underlying disorder such as intestinal stenosis, gastrocolic fistula or idiopathic steatorrhoea.

In Addisonian pernicious anaemia vitamin B_{12} is as effective as liver extract even for the neurological manifestations. Certain non-Addisonian megaloblastic anaemias also respond to vitamin B_{12}, but there are others that do not. In these the missing nutrient may be folic acid or possibly Wills's factor. In rare cases lack of one nutrient seems to inhibit the response from another, as though dual deficiencies were present.

In the past 100 years theories about megaloblastic anaemias have swung from 'nutritional' to 'toxic' and back to 'nutritional' again. Now the opposing theories can be united in a single working hypothesis which postulates an interplay between nutritional and toxic or inhibiting factors. The future lies in the integration of work in many fields, and it is a pleasure to thank all those who are collaborating to this end.

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The Relationships between Vitamin B_{12}, Folic Acid and Folinic Acid

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The interrelationships between the various substances used for the treatment of the megaloblastic anaemias are complex and little understood. The vast amount of experimental work carried out in recent years has given us so many papers that it is almost impossible to keep track of them, but the jig-saw is as yet incomplete. In the short time available it is not possible to deal fully even with one aspect of the problems, which have been considered in more detail in a recent review (Girdwood, 1952*a*); to-day I shall refer briefly to some of the reported work and add data derived from a few metabolic studies carried out on patients.

Chemical interrelationships

Dr Lester Smith has discussed the formula of vitamin B_{12} and related forms (Smith, 1952). As is known, the name folic acid is usually given to synthetic pteroylglutamic acid, although it is not certain that the two are identical (Robinson, 1951). Pteroylglutamic acid is not related chemically to vitamin B_{12}. It has long been considered that the chief form of folic acid present in foodstuffs is that found in yeast, pteroylhexaglutamylglutamic acid, a γ-linked peptide which has not been synthesized. Satisfactory experiments cannot be carried out with this substance because of the presence of 'conjugase inhibitors' in the yeast concentrates used as a source of this natural folic-acid conjugate.

Synthetic conjugates of pteroylglutamic acid are pteroyldiglutamic acid and pteroyltriglutamic acid (Diopterin and Teropterin, Lederle Laboratories Inc.). As far as is known, these do not occur in food and, in fact, the former does not occur naturally.

Folinic acid (Bond, Bardos, Sibley & Shive, 1949) appears to be the same substance as the citrovorum factor (Sauberlich & Baumann, 1948), so called because it will support the growth of the streptococcus *Leuconostoc citrovorum*. The chemical