

Serum Vitamin B₁₂ Concentration in Dietary Deficiency

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MANY farmers in the villages of Iran are very poor, subsisting chiefly on a diet of whole wheat bread, tea and sugar. Some of them rarely, if ever, consume meat, chicken, eggs or other animal protein foods which are the source of vitamin B₁₂. Others are able to obtain these foods in small although regular quantities. The daily caloric intake from a diet consisting exclusively of wheat is estimated at approximately 2,400, with about 90 gm. of protein. However, vitamin B₁₂ is not present in cereals or other vegetable products. The purpose of this investigation was to determine whether the serum vitamin B₁₂ concentration was unusually low in these persons.

METHODS

Two groups of villagers were studied. The first group subsisted on a diet deficient in animal protein. They ate whole wheat bread almost exclusively with a small amount of milk product called mast, similar to yogurt, once or twice a week, and a little meat or chicken not more than once a month.¶ Many of this group had even less animal protein than

this, some had none at all. The second group subsisted on a diet which contained meat, chicken or eggs as well as mast in regular amounts, at least every other day. There were twenty-three subjects in each group; all were patients in the Saadi Hospital, a free government institution. None had hepatic disease, renal insufficiency, gastrointestinal disease or leukemia, each of which might result in abnormalities in vitamin B₁₂ metabolism and serum levels. The hemoglobin in all patients was at least 11 gm. per 100 ml. Blood samples were obtained at mid-morning, the serum being separated after clotting, and frozen. When all the samples were collected they were sent by air mail to Syracuse, New York, with an elapsed time of eight days. There the vitamin B₁₂ concentration was determined using *L. leichmannii* as the test organism. The personnel and technic in this laboratory were the same as in a previous study of vitamin B₁₂ serum concentration in various chronic disease states.¹ It was determined that the vitamin B₁₂ level of serum frozen for ten days, then exposed to room temperature for ten days did not change significantly. This would simulate conditions obtaining in the present study, which was carried out during the winter months.

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¶ Five samples of wheat from different villages in the area were assayed for vitamin B₁₂ using *L. leichmannii*. These samples showed no vitamin B₁₂ activity but did show desoxyribose activity of 55, 22, 55, 46 and 52 µg. per gm., respectively, in terms of thymidine. Because this desoxyribose activity interferes with vitamin B₁₂ activity in the *L. leichmannii* assay, the five samples were assayed with *Ochromonas malhamensis*. These assays showed no vitamin B₁₂ activity in any of the samples.

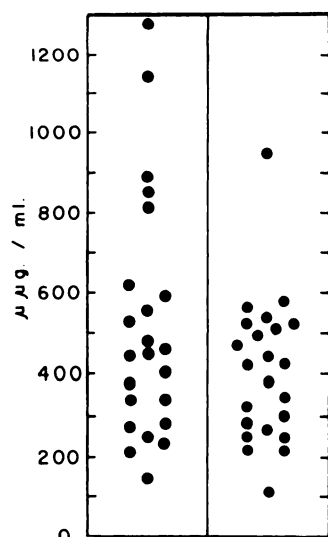


FIG. 1. Serum vitamin B₁₂ concentration in twenty-three persons with an adequate diet (*left*) and twenty-three without an adequate diet (*right*).

RESULTS

Figure 1 shows the results of the serum vitamin B₁₂ levels in the two groups. The mean level in the group with an "adequate" diet was 518 μg . per ml. with a range of 133 to 1,300, and a standard deviation of 297. The mean level in the group with an "inadequate" diet was 411 μg . per ml. with a range of 117 to 960 and a standard deviation of 167. There was no significant difference in the two groups ($t = 1.5$, $p = 0.1-0.2$).

COMMENTS

Although malnutrition is prevalent among the villagers of Iran who make up the bulk of the patient population of the Saadi Hospital, we have not seen any patient with megaloblastic anemia caused by dietary deficiency. The serum vitamin B₁₂ concentration in these villagers who were the subjects of the present study did not differ significantly from that reported in 333 normal subjects from Syracuse, New York.¹ Furthermore, there was no significant difference between the group subsisting on a diet extremely low in animal protein from those whose diets contained adequate amounts. Animal protein is the only source of dietary vitamin B₁₂.

These findings indicate that vitamin B₁₂

deficiency is not likely to occur even on a diet with extremely inadequate vitamin B₁₂ intake. Three possibilities come to mind which might explain this situation

The first possibility is that the small amount of vitamin B₁₂ present in the diet of the group on an inadequate intake was actually sufficient for the body requirement. This is estimated at 0.5 to 1 μg . per day. A group of extremely strict vegetarians known as Vegans has been studied from the standpoint of vitamin B₁₂ deficiency in England. Although megaloblastic anemia was not observed, presumably due to a high folic acid content in their food supply, neurologic lesions and abnormally low serum vitamin B₁₂ levels were present.²

A second explanation could be that the bacterial flora of the intestine in the patients studied might differ from the usual owing to the high carbohydrate cereal diet. Under these circumstances organisms capable of synthesizing vitamin B₁₂ might ascend into the ileum. Although vitamin B₁₂ is not absorbed from the colon it can be absorbed from the ileum.³ Very little information is at hand respecting the bacterial flora of the small intestine. Although it is presumed to be sterile under normal conditions, the bacterial flora of the gut has not been studied adequately, especially in relation to various types of dietary intake. Some bacteria are known to synthesize vitamin B₁₂, and others require it for their growth. For example, Foy et al.⁴ have found that non-addisonian megaloblastic anemia which occurs in Africans on inadequate diets may respond to penicillin given orally. These subjects have very low serum vitamin B₁₂ levels. They speculate that in these subjects the diet encourages the growth of bacteria competing for vitamin B₁₂, but that other subjects without anemia depend on bacterial synthesis for their supplies of vitamin B₁₂, folic acid and perhaps some essential amino acids.

The third possible explanation for the lack of vitamin B₁₂ deficiency in the presence of such inadequate diets is that the villagers literally live with their animals (donkeys, cows, sheep and goats), because they are driven into the village compound at night. The yards of the village are constantly littered with manure

and it is not difficult to imagine that the inhabitants might ingest a certain quantity of it through such close contact. Feces of ruminant animals contains large amounts of cobalamins including cyanocobalamin (vitamin B₁₂).

SUMMARY

Serum vitamin B₁₂ concentrations in twenty-three villagers of Iran consuming a diet extremely low in animal protein and presumably in vitamin B₁₂ content did not differ significantly from that found in twenty-three villagers who ate animal protein and who presumably received adequate amounts of the vitamin. Possible explanations are offered for this finding. The serum vitamin B₁₂ concentrations were not significantly different from those found in normal persons in the United States. This study indicates that vitamin B₁₂

deficiency caused only by a deficient diet must be very rare.

ACKNOWLEDGMENT

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