Human Dietary Deficiency of Vitamin B_{12}*

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EXPRESSED as percentage of the total dietary calories provided by the animal protein in the diet, the intakes of animal protein range widely in different countries; values vary from as high as 8 in parts of America down to less than 1 in India and

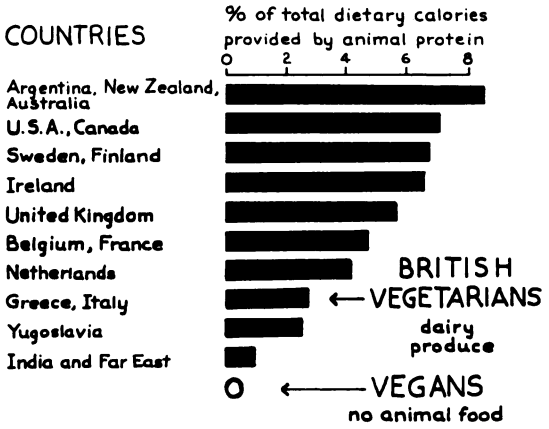

Fig. 1. Post-war animal protein intakes in different countries.

the Far East (Fig. 1). The high animal protein intakes in much of the Western world are indeed only possible because more than half the world's inhabitants are living on much lower intakes. Animal proteins on the whole are much more expensive than vegetable proteins; hence numerous workers have tried to replace animal protein by vegetable protein.[1,2] In Great Britain, during the last hundred years, there have been many thousands of vegetarians, who have refrained from eating flesh foods, though they still consume dairy produce. This has involved a reduction in their intake of animal protein to about half the normal British intake, without any visible ill effects. During the last ten years or so, some hundreds of these vegetarians have eliminated dairy produce from their diet, and called themselves *vegans*. After several years on this diet, which contains no animal protein, definite illnesses have gradually developed in some but not all of these vegans.[3,4] These illnesses are apparently due to a dietary deficiency of vitamin B_{12}.

Staple foods containing animal protein usually also contain vitamin B_{12}. On the other hand, this vitamin seems to be very scarce in vegetable foods, having so far been detected only in one or two, such as seaweeds[5] and ground nuts,[6] which are not consumed by British vegans. Their diets must therefore have been deficient in vitamin B_{12} and it is not surprising that their deficiency symptoms have been alleviated by administration of the vitamin.

Composition of Reported Vegan Groups

Before describing the occurrence of the deficiency symptoms in the group of British vegans, the composition of this group should be compared with that of a group of Dutch vegans recently studied at Leyden[7] and that of a smaller group of American vegans[8] (Table I). In both the Dutch and American vegans deficiency symptoms were much less in evi-

TABLE I

Composition of Groups of Vegans Studied

		Children	Adults
		0–18 years	19–83 years
American	males	2	14
	females	–	11
Dutch	males	–	39
	females	–	20
British	males	13	54
	females	19	63

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dence than among the British vegans. The British groups, however, contained more young people, who are probably more susceptible to vitamin B_{12} deficiency. The Dutch group contained four women and nine men aged 33 to 69 years who had been vegans for 2 to 15 years, 15 who had been vegans for 6 to 18 months, and 32 vegetarians who had lived on the vegan diet for short periods ranging from several months to a year.

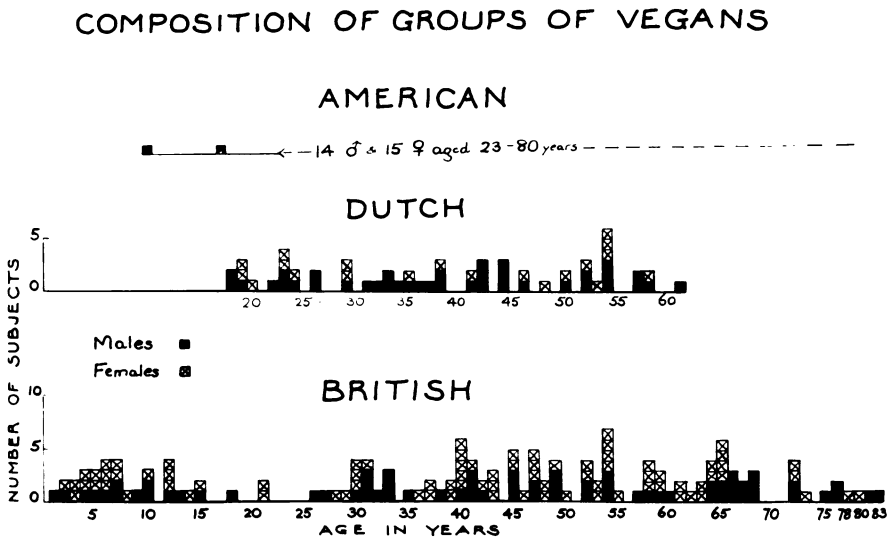

Figure 2.

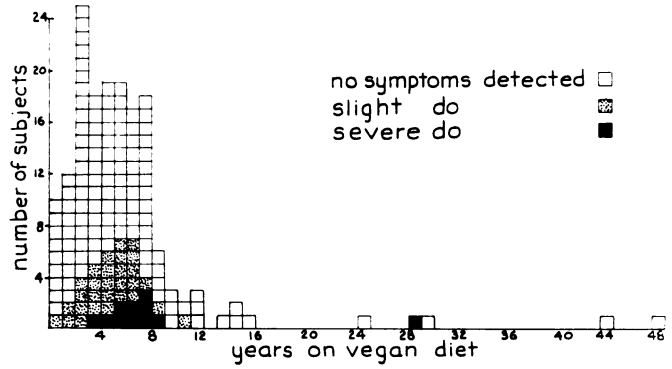

Fig. 3. Health in relation to period of veganism.

Health and Duration of Veganism

Another probable reason for the higher incidence of deficiency symptoms among the British vegans was their longer period of veganism. Even slight symptoms usually did not become prominent until they had been on the vegan diet for several years, and the more serious symptoms took still longer to develop (Fig. 3). Quite a number seemed to escape definite symptoms, even after more than five years on the diet. Children were rather more susceptible than adults, but some of them have continued to grow and develop satisfactorily for several years. The vegan mothers who have been more successful in rearing their children on the vegan diet often breast fed them for longer periods than usual.

Rate of Development of Deficiency Syndrome

The commonest and earliest symptoms have

been in the mouth; sore tongues were especially common. These *oral symptoms* generally cleared up after a month or two. *Paresthesia* was another fairly common symptom which developed more slowly and persisted rather longer. *Amenorrhea* and menstrual dis-

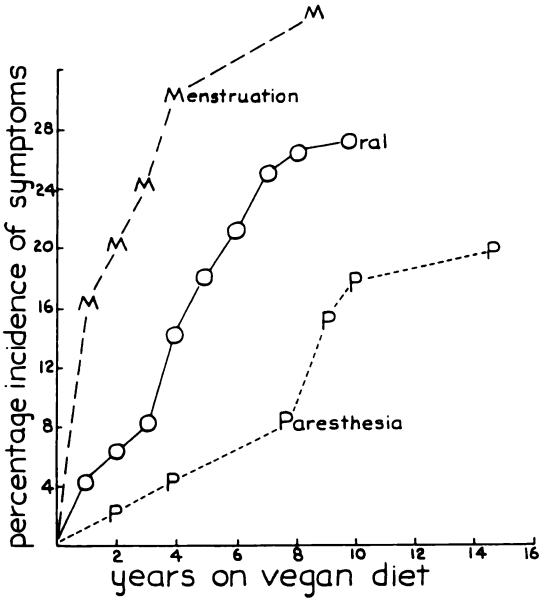

Fig. 4. Rate of development of different deficiency symptoms in vegans.

turbances were encountered in eight out of 22 women aged 15 to 45. They were also encountered among the Dutch vegans,[9] and are of interest in view of the fact that the main vitamin B_{12} deficiency symptoms observed in experimental animals are related to the reproductive processes (Fig. 4). *Nervous symptoms* were common among the vegans, but have not been plotted because their subjective nature makes their precise rate of development difficult to determine.

About 20 per cent of the subjects complained of pains in the back and spine. Some of these had stiff "poker" backs, so characteristic that they were termed "vegan" backs. Three deaths occurred, all among vegans of several years' standing. One of these was due to cancer which was present before the vegan diet was adopted. The other two deaths were associated with mental disease.

The percentage incidences of symptoms were lower in the Dutch than in the British vegans, e.g., oral symptoms—Dutch 12, British 27; paresthesia—Dutch 6, British 20. This was probably because 72 per cent of the Dutch vegans on whom these percentages were calculated had been on the vegan diet for less than two years, as compared with an average of five to six years for British vegans.

No clinical deficiency symptoms were observed among the 26 American vegans,[8] all of whom had maintained the animal protein-free diet for five years or more.

It has not yet been possible to arrange for some of the vegans with more serious symptoms to undergo detailed biochemical examination. The biochemical results which follow were obtained on vegans apparently in good health, most of whom had, however, experienced the less serious deficiency symptoms, especially those in the mouth, at some time during their period of veganism.

Serum Vitamin B_{12} Levels and Duration of Veganism

The serum vitamin B_{12} concentrations in the vegans so far examined have ranged from

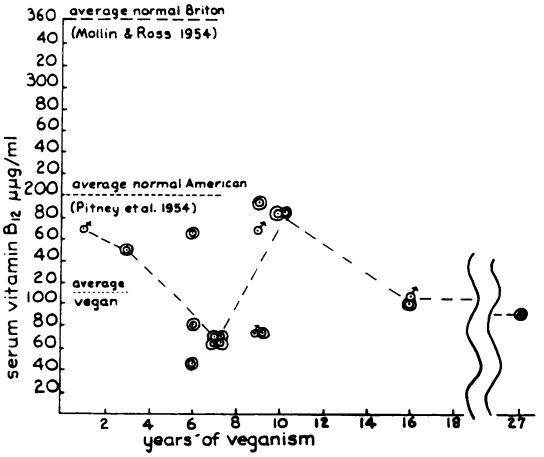

Fig. 5. Serum vitamin B_{12} levels compared with periods of veganism.

45 to 193 $\mu\mu$g/ml, and averaged 111 $\mu\mu$g/ml, as compared with the average normal values of from 200 to 320 $\mu\mu$g/ml obtained by several workers using the same method.[10,11] In more than half the vegans the average concentration was below 120 $\mu\mu$g/ml. In two of these it lay between 120 and 100 $\mu\mu$g/ml, suggestive of vitamin B_{12} deficiency.[12] In

the other nine it was below 100 $\mu\mu$g/ml, indicating definite deficiency, but this did not occur until six years on a vegan diet.

A curve drawn through the average vitamin B_{12} levels for each year of veganism for which data are available shows a steady fall during the first 7 years, followed by a recovery up to the original fairly high value between 7 and 10 years, then a more gradual descent (Fig. 5).

Serum Vitamin B_{12} Levels and Red Cell Counts

The red blood cell counts on some of the vegans indicated a slight anemia. However, correlation of the vitamin B_{12} concentrations with the red cell counts gave an average curve for the vegans which was quite distinct from the average curve in pernicious anemia, and was closer to the vitamin B_{12} levels associated with subacute combined degeneration of the cord (Fig. 6). This condition can be precipitated in vitamin B_{12} deficiency by administration of an excess of folic acid.[13] This might conceivably happen in vegans with low serum vitamin B_{12} levels who ingest large amounts of folic acid in vegetables. One of us[14] has previously described a vegan boy of 15 who developed dietary deficiency of vitamin B_{12} and suffered from classical subacute combined degeneration of the cord. This responded dramatically to treatment with vitamin B_{12} alone.

Serum Vitamin B_{12} Levels and Hemoglobin Levels

The hemoglobin levels in the vegans showed even less deviation from normal than had been shown by the red cell counts (Fig. 7). This might have been partly explained

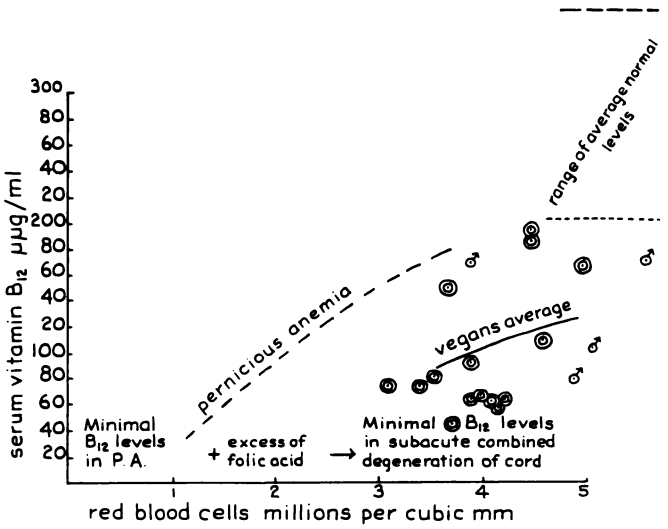

Fig. 6. Serum vitamin B_{12} levels and red blood cell counts in vegans.

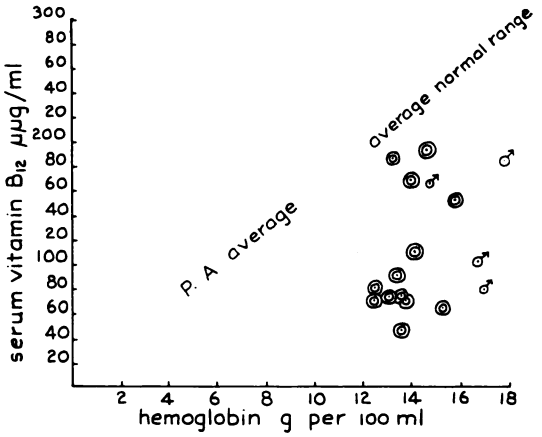

Fig. 7. Serum vitamin B_{12} levels and hemoglobin levels in vegans.

by an increase in the size of the red cells, such as was found by determination of their average diameters in the Dutch vegans. However, the mean corpuscular volume showed a

definite increase above the normal value, comparable with the increase found in the Dutch vegans.

No correlation was established between the serum vitamin B_{12} levels and any of the other blood values measured, which included plasma Fe, platelet, leukocyte and reticulocyte counts, and erythrocyte sedimentation rate.

Tyrosyl Derivatives and Serum Vitamin B_{12} Levels

The deficiency symptoms observed in the nervous systems of some of the vegans might have been due to chronic toxicity caused by a keto-acid such as parahydroxy phenylpyruvic acid (PHPP), which is derived from tyrosine, and in the metabolism of which vitamin B_{12} may be implicated because of its relationship to thyroid activity (Fig. 8).

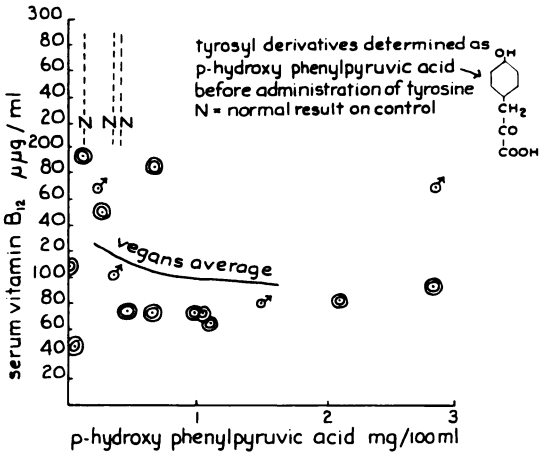

Fig. 8. Serum vitamin B_{12} levels and tyrosyl derivatives in vegans.

The concentration of PHPP was therefore determined in the blood and in the urine of the vegans, and in controls on normal diets, before and after oral administration of tyrosine and glucose (54 mg and 1.8 g, respectively, per kg body weight). The initial concentrations, before administration of tyrosine, tended to be higher in the vegans with lower serum vitamin B_{12} levels.

The concentration of the tyrosyl derivative PHPP in the blood appeared to increase with the period of veganism up to the first 6 to 10 years, after which it may level out, but many more data will be needed to confirm these findings (Fig. 9).

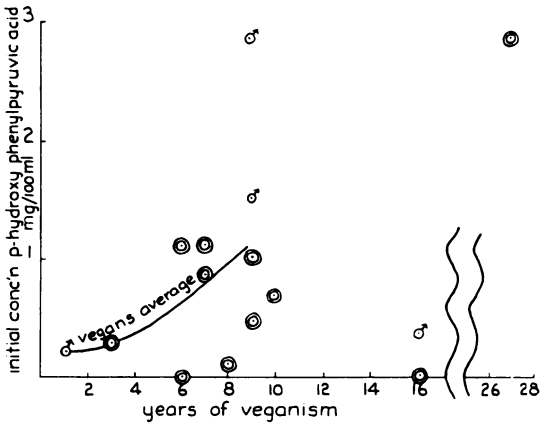

Fig. 9. Serum tyrosyl derivatives and period of veganism.

Cyanide and Thiocyanate Metabolism and Vitamin B_{12}

Another possible toxic agent is cyanide, which can cause demyelination in the central nervous system.[15] In the liver, cyanide is detoxicated by the enzyme rhodanese to thiocyanate, which is excreted in the urine. A measure of this detoxication is therefore provided by the urinary thiocyanate (Fig. 10).

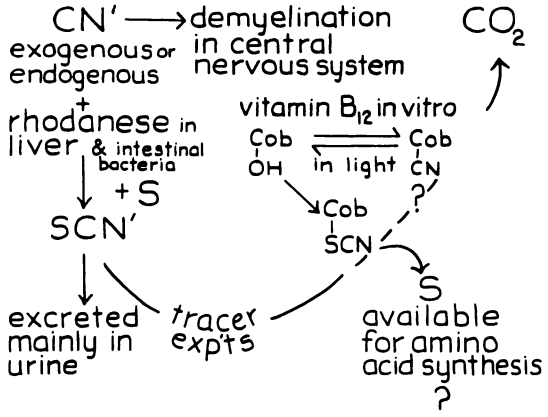

Fig. 10. Cyanide and thiocyanate metabolism and vitamin B_{12}.

Tracer studies[16] have shown that some cyanide is converted to carbon dioxide in the expired air. It may perhaps perform an essential function by acting as a "brake" on

biological oxidations. Vitamin B_{12} as hydroxocobalamin also can detoxicate cyanide, and serve as a carrier of cyanide and thiocyanate. Vitamin B_{12} has not yet been found in rhodanese, though closely associated with it in different organs and organisms. However, the urinary thiocyanate which is derived from cyanide may be associated with vitamin B_{12} in certain hypothetical systems which are discussed in detail elsewhere.[17]

The urinary excretion of thiocyanates may be increased by heavy smoking[18] or heavy consumption of beer or strong tea, or milk, eggs, and other sources of animal protein, all of which contain preformed thiocyanate.[4,19] None of these factors was significant in the vegans we have studied. Although cyanides and thiocyanates may be obtained from some raw vegetables such as cabbages,[20] which vegans sometimes eat, we did not detect significant amounts in samples of their diet, and therefore believe that the thiocyanate they excreted was mainly endogenous in origin.

Urinary Metabolic Data on Vegans and Non-vegans

The average daily excretion of thiocyanates per kg body weight was 135 μg among the vegans and 96 among the non-vegans, all being teetotal non-smokers. The vegan rate of excretion was 141 per cent of the non-vegan rate, but the difference was not significant, because complete 24-hour samples could not always be obtained to eliminate errors caused by the daily rhythm. This rhythm in thiocyanate excretion is, however, more or less parallel with the rhythm in total nitrogen excretion. By determining the thiocyanate/total nitrogen ratio, expressed in μg and mg, respectively, the standard error is much reduced, and complete collection of 24-hour samples is avoided.

The average thiocyanate/total nitrogen ratio in the vegans was 188 per cent of that in the non-vegans. However, part of this difference was due to the average total nitrogen excretion being lower in the vegans. When allowance was made for this, the corrected thiocyanate excretion in the vegans was still appreciably higher than in the non-vegans (Table II).

The average daily excretion of creatinine was significantly lower in the vegans. No creatine was detected. The Dutch vegans also had a lower creatinine excretion than the Dutch controls.

TABLE II

Urinary Metabolic Data on British Vegans and Non-vegans

	Average daily excretion (per kg body weight) of			
	SCN' μg	Total N_2 mg	Creatinine mg	Average SCN' ratio
Vegans*	135 ± 31	132 ± 15	17.3 ± 1.2	0.94 ± 0.05
Non-vegans*	96 ± 8	185 ± 27	27.1 ± 1.3	0.526 ± 0.01
Vegans as % of non-vegans	141	71	64	188

* All teetotal non-smokers.

SCN' ratio $= \frac{\text{SCN in } \mu g}{\text{total } N_2 \text{ in mg}}$ was determined on 181 vegan samples and 178 non-vegan samples.

Serum Vitamin B_{12} Levels and Thiocyanate Ratios

Turning to individual results, it has not yet been possible to establish a definite correlation between serum vitamin B_{12} levels and thiocyanate ratios in vegans, though there seems to be a tendency for lower vitamin B_{12} levels to be associated with higher thiocyanate ratios. Even after correction for the lower nitrogen excretions in the vegans, most of their thiocyanate ratios were above the normal range for teetotal non-smokers. Large doses (0.5 to 4 mg) of vitamin B_{12} can reduce the excretion of thiocyanates in both vegans and non-vegans (Fig. 11).

In one of the vegans the thiocyanate ratio increased from 1.02 before administration of tyrosine to 2.08 two hours later. In previous experiments without tyrosine, this vegan had shown an average ratio of 0.88; it seemed possible that administration of tyrosine might under certain conditions increase the excretion of thiocyanates. We have therefore carried out on each of the vegans who had received tyrosine a further experiment in which samples were taken of all the urine passed for at least 24 hours, no tyrosine being admin-

istered. The average thiocyanate ratio in each of these experiments was compared with the ratio obtained on the same vegan after tyrosine. The results indicated a stimulating effect of tyrosine on thiocyanate excretion in two of the other vegans. One of these had marked macrocytosis with serum vitamin B_{12} levels of 45 to 80 $\mu\mu$g/ml; this subject also had amenorrhea of several years standing and had experienced considerable loss of body weight. The other had slight macrocytosis with more normal serum vitamin B_{12} levels.

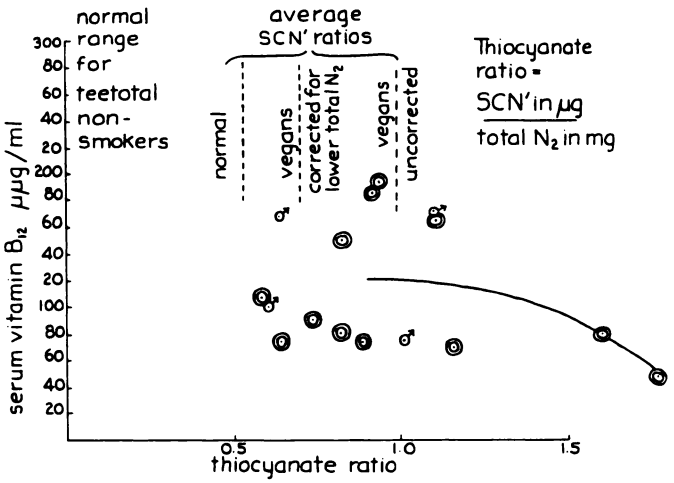

Fig. 11. Serum vitamin B_{12} levels and thiocyanate ratios in vegans.

Comparison of British, Dutch, and American Vegans

As this investigation is concerned primarily with vitamin B_{12}, we have not undertaken the detailed investigation of all the nutrients in the diets of all the vegans, nor endeavored to determine their status in regard to any vitamin except B_{12}. However, some data we have obtained on a few of the British vegans confirm the findings on the Dutch and American vegans, in that the vitamin and mineral intakes seemed satisfactory, except perhaps for calcium and vitamin D, and of course also for vitamin B_{12}. The dietary intake of this vitamin must have been extremely low, and it seems justifiable to assume that the differences in serum vitamin B_{12} values among the British vegans were due largely to differences in the supply of this vitamin from their intestinal flora.

The average body weight of the British adult male vegans was higher than that of the Dutch but lower than that of the American male vegans. In the adult females, body weight of British vegans was lower than that of the Dutch and American vegans. Data still being collected on the British vegan children indicate a subnormal rate of growth.

Unpublished data[21] obtained in a comprehensive study of the rate of growth and development of British vegetarian boys shows this not to be significantly different from that of non-vegetarian boys living under the same conditions. The vitamin B_{12} intakes of the vegetarian boys were no doubt considerably lower than the intakes of the non-vegetarian boys, but would be considerably higher than the vegan boys and girls.

The general blood picture in the British vegans did not differ significantly from that in the Dutch and American vegans. The only clear indication of dietary deficiency was provided by the serum vitamin B_{12} levels (Table III).

The results obtained in this investigation indicate the occurrence in man of a dietary deficiency of vitamin B_{12} similar to that encountered in animals also living on a diet free from animal protein. The poor rate of growth and development in animals on such a diet can be improved by addition of vitamin B_{12}, provided that their diet is not deficient in total protein.

TABLE III

Comparison of British, Dutch, and American Vegans

		British	Dutch	Amer.
% of dietary cal from protein		7*	9	10.4
Av. adult wt (kg)	♂	69.4	65.9	77.3
	♀	53.2	60.0	64.5
Red blood cells	♂	4.92	4.75	4.84
(m/cu mm)	♀	4.06	4.30	4.48
Hb %	♂	16.2	15.1	15.1
	♀	14.0	14.3	13.8
Serum vit. B_{12} $\mu\mu$g/ml		116	–	–

* Calculated by Worrall's method.[22]

The total protein intakes of the vegans must therefore be taken into consideration. This in the American vegans was 10.4 per cent, and in the Dutch long-term vegans about 9 per cent, on the calorie basis. In the British vegans it appears to have been about 7 per cent. This lower protein intake among the British vegans may indeed have been an important factor in causing the greater incidence of deficiency symptoms.

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