Evidence regarding the minimal daily requirement of dietary vitamin B\(_{12}\)\(^1\)\(^-\)\(^3\)

S. J. Baker,\(^4\) M.D., F.R.A.C.P., F.R.C.P.(C.) and V. I. Mathan,\(^5\) M.D., Ph.D.

ABSTRACT

The minimal daily requirement of dietary vitamin B\(_{12}\) is defined as that amount which must be ingested in the diet to prevent the occurrence of megaloblastosis, anemia, or other manifestations of vitamin B\(_{12}\) deficiency. An attempt has been made to define this requirement by studying hematological responses to varying amounts of the vitamin in five south Indian subjects with vitamin B\(_{12}\) deficiency anemia (one due to gastric atrophy and four due to dietary deficiency). Amounts ranging from 0.07 to 0.25 \(\mu\)g of dietary vitamin B\(_{12}\) were shown to be inadequate but amounts ranging from 0.3 to 0.65 \(\mu\)g were adequate or possibly more than adequate. It is concluded that the minimal daily requirement of dietary vitamin B\(_{12}\) for the average south Indian adult is in the region of 0.5 \(\mu\)g/day and that an intake of 1.0 \(\mu\)g/day would cover the needs of the vast majority of the population and allow a wide margin of safety.

KEY WORDS  Vitamin B\(_{12}\) requirements, megaloblastic anemia

Introduction

The amount of vitamin B\(_{12}\) which must be ingested each day by the average adult to maintain health has been variously estimated as between 10 \(\mu\)g (1) and 0.1 \(\mu\)g (2). A group of patients with vitamin B\(_{12}\) deficiency anemia, one due to atrophic gastritis and four due to apparent dietary deficiency, provided an opportunity to investigate this problem further.

Materials and methods

Five patients, with six episodes of anemia, were studied. All subjects gave their informed consent to the investigations and were admitted in a metabolic ward where there was total dietary control. Each subject's diet was studied throughout their stay in hospital by the duplicate meal technique. Double portions of all dietary items were prepared; the amount of each item the subject consumed was measured, and from the other portion an exact duplicate of the total 24-h intake was made, including the drinking water. Each 24-h pool was then homogenized and aliquots taken for assay.

Hematological investigations were carried out by standard techniques (3). Hb was measured by the cyanmethemoglobin method using a photoelectric colorimeter checked at frequent intervals against an international reference standard. Bone marrow smears were stained with May-Grunwald-Giemsa stain, these were mixed with other bone marrow slides and interpreted without knowledge of their origin. Megaloblastic changes were arbitrarily graded 1 to 4, the most severe being termed grade 4 and the least grade 1 (4). Serum and food vitamin B\(_{12}\) concentrations were estimated by microbiological assay using Euglena gracilis Z strain as the test organism (5). Vitamin B\(_{12}\) in food was estimated after papain digestion followed by autoclaving for 15 min at 120°C in the presence of cyanide (6). Serum, red cell, and food folate were estimated by microbiological assay using Lactobacillus casei (7). Food folate was estimated both directly, i.e., "free folate," and after conjugase treatment, i.e., "total folate."

Vitamin B\(_{12}\) absorption tests were usually carried out after the study of the hematological responses. In all cases they were done using a 1 \(\mu\)g dose of the vitamin labelled with 0.5 Ci of Co\(^{60}\). Absorption was measured either by the fecal balance technique (8), estimation of radioactivity in the serum at 8 h after the dose (9), or by measurement of whole body retention on a shadowshield whole body counter.

Stool fat excretion was measured daily by the method of van de Kamer et al. (10) while the patient was on a measured intake of 50 g/day. Results were expressed as a 3-day running mean. The values given in Table 1 represent the highest excretion for a 3-day period.

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\(^2\) Supported by the Wellcome Trust and the World Health Organization.

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KEY WORDS  Vitamin B\(_{12}\) requirements, megaloblastic anemia
Results

Patient 1

C. G., a 57-yr-old male power loom operator, was well until about 2 yr before admission when he noticed the gradual onset of generalized weakness, a feeling of numbness in the hands and feet, and increasing black discoloration of his hands, feet, and face. He was a nonvegetarian who ate a good diet that frequently included meat, fish, eggs, milk, and curds. There was no family history of anemia.

Examination revealed a man with marked pallor and hyperpigmentation of the hands, feet, face, tongue, and over the pressure points, similar to that previously described in Indians with megaloblastic anemia (11). There was an ejection systolic murmur at the base of the heart and a mild peripheral sensory neuropathy.

The initial investigations are shown in Table 1. He had no detectable intrinsic factor in his gastric juice and defective vitamin B12 absorption. The chest x-ray and microscopy of the urine were normal and there was no evidence of any infection throughout his stay in hospital. He was placed on a vegetarian diet that provided an average daily intake of 0.38 μg of vitamin B12. After 11 days on this diet there was no hematological response (Fig. 1). After a daily injection of 0.1 μg of vitamin B12 there was a slight increase in reticulocytes, but despite this, his Hb continued to fall and on the 17th hospital day he was given a transfusion of packed red cells which raised his Hb to 5.8 g/dl. Over the next 5 days, although his serum vitamin B12 concentration rose to 80 ng/l, his Hb concentration again fell and the appearance of his bone marrow remained unchanged. On the 23rd hospital day the amount of parenteral vitamin B12 was increased to 0.3 μg/day. This was followed by an irregular reticulocytosis, a further rise in serum vitamin B12 concentration, a slow rise in Hb concentration and hematocrit, and ultimate conversion of the bone marrow to a normoblastic pattern of erythropoiesis. The serum iron and folate concentrations remained within the normal range throughout. Coincidently with his hematological improvement, his signs and symptoms of peripheral neuropathy improved and his hyperpigmentation gradually faded. His defect in vitamin B12 absorption persisted, but the provision of exogenous intrinsic factor normalized the absorption. There were no intrinsic factor antibodies demonstrable in his serum. A jejunal and gastric biopsy were obtained after his Hb had improved. The jejunal biopsy was within normal limits for southern Indian subjects. The gastric biopsy showed a marked degree of atrophic gastritis. Before discharge from hospital the patient was given an injection of 500 μg of cyanocobalamin and advised to have regular parenteral therapy.

Comment

The basis of this patient’s vitamin B12 deficiency was undoubtedly the absence of intrinsic factor preventing the absorption of the

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* Marrow grade = severity of megaloblastic change 3 < 4.
† AG atrophic gastritis.
‡ N within limits of normal for southern India; (1) μg retained measured by whole body counting (normal ≥ 0.3 μg); (2) percentage of dose per liter of plasma at 8 h (normal ≥0.2%); (3) μg retained measured by fecal collection (normal ≥ 0.3 μg).

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vitamin. Although there were no intrinsic factor antibodies in the serum it is possible that this in fact represents a case of Addisonian pernicious anemia. However, in a region where tropical sprue is common, it is impossible to exclude the possibility that the gastric lesion was the end result of subclinical tropical sprue where the intestinal lesion had healed (12).

Because of the absence of intrinsic factor it can be assumed that negligible amounts of the vitamin were absorbed from the daily intake of 0.38 μg in the food. It is not surprising, therefore, that during the first 11 days, before parenteral therapy was started, the patients' condition deteriorated. With a daily injection of 0.1 μg of vitamin B₁₂ the Hb and hematocrit continued to fall necessitating a transfusion of packed red cells. When the amount of parenteral vitamin was increased to 0.3 μg/day there was a slow hematological response. The bi- or tri-phasic reticulocyte response that followed the increased dose of vitamin B₁₂ suggests sequential responses to a more effective dose of the same hemopoietic substance or to some other hemopoietic substance. However, the amount of vitamin B₁₂ given each day was carefully checked and was constant, as was his dietary intake. This irregular reticulocytosis must therefore presumably be a rather unusual response to a suboptimal dose of vitamin B₁₂.

It may be concluded that, for this individual, 0.1 μg of parenteral cyanocobalamin per day was not adequate to maintain even a very low Hb concentration, but that 0.3 μg daily was adequate to restore the marrow to a normoblastic pattern of erythropoiesis, raise the Hb concentration to an acceptable, although possibly still suboptimal level, and restore the serum vitamin B₁₂ concentration to normal.

**Patient 2**

Th., a 30-yr-old unemployed man, presented with a history of increasing debility for the previous 2 to 3 months. He ate a vegetarian diet of very poor nutritional quality. The only animal products he ever ate were, on very rare occasions, when he drank...
a small amount of milk or buttermilk. Physical examination revealed a pale looking individual with hyperpigmentation over the dorsum of the fingers, especially over the joints and terminal phalanges.

The initial investigations are shown in Table 1. He was severely anemic with a Hb concentration of 3.4 g/dl. Serum iron concentration was 46 μg/100 ml and unsaturated iron binding capacity 245 μg/100 ml. He was given an exchange transfusion of 1 unit of packed cells which raised his Hb concentration to 4.4 g/dl (Fig. 2). He was placed on a diet that simulated the one he had been accustomed to, which contained an average of 0.07 μg of vitamin B₁₂ and 290 μg of free and 440 μg of total folate. In addition, in an endeavor to ensure that neither iron or folate would limit the hematological response to vitamin B₁₂, he was given 100 μg of pteroylmonoglutamic acid daily by injection and an oral dose of 100 mg of elemental iron as ferrous sulphate three times a day. The iron was continued throughout his stay in the hospital. With this regime, despite a reticulocyte response with a peak on the 17th hospital day, his Hb concentration remained constant. By the 28th hospital day the marrow had improved slightly to a grade 3 megaloblastic pattern of erythropoiesis. Because his serum folate concentration was not rising, on the 30th hospital day the amount of folic acid given by injection was doubled. This resulted in a further small reticulocyte peak and the bone marrow on the 40th day had improved to a grade 2 megaloblastic pattern, but there was little change in the Hb concentration and the serum vitamin B₁₂ concentration was stationary at about 70 ng/l. On the 41st day the diet was changed by adding milk and curds so that the total dietary vitamin B₁₂ intake now averaged 0.30 μg/day. After this there was a steady rise of Hb concentration, a delayed reticulocyte response, a rise in serum vitamin B₁₂ concentration which reached a new plateau of about 100 ng/l and the mar-

![Graph](https://via.placeholder.com/150)

**FIG. 2.** Hematological response of patient 2 (Th). One-hundred milligrams of iron was given thrice daily during his stay in hospital. • transfusion; marrow 4, 3, 2, 1 grades of megaloblastosis; N, normoblastic; •••••• serum vitamin B₁₂; ——O—O, serum folate; •••••• Hb; ——O—O, reticulocytes.
row became normoblastic. Before discharge, the patient was given 500 μg of vitamin B₁₂ by injection and instructed regarding his diet. He has been seen periodically since then and has remained well.

**Comment**

The role of folate deficiency in this patient's anemia is difficult to assess. Although he developed a reticulocyte response when the amount of folate given parenterally was increased from 100 to 200 μg/day, his serum folate was not very low and his red cell folate was in the normal range. With an intake of 0.07 μg of vitamin B₁₂ daily in the diet there was no evidence of a rise in Hb. The marrow improved from grade 4 to grade 2 megaloblastic pattern, but whether this was because of the folate or the vitamin B₁₂ cannot be determined. As soon as the dietary intake of vitamin B₁₂ was increased to 0.30 μg daily, there was an increase in Hb concentration, a further rise in serum vitamin B₁₂, and a reversion of the marrow to a normoblastic pattern of erythropoiesis.

In this individual, 0.07 μg of vitamin B₁₂ in the food produced little response and was clearly inadequate; however, 0.3 μg/day was sufficient to produce normalization of the bone marrow and establish a reasonable Hb concentration, even though at this level of intake the serum vitamin B₁₂ concentration remained suboptimal.

**Patient 3**

Mu., a 44-yr-old male laborer, complained of increasing weakness and fatigue for the previous 3 months. He was a vegetarian, but consumed a few ounces of buttermilk two or three times per week.

Physical examination revealed no abnormality apart from pallor. The initial investigations are shown in Table 1 (patient 3a). Serum iron was 72 μg/100 ml and unsaturated iron binding capacity 244 μg/100 ml. He was put on a standard low vitamin B₁₂ diet which was calculated to contain about 0.1 μg of vitamin B₁₂ per day, but the actual content was not measured on this occasion. As there was no hematological response (Fig. 3A), on the 12th hospital day onward he was given a daily injection of 1 μg of cyanocobalamin. This produced a prompt hematological response, the reticulocytes rising to 39% on the 8th day of parenteral therapy. However, the rate of rise in Hb concentration was not quite optimal, probably due to the development of concomitant iron deficiency, the se-
rum iron on day 30 having fallen to 42 μg/100 ml and the unsaturated iron binding capacity having risen to 320 μg/100 ml. Serum folate remained within the normal range throughout.

On the 47th hospital day he was discharged and advised to take a course of iron tablets and to take regular vitamin B₁２ supplements.

Five years later, at the age of 49, Mu returned again complaining of symptoms of anemia which he had noticed for the previous 3 months. He was vague about how much treatment he had had after his previous admission, but he had not had any medication for at least several years. His dietary habits had not changed since his previous admission.

Physical examination showed pallor, a hyperdynamic circulation, and an ejection systolic murmur in the heart. The initial laboratory investigations are shown in Table 1 (patient 3b). He was given a diet that contained an average of 0.16 μg of vitamin B₁₂ per day as measured by microbiological assay. There was a reticulocyte response of 10% on the 10th day and a very slow rise in Hb concentration (Fig. 3B). Even though the red cell folate was normal, it was believed that the low serum folate concentration might be inhibiting the response to food vitamin B₁₂, so an injection of 200 μg of pteroylglutamic acid was given every day from the 42nd day onward. However, after the commencement of the folate injections there was no reticulocyte response or change in marrow morphology. On the 61st hospital day the diet was changed to include more milk and curds and thereafter he received an average of 0.39 μg of the vitamin per day. This was followed by a small reticulocyte peak, the Hb concentration increased, the bone marrow returned to a normoblastic pattern of erythropoiesis and the serum vitamin B₁₂ concentration rose to about 100 ng/l. Throughout the period of treatment, the serum iron concentration remained within normal limits. The patient was finally given an injection of 500 μg of hydroxocobalamin and instructed regarding necessary changes in the diet. He was seen again 2 yr later when he remained well.

Comment

At his first admission the patient showed no response to a diet thought to contain about 0.1 μg, but a prompt response to the daily parenteral administration of 1 μg. The serum vitamin B₁₂ concentration on this regime rose and plateaued at between 200 and 300 ng/l. On the second admission he showed a slight response to a diet containing an average of 0.16 μg of vitamin B₁₂ per day and his serum vitamin B₁₂ concentration plateaued at about 50 ng/l. However, the bone marrow only became normal when the intake was increased to 0.39 μg/day. This increased intake of vitamin B₁₂ also produced a slight secondary reticulocyte response and the serum vitamin B₁₂ increased to over 100 ng/l.

Patient 4

Nu., a 35-yr-old housewife, presented at the hospital complaining of anorexia, increased pigmentation, and puffiness of the face of 6 months duration. During this time her diet had been completely vegetarian although before the onset of her symptoms she had very occasionally eaten small amounts of meat or fish.

Physical examination showed no abnormality apart from pallor and hyperpigmentation. Initial hematological investigations are shown in Table 1. Gastroenterological investigations were all normal. She was given a diet containing an average of 0.45 μg of vitamin B₁₂ per day (Fig. 4). On the 14th day there was a small reticulocyte peak followed by a gradual rise in Hb concentration and some improvement in bone marrow morphology but only a slight change in serum vitamin B₁₂ concentration. The dietary intake was therefore increased by the addition of curds, so that an average of 0.55 μg of vitamin B₁₂ was ingested each day. With this intake the Hb concentration continued to rise, the marrow became normoblastic, and the serum vitamin B₁₂ concentration rose to 100 ng/l. Serum iron and folate concentrations remained in the normal range throughout. At this stage she was given instructions regarding her diet and discharged.

Comment

This woman showed a gradual response to a dietary intake of 0.45 μg of vitamin B₁₂ per day. When the intake was increased to 0.55 μg/day there was no further reticulocyte response and it seems probable that a normoblastic bone marrow and a reasonable Hb
Patient 5

Iu., a 24-yr-old housewife, was admitted withamenorrhea increasing weakness, jaundice, and swelling of the feet that she had noticed for the previous 5 months. She ate a vegetarian diet but consumed a little curd or buttermilk two or three times per week.

Examination showed marked pallor, icterus, pedal edema, patchy hyperpigmentation of the skin, and free fluid in the peritoneal cavity. Initial investigations are shown in Table 1. She was given a diet that contained an average of 0.25 μg of vitamin B$_{12}$ daily. Her Hb and hematocrit fell further so that on the 10th hospital day she required a transfusion of packed red cells (Fig. 5). Because her Hb concentration did not rise and her general condition was deteriorating, another packed cell transfusion was given on the 22nd hospital day. Although the serum vitamin concentration had risen slightly and there was a slight increase in reticulocytes, it was apparent that the amount of the vitamin concentration might well have been achieved with the original intake of 0.45 μg.

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she was receiving was inadequate. On the 23rd hospital day, her diet was therefore changed to one that provided an average intake of 0.65 μg of vitamin B₁₂ per day. This was followed by a sustained increase in Hb concentration, a delayed suboptimal reticulocyte response that was maximal on the 34th hospital day, 11 days after the change in the diet, and reversion of the bone marrow to a normoblastic pattern of erythropoiesis. Her serum vitamin B₁₂ concentration rose to 70 ng/l. As her Hb concentration rose, her jaundice, fluid retention, and hyperpigmentation disappeared. Serum iron and folate concentrations remained in the normal range throughout. She requested discharge on the 52nd day and was given an injection of 1000 μg of hydroxocobalamin and instructed regarding appropriate changes in her diet.

Comment

During the first 10 days in hospital this woman was not able to absorb enough vitamin B₁₂ from the 0.25 μg present in the food to prevent further fall in Hb concentration. However, after the first transfusion, her Hb concentration did not drop to the same extent as previously and on the 17th hospital day there was a slight reticulocytosis. If the same vitamin B₁₂ intake had been continued longer it is possible she might have shown further slow improvement. When she was given a diet that contained 0.65 μg of vitamin B₁₂ per day she showed a good hematological response indicating that this amount of the vitamin may have been more than adequate.

Discussion

The minimal daily dietary requirement of a given nutrient, for an individual, may be defined as that amount which must be ingested each day to maintain that individual in a state of health. This amount will depend on a number of factors, such as the metabolic requirements and the losses from the body on the one hand and the efficiency of the absorptive processes on the other. In a healthy population the minimal individual daily requirement of a given nutrient will form a range, the frequency distribution of which will often be Gaussian (13) or, as in the case of iron in women, skewed (14). The recommended daily intake of any nutrient for a population has been defined as “the daily amount considered to be sufficient for the maintenance of health in nearly all people” (15) (i.e., that amount which will cover the minimal daily requirement of all but the extreme right hand side of the frequency distribution curve). Although this is a relatively simple concept, with vitamin B₁₂, as with many nutrients, problems arise when an attempt is made to critically define it.

One approach is to determine how much vitamin B₁₂ is needed to maintain body stores constant in apparently healthy individuals. By radioisotopic tracer studies it has been shown that the rate of loss of vitamin B₁₂ from the body is exponential, 0.1 to 0.2% of the body stores being lost each day (1, 16–20). This means that the bigger the stores the greater are the absolute daily losses of the vitamin and the bigger the amount that must be ingested and absorbed each day to maintain the stores constant. On this basis, Gräsbeck (1) has estimated the requirement of vitamin B₁₂ as being in the region of 10 μg/day. However, body stores of the vitamin differ widely. Among nonvegetarian English subjects total stores are estimated to range from 1 to 5 mg (6, 21) and in vegetarian subjects they are much lower (22). There is presently no evidence that an individual with higher body stores is any more “healthy” than someone with lower body stores, except that the latter will develop overt vitamin B₁₂ deficiency sooner than the former if absorption of the vitamin is interfered with.

A more rational way of arriving at the recommended intake would be to determine the minimal amount which must be present in the diet to prevent the appearance of megaloblastosis, anemia, or other manifestations of vitamin B₁₂ deficiency. However, to determine this directly would require a very long-term prospective study of a number of individuals on controlled dietary intakes—an approach applicable to experimental animals but not to humans. An approximation to this has been attempted by studying individual subjects with vitamin B₁₂ deficiency anemia and endeavoring to determine the minimal amounts of the vitamin that are needed to improve or cure their hematological abnormalities.
Darby et al. (23) obtained good, although hematologically suboptimal responses to the daily parenteral administration of 0.25 μg of cyanocobalamin in seven patients with pernicious anemia. Long-term observation of these patients led these authors to suggest that their minimal daily requirement of the vitamin was 0.5 to 1.0 μg when given parenterally or 0.6 to 1.2 μg in the diet. Sullivan and Herbert (2) obtained suboptimal hematopoietic responses to 0.1 μg of parenterally administered vitamin B₁₂ in six of eight patients with pernicious anemia in relapse. The two patients who showed no response had infections and the authors postulate that this may have suppressed the response to minimal therapy. The authors termed this dose (0.1 μg) "the minimal effective daily dose of vitamin B₁₂" and suggested that "the minimal adult daily requirement is in that range". In these six patients the administration of 0.1 μg of the vitamin clearly produced a hematological response, but since in all the bone marrow was still megaloblastic when the experiments were terminated, it is not known whether these individuals would have remained healthy with no manifestations of vitamin B₁₂ deficiency if the administration of this amount of vitamin had been maintained indefinitely.

Information derived from patients with pernicious anemia in relapse (or our patient 1) must be interpreted with caution. The vitamin has to be given parenterally and, because there is very little information regarding the availability of vitamin B₁₂ in different foodstuffs, it is difficult to interpret the results in terms of vitamin B₁₂ in food. Furthermore, since such individuals lack intrinsic factor, they cannot absorb the vitamin B₁₂ which is normally excreted in the bile, resulting in greater losses and higher requirements than in normal subjects.

Winawer et al. (24) describe a 64-year-old American vegan, whose diet was said to be "free of vitamin B₁₂" although it does not appear to have been assayed. No response was observed to an oral supplement of 0.2 μg of vitamin B₁₂ but a prompt rise in reticulocytes and serum vitamin B₁₂ concentration occurred when the supplement was increased to 1 μg, suggesting that the latter was more than adequate therapy. Stewart et al. (25) describe a south Indian woman in London who developed a vitamin B₁₂ deficiency megaloblastic anemia while consuming a vegetarian diet that provided 0.5 μg of the vitamin per day. This patient showed an optimal hematological response when an additional 1 μg of cyanocobalamin was given by mouth. However, there was no attempt made to determine the smallest addition needed to her intake of 0.5 μg, so that it is not possible to estimate her minimal dietary requirement any more precisely.

The present investigation studied the response to different amounts of parenteral vitamin B₁₂ in patients 1 and 3a, and of dietary vitamin B₁₂ in patients 2 to 5. The results are perhaps best summarized in tabular form (Table 2). Column A represents amounts that are judged as being inadequate to maintain health in that the Hb concentration was falling or remaining stationary, the serum vitamin B₁₂ concentration was below 100 ng/l and usually below 80 ng/l and the marrow remained megaloblastic. Column B represents the amount that was judged adequate in that on these intakes the Hb concentration rose, the serum vitamin B₁₂ concentration rose, usually to above 100 ng/l, and the bone marrow became normoblastic. It seems a reasonable assumption that if these latter intakes had been continued indefinitely the subjects would have maintained good health without any manifestations of vitamin B₁₂ deficiency. It is also possible that some of these intakes may have been higher than the minimal amounts needed to maintain health. This is particularly so in patient 3a where 1 μg of parenteral therapy daily produced an almost optimal hematopoietic response. In pa-
tient 4 there was little detectable benefit obtained by increasing the intake from 0.45 to 0.55 μg and in patient 5 the brisk reticulocyte response to 0.65 μg/day suggests that a smaller amount may well have produced similar results. It should be noted that the serum vitamin B₁₂ concentration did not reach the usually accepted normal range of 140 ng/l or more, except in patients 1 and 3a where the vitamin was given parenterally. However, in patients 3b, 4, and 5 the serum vitamin B₁₂ concentration had not reached a plateau when the patients were discharged and it is possible that the concentrations would have risen higher if the experiment had been continued longer. Moreover, serum vitamin B₁₂ concentrations below 140 ng/l are not infrequently found in otherwise apparently healthy subjects (26). It may be concluded that in the patients in the present study, a dietary intake of 0.5 μg of vitamin B₁₂ would probably have been adequate to maintain them free of manifestations of vitamin B₁₂ deficiency.

It is of interest that measurements of the amount of vitamin B₁₂ actually eaten by apparently healthy south Indian villagers show the daily intake to be in the range of 0.3 to 0.5 μg (S. J. Baker and V. I. Mathan, unpublished observations). Subject 2 in the present study ate a very poor diet due to poverty, but subjects 3, 4, and 5 ate a diet that was not significantly different from that of the majority of the population who do not develop vitamin B₁₂ deficiency megaloblastic anemia. These subjects therefore presumably came from the right hand side of the frequency distribution curve of minimal daily requirements.

It has recently been shown that some bacteria isolated from the small intestine of south Indian subjects can produce vitamin B₁₂ in vitro (27). It has also been shown that free intrinsic factor may be present in the jejunum and ileum (28). If small bowel bacteria produce vitamin B₁₂ in vivo it may combine with free intrinsic factor and be available for absorption, thus reducing the amount of dietary vitamin B₁₂ that has to be ingested to meet metabolic requirements. Unfortunately there is no easy way of determining whether endogenous production of the vitamin is occurring and, if so, its extent. This therefore remains an unknown factor in studies of this type. It is possible that decreased endogenous production of vitamin B₁₂ in cases 3, 4, and 5, as compared with the general population, could be one explanation of why these subjects developed vitamin B₁₂ deficiency while others eating a similar diet, do not.

Another possible explanation for the development of vitamin B₁₂ deficiency in subjects 3, 4, and 5, is that while in each, the absorption of a test dose of radioactive vitamin B₁₂ was within the normal range, absorption of food vitamin B₁₂ may have been abnormal. Such a situation has been demonstrated by Carmel (29) in a 52-yr-old man with vitamin B₁₂ deficiency, who had a normal Schilling test, but a "subnormal" absorption when the test dose was given with ovalbumin (30). However, if this be so, it would tend to make our estimates of the daily dietary requirement for healthy individuals too high rather than too low.

The WHO recommended dietary intake of vitamin B₁₂ for adult males or nonpregnant females is 2 μg/day (31). From the previous studies of Sullivan and Herbert (2), and Darby et al. (23) and from the present study, it would appear that this recommendation could well be reduced to 1 μg/day, and still allow an adequate margin of safety.

The painstaking work of Mr. S. P. Swaminathan and the other technical staff, and the nursing and dietetic staff of the Unit, which made this study possible, is gratefully acknowledged.

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