Haematological studies on vegans

BY T. A. B. SANDERS AND F. R. ELLIS

Department of Pathology, Kingston Hospital, Kingston-upon-Thames, Surrey, KT2 7BD

AND J. W. T. DICKERSON

Department of Biochemistry, Nutrition Division, University of Surrey, Guildford, Surrey, GU2 5XH

(*Received* 19 *May* 1977 – *Accepted* 29 *November* 1977)

1. The concentrations of vitamin B_{12} and folate in the serum and folate in the erythrocytes were determined and full blood counts made on a series of caucasian vegans and omnivore controls.

2. The blood counts and films were normal in all the vegans and no subject had a haemoglobin concentration below the lower limit of normality.

3. Although within the normal range, male but not female vegans had lower values for erythrocyte counts and higher values for mean corpuscular volume and mean corpuscular haemoglobin than their controls regardless of whether they were taking vitamin B_{12} supplements or not.

4. The mean serum vitamin B_{12} concentration was lower in the vegans not taking vitamin B_{12} supplements and in those using foods supplemented with the vitamin than in the controls, but in no subject was it below 80 ng/l.

5. The serum folate concentrations were higher in the vegans than in their controls. The mean value for erythrocyte folate tended to be greater in the vegans not taking vitamin B_{12} supplements. No subject had an erythrocyte folate concentration of less than 100 μg/l.

6. It is concluded that megaloblastic anaemia is very rare in caucasian vegans and that a diet consisting entirely of plant foods is generally adequate to promote normal blood formation providing it is composed of a mixture of unrefined cereals, pulses, nuts, fruit and vegetables and is supplemented with vitamin B_{12}.

Vegans are a minority group who for ethical reasons eat no foods of animal origin. Vitamin B_{12} is a product of microbial synthesis and is not found in plant foods (Lester Smith, 1965) and therefore should be absent from vegan diets. Rose (1976) claims that megaloblastic anaemia is a predictable consequence of vegan dietary habits adopted in Britain. Neurological symptoms (Badenoch, 1952; Wokes, Badenoch & Sinclair, 1955; Smith, 1962) and megaloblastic anaemia (Hines, 1966) apparently due to a dietary deficiency of vitamin B_{12} have been reported in caucasian vegans. Megaloblastic anaemia, attributed to a dietary deficiency of vitamin B_{12}, in vegetarian Asian immigrants to this country has been reported (Stewart, Roberts & Hoffbrand, 1970; Britt, Harper & Spray, 1971; Roberts, James, Petrie, Morgan & Hoffbrand, 1973). On the other hand, Hardinge & Stare (1954*a*), West & Ellis (1966) and Ellis & Montegriffo (1970) failed to find any clinical or haematological evidence of vitamin B_{12} deficiency in their studies of caucasian vegans, although the serum vitamin B_{12} concentrations of some of their subjects indicated deficiency. In view of the trend towards vegetarianism in this country, and because many people in the developing countries, especially those of Asia, eat a mainly vegetarian diet, it is important to establish whether a vegan diet can promote normal blood formation.

SUBJECTS, MATERIALS AND METHODS

Vegan subjects were contacted through the Vegan Society; these were all caucasians and were mainly from the professional and middle classes. A total of thirty-four vegans were examined (twenty male and fourteen female). The average age of the vegan subjects was

Table 1. *Details of the vegan subjects*

Sex	Age (years)	Previous period on vegetarian diet (years)	Period on vegan diet (years)	Type of vitamin B_{12} supplement taken	Vitamin B_{12} concentration (ng/l)	Erythrocyte count (1×10^{12}cells/l)	Haemoglobin concentration (g/dl)
Male	5	—	5	Food	375	4·38	13·2
Male	5	—	5	Food	—	4·49	13·6
Male	10	—	3	None	130	4·32	13·0
Male	21	5	2	None	200	4·45	14·3
Male	22	3·5	4	Food	160	5·05	16·1
Male	22	2	2	Food	210	5·53	15·6
Male	25	3	1	Food	245	4·26	13·1
Male	25	—	3	Food	240	4·46	14·0
Male	28	—	28	Food	322	3·91	14·0
Male	30	3	2	Tablet	675	3·90	13·3
Male	31	2	8	Tablet	500	4·47	14·4
Male	31	1	6	None	230	4·72	14·1
Male	35	3	0·5	None	94	3·96	14·3
Male	46	—	10	Food	185	4·65	15·2
Male	46	30	5	Tablet	375	4·94	16·0
Male	48	3	5	Food	150	4·43	13·4
Male	49	1	13	None	120	4·81	13·9
Male	57	—	17	Tablet	215	4·77	16·1
Male	64	20	30	Food	250	4·49	15·1
Male	84	—	3	None	100	4·19	14·3
Female	3	—	3	Food	—	4·27	12·2
Female	7	—	7	Food	155	4·47	12·6
Female	12	—	3	None	220	4·25	13·2
Female	22	4	3	Food	375	4·35	13·2
Female	28	3	3	Food	325	4·20	13·9
Female	29	2	8	Tablet	500	4·39	13·6
Female	33	6	6	None	180	4·73	14·8
Female	39	—	3	None	130	4·64	14·8
Female	39	12	12	Food	220	4·59	12·9
Female	39	5	3	Food	280	4·07	13·5
Female	43	17	26	Tablet	260	4·41	13·6
Female	50	4	1	Food	225	4·58	14·4
Female	58	0·5	4	None	235	4·30	13·7
Female	66	—	17	Food	330	4·31	12·5

thirty-four (range 3–84 years). The length of time the vegans had been on the diet was noted and dietary information was collected from all subjects by questionnaire; in the case of young children the information was provided by their parents. In addition 7-d weighed dietary survey information on four vegan subjects was available. Omnivore control subjects were healthy hospital staff, their relatives and friends. Blood samples were drawn by venepuncture with stasis between 9.00 hours and 12.00 hours from subjects after an overnight fast. Portions anticoagulated with EDTA were taken for full blood counts and measurement of haemoglobin concentration on a Coulter Model S cell counter (Coulter Instruments, Luton, Beds.) and stained blood films were examined. Serum vitamin B_{12} concentrations (Spray, 1955) and serum and erythrocyte folate concentrations (Chanarin, 1969) were assayed microbiologically. The Fisher-Behrens test was used for testing differences between mean values (Campbell, 1967).

RESULTS

All the vegan and omnivore subjects seemed healthy when studied. The vegan subjects had been on the diet for an average of 7 years (range, 6 months–30 years; see Table 1).

Table 2. *Blood counts and haemoglobin concentrations in vegan and omnivore control subjects*

	No. of subjects	White blood cell count (1×10^9 cells)		Erythrocyte count (1×10^{12} cells)		Haemoglobin (g/dl)		Packed cell volume		Mean corpuscular volume (fl)		Mean corpuscular haemoglobin (pg)		Mean corpuscular haemoglobin concentration (pg/dl)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Males															
Vegans not taking vitamin B_{12}	6	5·5	0·43	4·41*	0·13	14·0*	0·21	0·41*	0·007	93*	2·4	31·4	0·96	33·9	0·36
Vegans taking vitamin B_{12}	14	4·5	0·60	4·55**	0·11	13·8	0·84	0·41*	0·009	92**	1·7	31·9**	0·45	34·9	0·33
Controls	12	5·8	0·27	5·08	0·06	14·9	0·26	0·44	0·006	86	1·2	29·2	0·32	34·0	0·31
Females															
Vegans not taking vitamin B_{12}	4	5·0	1·07	4·48	0·12	14·1	0·40	0·41	0·012	91	1·3	31·4	0·25	34·0	0·18
Vegans taking vitamin B_{12}	10	6·7	0·66	4·36	0·05	13·2	0·22	0·38	0·060	88	1·3	30·2	0·57	34·4	0·35
Controls	11	6·9	0·48	4·36	0·10	13·0	0·21	0·38	0·007	88	0·4	30·3	0·41	34·3	0·43

Statistical significance of difference between means * $P < 0·05$, ** $P < 0·01$.

Table 3. *Concentrations of serum vitamin B_{12} and serum and erythrocyte folate in vegans and omnivore control subjects*

	Serum vitamin B_{12} (ng/l)			Serum folate (μg/l)			Erythrocyte folate (μg/l)		
	No. of subjects	Mean	SE	No. of subjects	Mean	SE	No. of subjects	Mean	SE
Males									
Vegans not taking vitamin B_{12}	6	146*	23	5	12·9	2·4	4	379	106
Vegans taking vitamin B_{12}	13	300	42	11	15·4**	1·2	10	243	47
Controls	12	380	45	12	7·9	0·9	11	316	26
Females									
Vegans not taking vitamin B_{12}	4	191*	23	3	>18·0*	—	3	699*	104
Vegans taking vitamin B_{12}	9	297	34	8	16·4**	0·90	8	398	53
Controls	8	374	36	8	7·6	0·98	8	306	50
Both sexes									
Vegans not taking vitamin B_{12}	10	164**	17	8	14·8**	1·5	7	516*	81
Vegans taking vitamin B_{12}	22	299	28	19	15·2**	0·9	18	312	39
Controls	20	378	30	20	7·9	0·6	19	312	25

Statistical significance of difference between means $*P < 0·05$, $**P < 0·01$.

Six subjects had been born of and breast-fed by vegan mothers and weaned and reared on a vegan diet. None of the vegan subjects admitted eating meat, fish, eggs, milk products, or any other foods of animal origin. The results of the 7-d weighed dietary intake survey on four of the vegan subjects suggested an average daily iron intake of 23 mg/d (range 15–31 mg/d); protein on average accounted for 12% of the dietary energy (range 11–13%). Vegan subjects were divided into two groups depending whether they took vitamin B_{12} supplements or not. Six vegan subjects were taking vitamin B_{12} tablets (Cytacon; Glaxo Laboratories Ltd, Greenford, Middlesex) on a regular basis, eighteen subjects were using foods supplemented with the vitamin (Barmene, Velactin, Plamil, Granogen) and ten subjects were taking neither tablets nor foods supplemented with the vitamin. The mean serum vitamin B_{12} concentration was higher in those vegans taking vitamin B_{12} tablets than those using foods supplemented with the vitamin (421 ± 70 ng/l (mean ± SE) compared with 253 ± 19; $P < 0·05$). Four of the ten subjects who were not taking vitamin B_{12} supplements had normal serum levels of the vitamin (greater than 180 ng/l): their vitamin B_{12} values were 200, 230, 220 and 235 ng/l, and they had been on the diet for 2, 6, 3, and 4 years respectively.

Tables 2 and 3 summarize the results in thirty-four vegans and twenty-three omnivore subjects. No subject had a haemoglobin value below the lower limit of normality (13·0 g/dl for males, WHO 1968; 11·5 g/dl for females, Chanarin 1969). No subject had a serum vitamin B_{12} concentration indicative of deficiency (less than 80 ng/l, WHO 1968) or a serum folate value less than 2·5 μg/l. There was no evidence of macrocytosis or microcytosis, no polysegmented neutrophils were seen and all the blood films were normochromic when examined.

Although the blood films were normal, a number of statistically significant differences were noted between the vegans and their omnivore controls: in the male but not the female vegans the mean values for erythrocyte count and packed cell volume were lower ($P < 0·01$

and $P < 0.05$) and the mean values for mean corpuscular haemoglobin and mean corpuscular volume were higher (both $P < 0.01$) regardless whether they were taking vitamin B_{12} supplements or not; the mean values for serum vitamin B_{12} concentration was lower in the vegans not taking vitamin B_{12} supplements ($P < 0.01$) and in those using foods supplemented with the vitamin ($P < 0.01$) but not in those taking vitamin B_{12} tablets; the mean value for serum folate was much higher in the vegans ($P < 0.01$); the mean erythrocyte folate concentration tended to be higher ($P < 0.05$) in the vegans not taking vitamin B_{12} supplements.

DISCUSSION

The blood counts and films of the vegan subjects were essentially normal, in agreement with Hardinge & Stare (1954*a*), West & Ellis (1966) and Ellis & Montegriffo (1970). The findings that male but not female vegans tended to have lower values for erythrocyte counts and higher values for mean corpuscular volume and mean corpuscular haemoglobin are novel. Cotes, Dabbs, Hall, McDonald, Miller, Mumford & Saunders (1970) found no difference between the physiological response to exercise of female caucasian vegans and omnivores; no similar studies of male caucasian vegans appear to have been made and would be of interest.

The finding of lower serum vitamin B_{12} and higher serum folate values in vegans is in agreement with previous reports (West & Ellis, 1966; Ellis & Montegriffo, 1970). Erythrocyte folate concentrations in vegans do not appear to have been previously reported. The level of serum folate is often increased in patients with untreated pernicious anaemia, while the erythrocyte folate concentration is abnormally low (Chanarin, 1969) apparently because vitamin B_{12} is necessary for the uptake of folate into the erythrocyte (Nutrition Reviews, 1975). In this study none of the vegan subjects had an abnormally low erythrocyte folate concentration; this would suggest, firstly, that none of the subjects were suffering from vitamin B_{12} deficiency and, secondly, that the high serum folate concentrations found in many of the vegan subjects were due to high dietary intakes of folate. This might explain why megaloblastic anaemia was not encountered in our vegan subjects.

Vegans tend to have lower intakes of energy, protein, fat, calcium, riboflavin, saturated fatty acids and vitamin B_{12}, and higher intakes of unrefined carbohydrates, thiamin, vitamin C, vitamin A, polyunsaturated fatty acids, fibre, folate and iron than omnivores (Hardinge & Stare, 1954*a*; Hardinge, Crooks & Stare, 1962; Miller & Mumford, 1972; Mumford & Ellis, unpublished results).

Vitamin B_{12} is the vitamin most likely to be deficient in vegan and occasionally vegetarian diets. The present study has provided no evidence of pathologically low values of vitamin B_{12} in the serum of vegans. In twenty-four of the subjects this could be attributed to them taking vitamin supplements or foods supplemented with the vitamin. There were, however ten subjects who did not take supplements and it was therefore surprising that these subjects did not show evidence of vitamin B_{12} deficiency. Some vegans may obtain the vitamin through the accidental ingestion of insects or from micro-organisms, that produce the vitamin in their food or as a result of poor personal hygiene. Alternatively, some vegans may be able to absorb vitamin B_{12} which has been synthesized by their own gut microflora.

A few cases of vitamin B_{12} deficiency, sometimes resulting in neurological symptoms, have been reported in caucasian vegans apparently due to dietary deficiency (Badenoch, 1952; Wokes *et al.* 1955; Smith, 1962; Hines, 1966; Verjaal & Timmermans-van den Bos, 1967; Winawer, Strieff & Zamcheck, 1967; Ledbetter & del Pozo, 1969; Misra & Fallowfield, 1971). The results provided by Badenoch (1952), Wokes *et al.* (1955) and Smith (1962) are incomplete and their diagnoses of subacute combined degeneration of the spinal cord due to vitamin B_{12} deficiency are not convincing (Pallis & Lewis, 1974). However, this

and other studies (Hardinge & Stare, 1954*a*; West & Ellis, 1966; Ellis & Montegriffo 1970; Armstrong, Davies, Nicol, van Merwyk & Larword, 1974) failed to find symptoms attributable to a dietary deficiency of vitamin B_{12}. This would suggest that dietary vitamin B_{12} deficiency is rare among vegans. However, as there is a possibility of developing symptoms of vitamin B_{12} deficiency, vegans should supplement their diets with the vitamin. The maximum amount that can be absorbed is in the region of 3 μg at any one meal (Heyssel, Bozian, Darby & Bell, 1966), the proportion absorbed increasing the lower the dietary concentration of the vitamin. Massive oral dosage (Rose, 1976) is not an effective way of preventing dietary deficiency of vitamin B_{12}. A regular dietary intake of the vitamin is required or alternatively periodic massive dose by parenteral injection. The former can easily be achieved by the daily use of foods supplemented with the vitamin (Ellis & Wokes, 1967). Miller & Mumford (1972) calculated that a typical daily serving of some vegetable foods that are supplemented with the vitamin would provide 2–6 μg of the vitamin, thus meeting requirements.

The health of caucasian vegans appears to differ little from that of omnivores (Hardinge & Stare, 1954*a*; Ellis & Montegriffo, 1970; Ellis, West & Sanders, 1976; Sanders, 1977). Pregnancy in caucasian vegans and the health of children reared on vegan diets appear to be essentially normal (Thomas, Ellis & Diggory, 1977; Mumford & Ellis, unpublished observations; Sanders, 1977). Caucasian vegans tend to have lower concentrations of serum cholesterol and triglycerides and less body fat than omnivores (Hardinge & Stare, 1954*b*; Sanders, 1977) which suggest that they may be less prone to ischaemic heart disease than omnivores, and according to Aries, Crowther, Drasar, Hill & Ellis, (1971), caucasian vegans are probably less susceptible to cancer of the colon than omnivores. The vegan diet appears to be adequate provided it comprises a mixture of unrefined cereals, pulses, nuts, fruits and vegetables and is supplemented with vitamins B_{12} and D; such a diet will generally promote normal blood formation.

We are grateful to the South West Thames Regional Health Authority for a grant. This work incorporates results from a thesis for which T. A. B. Sanders was awarded the degree of Doctor of Philosophy in the University of London.

REFERENCES

Aries, V. C., Crowther, J. S., Drasar, B. S., Hill, M. J. & Ellis, F. R. (1971). *J. Path.* **103**, 54.
Armstrong, B. K., Davis, R. E., Nicol, D. J., Van Merwyk, A. J. & Larword, C. J. (1974). *Am. J. clin. Nutr.* **27**, 712.
Badenoch, A. C. (1952). *Br. med. J.* **2**, 668.
Britt, R. P., Harper, C. & Spray, G. H. (1971). *Q.J. Med.* New series. L, **160**, 499.
Campbell, R. C. (1967). *Statistics for Biologists.* Cambridge: Cambridge University Press.
Chanarin, I. (1969). *The Megaloblastic Anaemias.* Oxford and Edinburgh: Blackwell Scientific Publications.
Cotes, J. E., Dabbs, J. M., Hall, A. M., McDonald, A., Miller, D. S., Mumford, P. & Saunders, M. J. (1970). *J. Physiol., Lond.* **209**, 30.
Ellis, F. R. & Montegriffo, V. M. E. (1970). *Am. J. clin. Nutr.* **23** (3), 249.
Ellis, F. R., West, E. D. & Sanders, T. A. B. (1976). *Plant Foods for Man* **2**, 43.
Ellis, F. R. & Wokes, F. (1967). *Nutritio Dieta* **9**, 81.
Hardinge, M. G., Crooks, H. & Stare, F. J. (1962). *Am. J. clin. Nutr.* **10**, 516.
Hardinge, M. G. & Stare, F. J. (1954*a*). *J. clin. Nutr.* **2**, 73.
Hardinge, M. G. & Stare, F. J. (1954*b*). *J. clin. Nutr.* **2**, 83.
Heyssel, R. M., Bozian, R. C., Darby, W. J. & Bell, M. C. (1966). *Am. J. clin. Nutr.* **18**, 176.
Hines, J. D. (1966). *Am. J. clin. Nutr.* **19**, 260.
Ledbetter, R. B. & Del Pozo, E. (1969). *Acta haemat.* **42**, 247.
Lester Smith, E. (1965). *Vitamin* B_{12}. London: Methuen & Co. Ltd.
Miller, D. S. & Mumford, P. (1972). *Pl. Fds hum. Nutr.* **2**, 201.
Misra, H. N. & Fallowfield, J. M. (1971). *Postgrad. med. J.* **47**, 624.

Nutrition Reviews (1975). *Nutr. Rev.* **33** (4), 118.
Pallis, C. M. & Lewis, P. D. (1974). *The Neurology of Gastro-intestinal Disease*. London: W. B. Saunders Co. Ltd.
Roberts, P. D., James, H., Petrie, A., Morgan, J. O., Hoffbrand, A. V. (1973). *Br. med. J.* **3**, 67.
Rose, M. (1976). *Lancet* ii, 681.
Sanders, T. A. B. (1977). The composition of red cell lipid and adipose tissue in vegans, vegetarians and omnivores. Ph.D. thesis: University of London.
Smith, A. D. M. (1962). *Br. med. J.* **1**, 1655.
Spray, G. H. (1955). *Clin. Sci.* **14**, 661.
Stewart, J. S., Roberts, P. D. & Hoffbrand, A. V. (1970). *Lancet* ii, 542.
Thomas, J., Ellis, F. R. & Diggory, P. L. C. (1977). *Proc. Nutr. Soc.* **36**, 46A.
Verjaal, A. & Timmermans-Van Den Bos, A. H. (1967). *J. Neurol. Neurosurg. Psychiat.* **30**, 464.
West, E. D. & Ellis, F. R. (1966). *J. Neurol. Neurosurg. Psychiat.* **29**, 391.
WHO (1968). *Tech. Rep. Ser. Wld Hlth Org.* no. 405.
Winawer, S. J., Strieff, R. R. & Zamcheck, N. (1967). *Gastroenterology* **53**, 130.
Wokes, F., Badenoch, J. & Sinclair, H. M. (1955). *Am. J. clin. Nutr.* **3**, 375.

Printed in Great Britain