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Anaemia and vitamin B_{12} dietary deficiency

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Dietary deficiency of vitamin B_{12} with low serum levels occurred (Wokes, Badenoch & Sinclair, 1955*a,b*) in persons termed 'vegans' (Hill, 1952) after several years on a diet containing no animal food, and therefore probably deficient in this vitamin. This deficiency did not lead to the characteristic blood picture of pernicious anaemia, or indeed to any pronounced macrocytosis, but it was accompanied by marked changes in the nervous system, extending in some instances as far as subacute combined degeneration of the cord (J. Badenoch, 1954; A. G. Badenoch, 1956), and there were two deaths attributed to these changes. However, about half of the vegans escaped serious illness even after many years on the diet, and in some of them normal serum vitamin B_{12} levels indicated a normal vitamin B_{12} status. The wide variation in response to the dietary deficiency suggested significant variations either in dietary intake of the vitamin or in dietary conditions concerned with intestinal synthesis of the vitamin and its absorption from the intestine. These variations could have wide and far-reaching implications in many parts of the world where economic conditions necessitate adherence to diets very low in animal protein. Hence, although the vegans constitute a minute fraction of our population, data on their diets may help in tackling world food problems.

Previous findings with subnormal intakes by man of animal food, particularly animal protein, have been summarized in recent reviews (see Dean, 1953). McCance & Widdowson (1946*b*) at the beginning of World War II carried out on British adults an experimental study of rationing in which the intake of animal protein was gradually reduced, falling below 2% on the calorie basis in two of the subjects, and below 4% in another two subjects, during the last 2 weeks of the 3-month experimental period. These intakes supported severe muscular exertion in persons who normally consume much more animal protein. The diet was adequate in vitamins A, B_1 and C, nicotinic acid, calcium, phosphorus, iron and other minerals.

In an investigation by Widdowson & McCance (1954), 160 German children aged 4–15 years were fed for a year on diets in which the animal protein, half from

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dairy produce, provided only from 1·5 to 1·9% of the total calories. Widdowson & McCance concluded that a balanced diet, containing all the nutrients required for a high rate of growth and development, can be provided with minimal amounts of milk and meat if plenty of wheat and vegetables is available.

In a second investigation (Dean, 1953) on many hundreds of German children, from newborn babies up to children 11 years old, the animal protein provided 3–7%, and the total protein 9–18%, of the total calories. Most of the milk protein could be satisfactorily replaced by the protein of soya and malted cereals, but better growth was obtained with higher intakes of both animal and total protein. Supplements of vitamins A and D were given to almost all the children but the riboflavin content of the diet may have been marginal, depending on the conditions of malting of the cereals (cf. Klatzkin, Norris & Wokes, 1949; British Medical Association: Committee on Nutrition, 1950).

Results on vegetarian children (Widdowson, 1947) and on vegetarian children and adults (Wokes, 1941) indicate an average intake of animal protein, all from dairy produce, about two-thirds of that amongst people on a mixed diet. Most of the vegans had been vegetarians for some years before abandoning the use of dairy produce, but this preliminary period of subnormal vitamin B_{12} intake did not appear to make them more susceptible to vitamin B_{12} deficiency than others who went almost directly from a mixed diet containing meat and high in vitamin B_{12} to a vegan diet of presumably quite low vitamin B_{12} content.

Vitamin B_{12} status of vegans

Microbiological assays on vegan foods showed only slight traces of vitamin B_{12}, e.g. soya beans and peas 0, sprouted peas 1, peanut kernels 0·6, peanut butter 0·9, peanut meal 2·3, Marmite 5 mμg/g respectively, measured by the *Ochromonas malhamensis* method of Ford (1953). The vegans could not have obtained significant amounts of vitamin B_{12} from these foods. However, fermentation or bacterial contamination might sometimes have increased vitamin B_{12} intakes, producing variations in the serum vitamin B_{12} levels in the vegans from 45 to 193 $\mu\mu$g/ml. The average level was near the lower end of the normal range (200–720 $\mu\mu$g/ml.) and well below the average normal level of 358 $\mu\mu$g/ml. (Witts, 1956). The lowest levels were observed in nine vegans who had been on the diet for 6–10 years. In seven of these the signs of deficiency were well marked, whereas only slight signs were observed in two of all those with high vitamin B_{12} levels. Of three long-term vegans, two who had been on the diet for about 16 years were aged 59 and 63, respectively, when their serum vitamin B_{12} levels were found to be about 100 $\mu\mu$g/ml. A third long-term vegan who had been on the diet for 27 years was aged 65 when her serum level was found to be about 90 $\mu\mu$g/ml. The rather low levels in these long-term vegans may, in part at least, have been due to their ages, since Chow (1956) has recently observed that the serum level tends to fall to about half the normal in persons over 60 years of age.

When the effects of the vitamin B_{12} deficiency became apparent (A. G. Badenoch, 1952; Wokes, 1952), warnings were issued to the vegans, many of whom again resorted to dairy produce, especially milk. Others took vitamin B_{12} as a supplement to their diet. Hence, when estimations of serum vitamin B_{12} levels were made in the vegans, only a few remained in whom we could expect values not affected by such supplementation. The results for them are shown in Fig. 1. They fit in satisfactorily with the general picture of the incidence of signs of deficiency earlier observed in about 150 of the vegans.

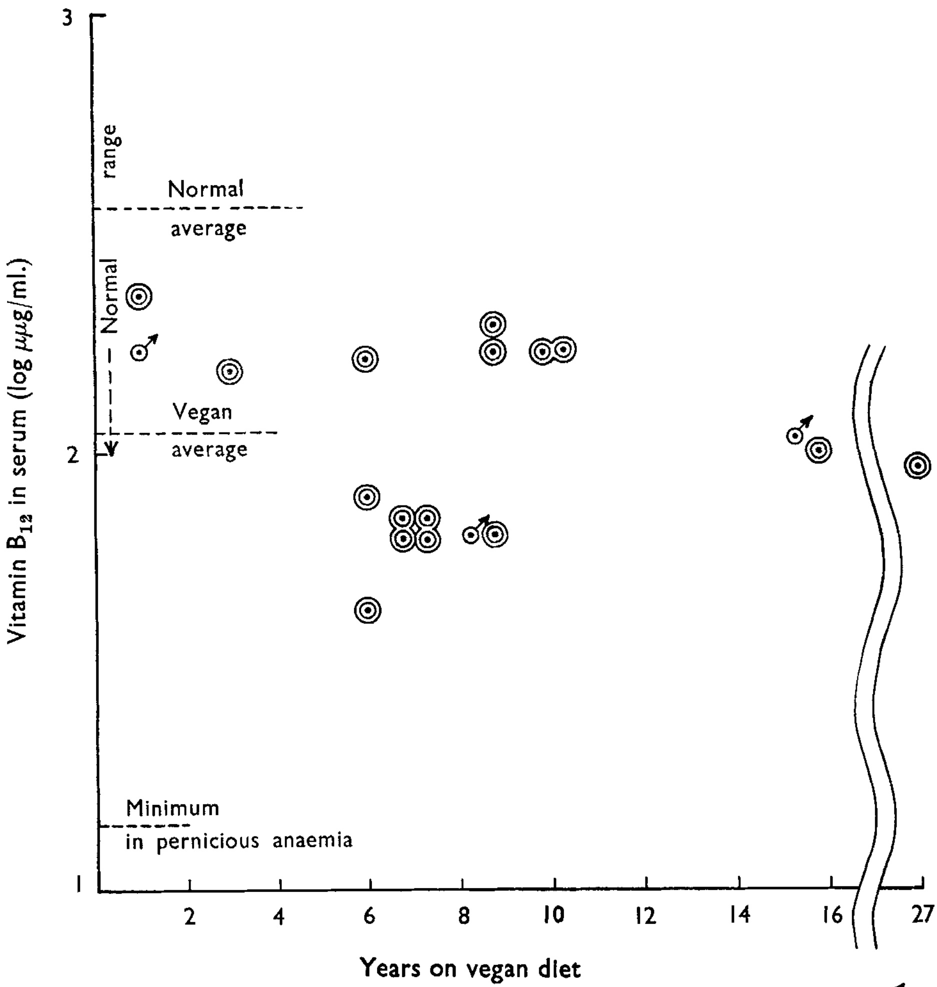

Fig. 1. Effect on serum vitamin B_{12} level of the length of time spent on a vegan diet. ♂, male vegans; ◎, female vegans.

Periodicity, apparently not connected with dietary changes, was observed with some of the signs of vitamin B_{12} deficiency. For example, sore tongues occurred at intervals with intermediate remissions. In a young female vegan, after $3\frac{1}{2}$ years on the diet, a rhythm developed in the body-weight, which, after falling sharply by about 10 kg, ranged from about 48·5 to 51·5 kg with about 2 years between each peak. She also developed amenorrhoea.

Table 1. *Sources of calories in average vegetarian and vegan diets*

Class of foodstuff	Percentage of total dietary calories provided by each class of foodstuff: Vegetarian diet	Vegan diet
Milk	15	None
Cheese	2	None
Eggs	2	None
Cereals	15	16
Potatoes	6	9
Pulses Other vegetables	20	20 4
Fruit Nuts	16	15 12
Sugar, sweets, beverages	11	10
Butter or margarine	11	13
Total	98	99

Sources of protein in vegan diets

Table 1, summarizing results obtained by Wokes (1941) and by Widdowson (1947), shows the general pattern of the vegetarian diet, also of the vegan diet as elucidated by questionnaires sent to individual vegans and to establishments catering for vegans. Change from vegetarian to vegan diet involves mainly replacement of milk and cheese by pulses and nuts, with an increase in the consumption of fruit, but almost no change in the consumption of cereals. On the calorie basis the ratio of pulses to cereals in the vegan diet is 1·25 as compared with about 1·0 in vegetarians. In Indian diets very low in animal protein consumption of cereals is very much higher than that of pulses (Patwardhan, 1956). Pulses contain much less methionine than cereals, and British vegans consuming unusually large amounts of pulses may well have experienced methionine deficiency. It might be partly overcome by consumption of sufficient quantities of green vegetables, in which the leaf protein is nutritionally complete (McCollum, Orent-Keiles & Day, 1939). However, the intakes of these green vegetables by the vegans could have supplied only a small fraction of their protein requirement (see Table 2). The bulky nature of green vegetables limits the amount that can be comfortably digested, as predicted by McCollum *et al.* (1939).

Soya is used by vegans not only as a vegetable but also in pastry and confectionery. Groundnuts replace butter as a spread and in savouries. Oatmeal is used as porridge, also mixed with roughly ground nuts and pulped raw fruit such as apples to produce *muesli*, a form of compote originating in Switzerland. Typical vegan diets contain much raw fruit and raw vegetables, which may perhaps modify the intestinal flora in the direction of providing more vitamin B_{12} to the host.

Biological value of proteins consumed by vegans

Apart from leaf proteins, the biological value of most individual plant proteins is lower than that of the animal proteins in milk and eggs. In suitable mixtures of different plant proteins, one protein may help to make good deficiencies in another.

Table 2. *Protein values and intakes of individual foods consumed by vegans*

Food	Calories supplied by protein (as percentage of total calories)	Protein content (g/100 g)	Amount eaten daily (g)	Protein content of daily portion (g)
Green vegetables:				
Turnip or broccoli tops	92	2·9	56	1·6
Watercress	79	2·9	28	0·8
Spinach	78	5·1	57	2·9
Spring greens or cabbage	60	1·4	120	1·7
Lettuce	38	1·1	85	0·9
Pulses				
Soya beans	38	40·9	57	23·3
Haricot beans	32	21·4	57	12·0
Peas, fresh	36	5·8	85	4·9
Lentils	31	23·8	28	4·9
Butter beans	28	19·2	57	10·9
Nuts				
Peanuts	19	28·1	57	16·0
Almonds	19	20·5	28	5·6
Cereals:				
Oatmeal	12	12·1	28	3·4
Wheat germ	34	30·0	14	4·2
Wheat, whole, English	11	8·9	62	5·5

For each food is given the average daily intake when that food is consumed. Generally speaking, only one or two foods are consumed from each class on any given day.

For example, the deficiency in methionine of soya protein may be partly met by blending with maize, the protein of which contains considerably more methionine. This supplementary action of maize on soya protein was made use of by Dean (1952) in Africa in some of his earlier work on kwashiorkor, in which some anaemia was encountered. In India commonly used pulses such as *dhal* may be blended with millet or rice containing several times as much methionine (Patwardhan, 1956). In these Indian diets the ratio of pulses to cereals was much lower than in the British vegan diet.

The high protein content (18% on the calorie basis) of Dean's (1952) successful diet raises the question whether deficiencies of proteins in essential amino-acids could be overcome by higher intakes of total protein. As the animal-protein intake is reduced the total protein intake usually has to be increased to obtain good results, but increases cannot be relied upon to make good gross deficiencies.

Individual protein intakes amongst vegans

Possible individual variations in protein intakes amongst vegans, which might explain the variation in incidence of dietary deficiencies, were explored by studying the urinary excretions of creatinine and non-protein nitrogen in each of the vegans with known serum vitamin B_{12} levels. From the NPN: creatinine ratio the protein contents of the diets were calculated by Worrall's (1954) method and found to

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average 7% on the calorie basis (Wokes *et al.* 1955*a,b*). Worrall's method has since been checked against samples of the diet consumed by a woman on whom Dr Ungley kindly carried out for us a week's metabolism experiment. Throughout this period, the protein content of her diet, as calculated by Worrall's method, varied widely, and often differed considerably from the protein content calculated by means of McCance & Widdowson's (1946*a*) data from her known intakes of foods. We therefore investigated in a number of vegans and non-vegans the rates of excretion of creatinine and of non-protein nitrogen respectively throughout the day, and found them to exhibit different rhythms, so that the NPN: creatinine ratio in any one person is not constant (Wokes & Picard, 1955*a*). Using results on urine collected after fasting overnight, we obtained by Worrall's method an average result for the protein intake of Dr Ungley's vegan patient during 5 days of 12% (results for individual days were 13·5, 12·8, 9·5, 11·8, 12·3), compared with 10·9% by analysis of a composite sample of the diet and 10·4% as calculated from the intakes of food. Applying the same method to nine other vegans, we obtained an average value of 11·3% for their protein intake, ranging from 8·7 in a school teacher with definite signs of deficiency to 16·9 in a police inspector with no signs of deficiency (see Table 3).

Table 3. *Calories derived from proteins by vegans (calculated by Worrall's* (1954) *method)*

Sex	Occupation	Age (years)	Body-weight (kg)	Calories derived from protein (as a percentage of total calories)
F.	Teacher	21	50	8·7
	Head teacher	54	47	9·0
	Housewife	63	65	9·9
	Housewife	52	55	10·1
	Student	15	47	11·0
	Housewife	40	57	11·7
	Secretary	54	51	12·0
M.	Unknown	30	63	12·4
	Police inspector	42	93	16·9

Vitamin values of vegan diets

Evaluations based on Fixsen & Roscoe's (1937–8) tables of vitamin values indicated no shortage of vitamins A, B_1 and C, riboflavin and nicotinic acid (cf. Kodicek, 1942). Analyses of the diet consumed by Dr Ungley's vegan patient confirmed this finding. The vitamin D intakes come entirely from margarine, of which some vegans eat very little and others as much as 2 oz. daily. Some blackening and crumbling of teeth has been observed amongst vegan children and adults, but no occurrence of rickets has been established. The folic-acid content, though not directly determined, must have been raised by the green vegetables consumed, and this increase in combination with low vitamin B_{12} intakes may have caused the nervous lesions sometimes observed.

Extradietary sources of vitamin B_{12}

The vegans may have acquired significant amounts of vitamin B_{12} from their intestinal flora. It is well known that the intestinal flora can be modified by the diet. Ingestion of raw starch may favour the growth of *Bacterium coli*. The large quantities of raw vegetables, including potatoes, consumed by vegans may therefore have enhanced their low dietary intakes of vitamin B_{12}.

Possible metabolic functions of vitamin B_{12}

Metabolic functions will be affected by variations in the amount of vitamin B_{12} from dietary or intestinal sources or by variations in the supply of essential amino-acids, especially methionine. There is, of course, much evidence that vitamin B_{12} plays a part in the *de novo* synthesis of labile methyl groups to convert homocysteine to methionine, and also in the reduction of disulphide to sulphydryl groups. The presence of sulphur in methionine led us to study in vegans and non-vegans the metabolism of thiocyanate, the sulphur-containing detoxication product of cyanide, in the metabolism of which vitamin B_{12} in the form of hydroxo- or aquo-cobalamin appears to be implicated. A hypothesis has recently been published (Wokes & Picard, 1955*b*) explaining on this basis a possible role of vitamin B_{12} in human nutrition.

It is suggested that vitamin B_{12} in the form of hydroxocobalamin can take up thiocyanate to form thiocyanatocobalamin, which then donates sulphur to an active intermediate, itself reverting first to cyanocobalamin and then to hydroxocobalamin, thus completing the cycle. The sulphur thus made available could enter into the synthesis of essential sulphur-containing metabolites, which might include amino-acids such as methionine, in which vegan diets tend to be deficient.

Vitamin B_{12} and the nervous system

Smith (1956) advances a hypothesis to explain why the neurological manifestations of pernicious anaemia can be corrected by vitamin B_{12} but not by folic acid, which may apply to the vegans in whom these manifestations occurred. Lack of vitamin B_{12} would not only inhibit methionine synthesis but might also by inhibition of a sulphydryl system lead to abnormal metabolism of tryptophan and tyrosine with accumulation of toxic products. Reference to alterations in sulphur metabolism in vitamin B_{12} deficiency is made also by Sinclair (1956), who mentions that these alterations are affected by large oral doses of tyrosine. Such doses have been found (Wokes *et al.* 1955*a,b*; Wokes & Picard, 1955*a*) to produce a transient increase in the urinary excretion of thiocyanates in vegans with vitamin B_{12} deficiency.

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