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Serum Vitamin B-12 Levels and Urinary Methylmalonate Excretion in Total Vegetarians

Shari H. Patchett

ABSTRACT

SERUM VITAMIN B-12 LEVELS AND URINARY METHYLMALONATE EXCRETION IN TOTAL VEGETARIANS

by

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The purpose of this research project was threefold: 1) to determine the present serum vitamin B-12 status in a select group of strict or total vegetarians all of whom had been vegetarian for six months or more. 2) to determine the daily intake of vitamin B-12, total calories, protein, % calories as fat, folic acid, and crude fiber on a total vegetarian diet. 3) to observe if subjects with low serum vitamin B-12 levels and low intakes of oral vitamin B-12 also had elevated methylmalonic acid (MMA) in the urine.

Six of the 19 serum vitamin B-12 levels were below normal. The mean daily intake of vitamin B-12 for the 16 subjects who completed their diet record was 0.4 micrograms/day (ug/day) (range 0.0-1.4 ug/day). Sources of vitamin B-12 included fortified cereals, meat analogues, tofu milk, and soy milk. The results of the urinary methylmalonic acid (UMMA) test from the casual and 24-hour urine specimens showed that 5 out of the 19 subjects (26%) had elevated UMMA indicating an inadequate vitamin B-12 to efficiently metabolize MMA that is produced. Finally, 5 out of the 6 subjects (83%) who had below normal serum vitamin B-12 levels also had elevated UMMA levels.

Based upon this study almost a third of the total vegetarians who did not actively take a cobalamin supplement had below normal serum vitamin B-12 levels. Of this group that had low serum vitamin B-12 levels 83% also had an elevated UMMA indicating a possible biochemical limitation in the body due to a lack of adequate vitamin B-12. It would therefore be prudent to strongly recommend that this

population supplement their diet with a reliable vitamin B-12 source on a regular basis.

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Each person whose signature appears below certifies that this manuscript in his/her opinion is adequate, in scope and quality, in lieu of a thesis for the degree Master of Science.

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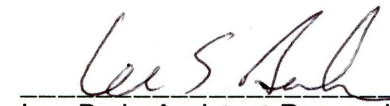

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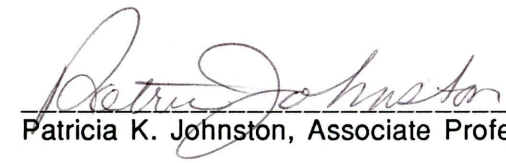

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INTRODUCTION

The popularity of vegetarian diets have been increasing in the last two decades (1). Vegetarian diets range from a lacto-ovo-vegetarian (LOV) diet that includes milk, eggs and cheese but no flesh foods, fish or fowl to a total vegetarian diet which includes only plant foods (2-4). Recently the term "new vegetarian" was introduced to describe persons who follow differing vegetarian dietary patterns with varying degrees of exclusion of animal foods. New vegetarians include Macrobiotics, fruitarians, Zen Buddhists, Krishnas, Sufis, and American Hindus (1,5,6).

The total vegetarian, sometimes called vegan, is of particular interest because the vegan diet includes no animal products, the only known practical source of vitamin B-12 (7,8). Vitamin B-12 is not present in ordinary plant foods (9,10). In view of this, it is noteworthy that the literature describes few cases of overt vitamin B-12 deficiency among total vegetarians (11-25).

While some total vegetarians have deficient levels of serum vitamin B-12, most appear to have normal levels. Normal serum vitamin B-12 levels are generally considered to be 200 picograms/milliliter (pg/ml) to 900 pg/ml (25,26), although there are some variations from laboratory to laboratory. In 1988, Crane and Sample studied 47 total vegetarians living in a small community in Northern California. Of the 47 subjects who had been on a total vegetarian diet for one year or more, it was reported that 42% had serum vitamin B-12 levels below 180 pg/ml. Fifty nine percent of the subjects who had been total vegetarians for two years or longer had serum vitamin B-12 levels below 180 pg/ml (11).

Because of a general concern for the overall health status of a local community of total vegetarians it appeared to be both educational as well as helpful to this population to conduct some additional studies in regards to their vitamin B-12 nutriture.

The purpose of this research project was to further investigate this total vegetarian community. The objectives were: 1) to determine the present serum vitamin B-12 status in a select group of strict or total vegetarians all of whom had been vegetarians for six months or more. 2) to determine the daily intake of vitamin B-12, total calories, protein, % calories as fat, folic acid, and crude fiber on a total vegetarian diet. 3) to observe if subjects with low serum vitamin B-12 levels and low intakes of oral vitamin B-12 also had elevated MMA in the urine.

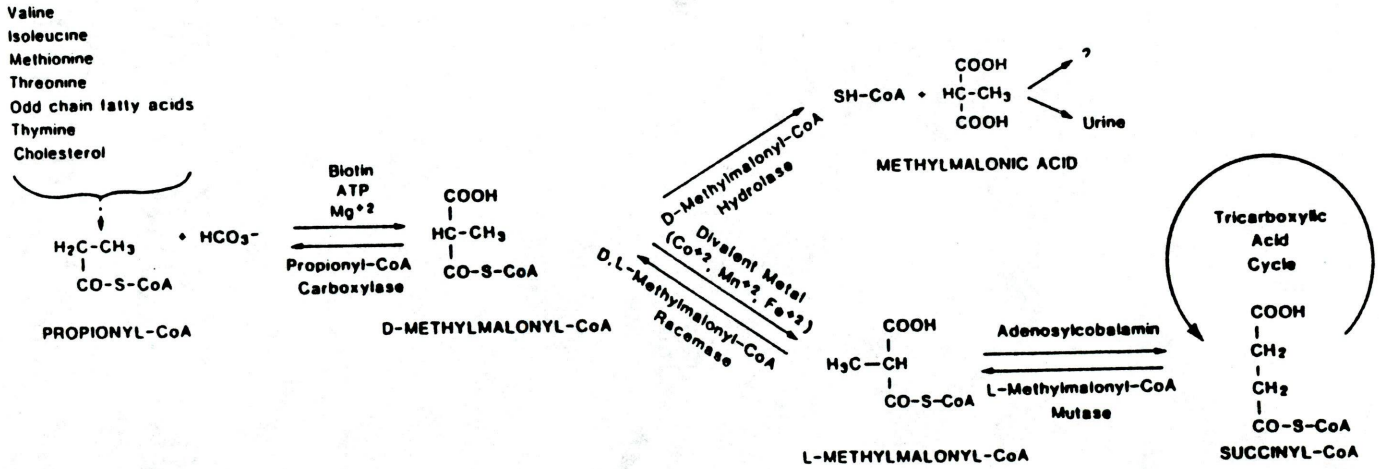
LITERATURE REVIEW

There are several known biochemical defects that develop in the body when there is a deficiency of vitamin B-12. One is an increased excretion rate of MMA in the urine. This particular biochemical defect takes place in cellular metabolism in the conversion of methylmalonyl-CoA to succinyl-CoA and Stabler et al (27) has described this defect (Figure 1). This enzymatic pathway requires both the specific enzyme L-methylmalonyl-CoA mutase and the cofactor adenosylcolbalamin. When there is a deficient amount of vitamin B-12 present this pathway can not function properly and there is a build up of L-methylmalonyl CoA which is then converted to D-methylmalonyl-CoA using two other enzymes (DL-methylmalonyl-CoA racemase and D-methylmalonyl-CoA hydrolase). It is known that "30% of serum MMA is excreted in the urine and 70% is metabolized to unknown products via undefined pathways (27-28)." The level of MMA in the urine is one method of assessing vitamin B-12 nutriture (27-32). Several studies have been conducted using the GC-MS method to measure UMMA (27,29,30,32) as a possible indicator of vitamin B-12 deficiency. One study concluded that the "overall test accuracy in the population they were studying was 99% (30)." Other studies concluded that UMMA would be a "good indicator" or very "useful" in the detection of vitamin B-12 deficiency (27,32).

The literature is scattered with studies done on persons following various vegetarian dietary patterns ranging from semi-vegetarian to LOV to total vegetarians. This literature review has been narrowed to those studies strictly done on or including total vegetarians. The specific focus of the literature review was on research findings related to serum vitamin B-12 levels, diet history, and symptoms of overt vitamin B-12 deficiency.

In 1954 Hardinge and Stare studied 26 total vegetarian subjects who had adhered

Figure 1. Pathway for the formation of methylmalonic acid


Valine
Isoleucine
Methionine
Threonine
Odd chain fatty acids
Thymine
Cholesterol
PROPIONYL-CoA
+ HCO3-
Biotin
ATP
Mg+2
Propionyl-CoA Carboxylase
D-METHYLMALONYL-CoA
D-Methylmalonyl-CoA Hydrolase
SH-CoA +
Urine
METHYLMALONIC ACID
Divalent Metal (Co+2, Mn+2, Fe+2)
D,L-Methylmalonyl-CoA Racemase
L-METHYLMALONYL-CoA
Adenosylcobalamin
L-Methylmalonyl-CoA Mutase
Tricarboxylic Acid Cycle
SUCCINYL-CoA

to the diet for at least five years and found that none of them had any symptoms associated with vitamin B-12 deficiency when compared with non-vegetarians (12).

In 1955 Wokes et al (13) compared 149 British vegans with two previous studies done on American vegans (12) and Dutch vegans (14). He found that the total vegetarian diet in all these studies appeared to be adequate in most vitamins and minerals except calcium, vitamin D and vitamin B-12. The mean serum vitamin B-12 concentration in over half of the vegans studied was below 120 pg/ml. There were more vitamin B-12 deficiency symptoms such as sore tongues, paresthesia and amenorrhea reported in the British vegans than in the Dutch vegans. There were no deficiency symptoms reported in the American vegans. From this study Wokes hypothesized that low serum vitamin B-12 could be associated with 1) lower intake of protein (later studies showed the opposite to be true) and 2) longer years spent on a total vegetarian diet. Wokes further suggested that the variance in serum vitamin B-12 could be due to differences in the synthesis of vitamin B-12 in the flora of the subjects' intestinal tract.

In 1962 Smith (15) studied 12 vegans with an mean serum vitamin B-12 of 128 pg/ml and found manifest symptoms of subacute combined degeneration of the spinal cord in only 2 out of the 12 subjects. However, nine out of eleven subjects had abnormal electroencephalograms (EEG's).

In 1966 West and Ellis (16) compared 17 vegans, 10 lacto-vegetarian (LV) or lacto-ovo-vegetarians, 10 patients with pernicious anemia, 1 patient with mixed vitamin B-12 and folic acid deficiency, 2 patients with folic acid deficiency, and 18 non-vegetarians (NV) as controls. The vegans had a mean serum vitamin B-12 of 240 pg/ml, and the vitamin B-12 and folic acid deficiency group (not including the 2 patients with folic acid deficiency) had a mean serum vitamin B-12 level of 57.1

pg/ml. Eleven of the 17 vegans supplemented their diet with vitamin B-12 (yeast, fortified milk substitutes or injections of pure vitamin B-12). Results indicated that EEG's of the pernicious anemia patients were "more abnormal" than the vegans, and the vegans EEG's were "slightly more abnormal" than the LV, LOV and NV patients . The only correlation coefficient with statistical significance in the study was "the EEG score in the vegans related to length of time as a vegan."

In 1967 Ellis and Mumford (17) found mean serum vitamin B-12 levels of 253 pg/ml in total vegetarian males and 221 pg/ml in total vegetarian females. There were "slight neurological abnormalities" reported in three male vegans, one of whom had pernicious anemia. A high serum folate level was found in the vegans compared to the controls and it was concluded that this was because of the high vegetable and fruit intake of the vegans.

In 1967 Ellis and Wokes (18) studied 31 vegans with a mean serum vitamin B-12 level of 166 pg/ml and found that 12 of the 31 vegan subjects had serum vitamin B-12 levels below 140 pg/ml. No overt deficiency symptoms were mentioned. Velactin, muesli, barmene, dried torula yeast, and cytacon tablets were reported to produce a marked rise in subjects' serum vitamin B-12 level. The conclusion was that vegans should probably take a vitamin B-12 supplement.

In 1970 Ellis and Montegriffo (19) reported that 26 vegans had a mean serum vitamin B-12 level of 236 pg/ml, and that 9 of the 26 subjects had serum vitamin B-12 levels of 140 pg/ml or less. None of these subjects displayed any clinical signs of a vitamin B-12 deficiency. It was noted in the study that mean serum folate levels in the 26 vegans were more than two and a half times higher than in the 21 controls It was suggested this may have prevented the development of megaloblastic anemia. Ellis and Montegriffo proposed that normal serum vitamin B-12 levels were maintained because

1) the vegans were absorbing vitamin B-12 synthesized by the intestinal tract or, 2) the vegans had a very efficient vitamin B-12 enterohepatic circulation system.

In 1975 Lee (20) reported a mean serum vitamin B-12 level of 440 pg/ml in 18 pure vegetarians. A radioassay was used to determine serum vitamin B-12 levels. She hypothesized that the serum vitamin B-12 levels determined by the radioassay were much higher than previous studies which used a microbiological assay because microbiological assays may yield false low results when the serum contains substances which inhibit the growth of microorganisms. Alternatively it may be that the intrinsic factor Lee used in the radioassay was not a "pure intrinsic factor" and that she measured both active vitamin B-12 and inactive vitamin B-12 analogues. Lee found that 1) serum vitamin B-12 levels did not appear to be related to length of time a subject had been a vegan, and 2) serum vitamin B-12 levels and protein intake were significantly correlated. Lee further found that as the intake of fiber increased in the diet, there was also an increase of vitamin B-12 in the feces. This could be due to an increase in bacterial synthesis or decreased absorption of vitamin B-12.

In 1978 Sanders and Ellis (21) studied 34 vegans whose serum vitamin B-12 levels were lower than the control group of omnivores. None of the subjects were found to have any symptoms associated with a vitamin B-12 deficiency. They hypothesized that vegans may not display any vitamin B-12 deficiency symptoms 1) because of a high dietary intake of folic acid which may mask megaloblastic anemia, 2) because of some "accidental ingestion of insects," 3) because of poor personal hygiene which may result in micro-organisms that produce vitamin B-12 or, 4) because the vegans own gut microflora may synthesize and absorb vitamin B-12. Sanders and Ellis concluded that the vegan diet appeared to be adequate if there was plenty of variety in the diet and the diet was supplemented with vitamin B-12 and vitamin D.

In 1979 Throssell (22) investigated 9 total vegetarians who had an average serum vitamin B-12 level of 381 pg/ml. The mean daily intake of vitamin B-12 determined from the 7-day diet history was 0.6 ug a day (from fortified cereals, soy milk, or an occasional multi-vitamin supplement). The paper concluded that vegans appear to be able to maintain an adequate serum vitamin B-12 level if they consume a well-balanced diet that is supplemented with foods fortified in vitamin B-12. Possible exceptions to this as stated by the author include individuals who have additional vitamin B-12 needs such as an infant or the elderly.

The growing infant or child has a higher vitamin B-12 requirement because "vitamin B-12 is an essential nutrient necessary for DNA synthesis and normal red blood cell development" (29). The elderly person may have a higher vitamin B-12 requirement because of: 1) malabsorption of vitamin B-12 due to pernicious anemia, gastric disease or small intestinal disease, 2) drugs they take which interfere with vitamin B-12 absorption, 3) chronic alcoholism, or 4) a restrictive and sometimes unbalanced food intake (9,33,34).

In 1981 Abdulla et al (23) studied 6 strict vegetarians whose daily intake of vitamin B-12 was reported to be 0.3-0.4 ug/day. This small amount of vitamin B-12 was presumed to be from fermented vegetables or food contaminated by B-12 producing microorganisms. There were no serum vitamin B-12 levels presented, and the conclusion of the study stated that there were no clinical or biochemical deficiency symptoms of vitamin B-12 observed in the subjects.

In 1982 Campbell et al (24) studied 10 vegan subjects from the Rastafarian cult. No serum vitamin B-12 levels were reported; however eight of the ten subjects were found to have moderate to severe macrocytic anemia with neurological manifestations of a vitamin B-12 deficiency. "There was a complete hematological

response to parenteral cyanocobalamin treatment in all patients" which seemed to indicate that these men were indeed vitamin B-12 deficient.

In 1982 Dong and Scott (25) investigated a group of 13 vegans, 28 lacto-vegetarians, and 15 lacto-ovo-vegetarians. They found a mean serum vitamin B-12 of 120 pg/ml in the vegans. Subjects showed minimal hematological abnormalities which were much less than was expected. Again, a high dietary intake of folic acid may have masked any hematological disorders and the "absence of macrocytosis does not necessarily mean an absence of neurological disorders." In this study Dong and Scott did not think that "natural hygiene" contributed to a higher serum vitamin B-12 level. The investigators encouraged the use of the supposedly vitamin B-12 rich food spirulina to maintain a normal serum vitamin B-12 level.

In 1988 Crane and Sample (11) studied 47 total vegetarians. Of the 47 subjects who had been on a total vegetarian diet for one year or more, 42% had serum vitamin B-12 levels below 180 pg/ml. Fiftynine percent of subjects who had been total vegetarians for two years or longer had serum vitamin B-12 levels below 180 pg/ml. No overt deficiency symptoms were reported; however, one subject noted in retrospect that his chronic symptoms of indigestion disappeared within a week after starting oral vitamin B-12. A second part of this study included 13 lacto-ovo-vegetarians who were placed on a total vegetarian diet for at least two months. A serum vitamin B-12 level was taken at one week and two months after the beginning of the vegetarian diet. The average serum vitamin B-12 levels decreased 11-53% (mean 32.7%) after two months.

METHODS

A general letter describing this study (Appendix A) was mailed to all persons (n=50) identified by one of the investigators as total vegetarians who did not use vitamin supplements. If the prospective subjects were interested in participating in the study they were requested to fill out a questionnaire (Appendix B) to verify their dietary practices. Twenty-two responses were received. Three of the respondents had been total vegetarians for less than six months leaving 19 subjects who qualified for the study. These subjects were then contacted and verbally asked if they would be willing to participate in the study. It was explained to them that they would be asked to give a blood sample, a casual urine sample, a 24-hour urine sample, a 7-day diet record, and signed the informed consent form (Appendix C).

Fasting blood samples were drawn between 7-8 am. Subjects were instructed not to consume any food after midnight the night before. Two tubes of blood were taken. The venoject dry edta (Na-2) sterile tube was gently mixed twice to insure that the blood would not clot for a complete blood count (CBC). The autosep inert separator gel and clot activator (silicone coated) sterile tube for the serum B-12 and folic acid was allowed to sit for 30 minutes and then spun for 10 minutes. The serum was then gently transferred into two small bottles. One set of samples was analyzed at the Loma Linda University Clinical Laboratory (LLUCL) using the Bio-Rad Quantaphase B-12/Folate radioassay (1000 Alfred Nobel Drive Hercules, California 94547). The duplicate set of samples was analyzed by Central Diagnostic Laboratory (CDL) (18408 Oxnard Street, Tarzana, California 91356).

Casual urine samples were also collected the morning of the blood drawing. An aliquot of the urine was transferred into a 10 ml bottle and frozen. After all the urine samples were received, the specimens were thawed and then inverted several times to

insure a good mix. An aliquot of each urine sample was transferred into another bottle and the samples were sent immediately to Dr. Norman (Norman Clinical Laboratory 1044 Sunwood Court Cincinnati, Ohio 45231) by U.P.S. Dr. Norman's laboratory assayed all the samples for urinary methylmalonic acid and creatinine. The methylmalonate was determined by a gas chromatography-mass spectrometry (GC-MS) method (31).

For the 24-hour urine collection, the subjects were provided with a 24-hour urine collection explanation sheet (Appendix D) and if the subjects came to pick up the collection jug themselves a short verbal explanation was also given. The urine samples were refrigerated during the daily collection period. When the 24-hour urine samples were brought in, the total volume of each sample was measured with a 500 ml graduated cylinder. Two 10 ml aliquots were sent to the Norman Clinical Laboratory for analysis, and the remainder of the urine was discarded.

For the 7-day diet record the subjects were again given an instruction sheet (Appendix E) with some additional verbal instruction. The 7-day diet record was analyzed with the software program The Professional Dietitian TM* (*Constellation Research). Approximately 100 personal recipes were added to the software program. These recipes were developed as a combination of foods already in the software program. Serving sizes of individual recipes were determined by taking the mean weight of three samples of a known volume of a recipe and weighing it on a Ohaus Triple Beam Balance (700 series 2610 g, Florham Park, N.J., 07932). Each sample of food was weighed twice to ensure accuracy. Some of the personal recipes called for foods not in the software program. These foods were left out of the computer developed recipe if the nutrient data could not be found in either Nutritive Value of American Foods (Agriculture Handbook No. 456) or Food Values Commonly Used (Bowes & Church's

Fifteenth Edition revised by Jean A.T. Pennington, Ph.D., R.D.). There were approximately four recipes that were added to the software program using nutritional information provided on the packaging by the manufacture.

RESULTS

The mean age (Table 1) of the subjects was 45.8 years (range 24-78) and they reported that they had been total vegetarians for an mean length (Table 1) of 5.7 years (range 0.83-25).

The mean serum vitamin B-12 level (Table 2) was 271 pg/ml (range 116-492 pg/ml) from LLUCL and 275 pg/ml (range <50-466 pg/ml) from CDL. There was no statistical difference between the results obtained at LLUCL and CDL (statistical analyses using paired T-test). Serum vitamin B-12 levels in relation to years following total vegetarian diet is shown in Figure 2.

Four out of 18 subjects for whom data were available had elevated MMA in the casual urine specimens (Table 2). One casual urine specimen could not be obtained, however, the 24-hour MMA in this subject was clearly elevated. The average MMA excretion for the casual urine specimens was 3.67 mcg/mg creatinine (range 1.1-12.8 mcg/mg creatinine).

Four out of 18 subjects had elevated MMA in the 24-hour urine specimens (Table 2). One 24-hour urine specimen was not obtained because the subject declined to collect it. The mean MMA excretion for the 24-hour urine sample was 4.39 mcg/mg creatinine (range 1.2-17.0 mcg/mg creatinine). The MMA of subject number seven was slightly elevated in the casual urine sample but was high normal in the 24-hour urine sample. Combining the data, five of the 19 subjects had elevated MMA values. The relationship between serum vitamin B-12 values and UMMA is shown in Figure 3.

Other blood parameters for the 19 subjects are presented in Table 3 and the results of the 7-day diet record are in Table 4. Only 16 diet records were returned from the 19 subjects who began the study and no specific reasons were stated as to why

TABLE 1

AGE, YEARS AS TOTAL VEGETARIAN AND
SUPPLEMENT USED IN MALE AND FEMALE SUBJECTS

Female

#	Age	Years Total Vegetarian	B-12 Supplement	Multi
1	42	6 years	N	N
2	59	20 years	N	Y
4	45	0.83 years	O	N
7	31	2.5 years	N	N
9	47	2 years	N	N
12	60	25 years	N	N
13	24	2 years	N	N
14	51	1 years	N	N
15	69	2 years	O	N
19	44	3 years	N	N

N=No Y=Yes O=Occasional

Male

#	Age	Years Total Vegetarian	B-12 Supplement	Multi
3	34	5 years	N	N
5	32	2.2 years	N	N
6	63	3 years	N	N
8	36	3.5 years	N	N
10	47	2 years	N	N
11	27	5 years	N	N
16	78	5 years	N	N
17	29	9 years	N	N
18	53	5 years	N	N

N=No Y=Yes O=Occasional

TABLE 2

DIETARY AND SERUM VITAMIN B-12 VALUES
AND URINARY METHYLMALONIC ACID LEVELS

	Dietary Vit B-12 intake ug/day	Serum B-12 CDL	Serum B-12 LLU	Methylmalonic acid Casual	Methylmalonic acid 24-hour
		200-850 pg/ml*		<5.0 mcg/mg creatinine*	
1	0.0	< 50	#	#	17.0
2	+	201	204	2.2	3.4
3	0.1	266	164	2.4	4.0
4	1.4	424	492	3.0	2.7
5	+	382	334	1.6	1.4
6	0.4	181	142	7.2	5.3
7	0.1	186	160	5.4	4.4
8	0.1	< 50	126	2.8	3.9
9	1.3	327	381	2.7	3.2
10	1.4	359	267	1.1	1.2
11	+	223	303	1.6	~
12	1.0	333	257	4.3	2.9
13	0.0	314	280	4.0	3.7
14	0.0	414	321	1.7	2.2
15	0.0	374	355	2.1	2.5
16	0.1	380	356	2.8	2.3
17	0.2	466	430	2.9	2.5
18	0.0	101	116	12.8	14.8
19	0.0	188	196	5.5	6.0

MMA = Methylmalonic acid (Tests done at Normal Clinical Laboratory, Cincinnati, Ohio)
CDL = Clinical Diagnostic Laboratory (Serum B-12)
LLUCL = Loma Linda University Clinical Laboratory (Tests performed by S.H. Patchett)
*Range of normal values
#Unable to obtain another blood sample before the subject took a Vitamin B-12 supplement
~Subject refused to submit a 24 hour urine sample
+Dietary record not taken

Figure 2. Serum vitamin B-12 levels in relation to number of years following a total vegetarian diet.

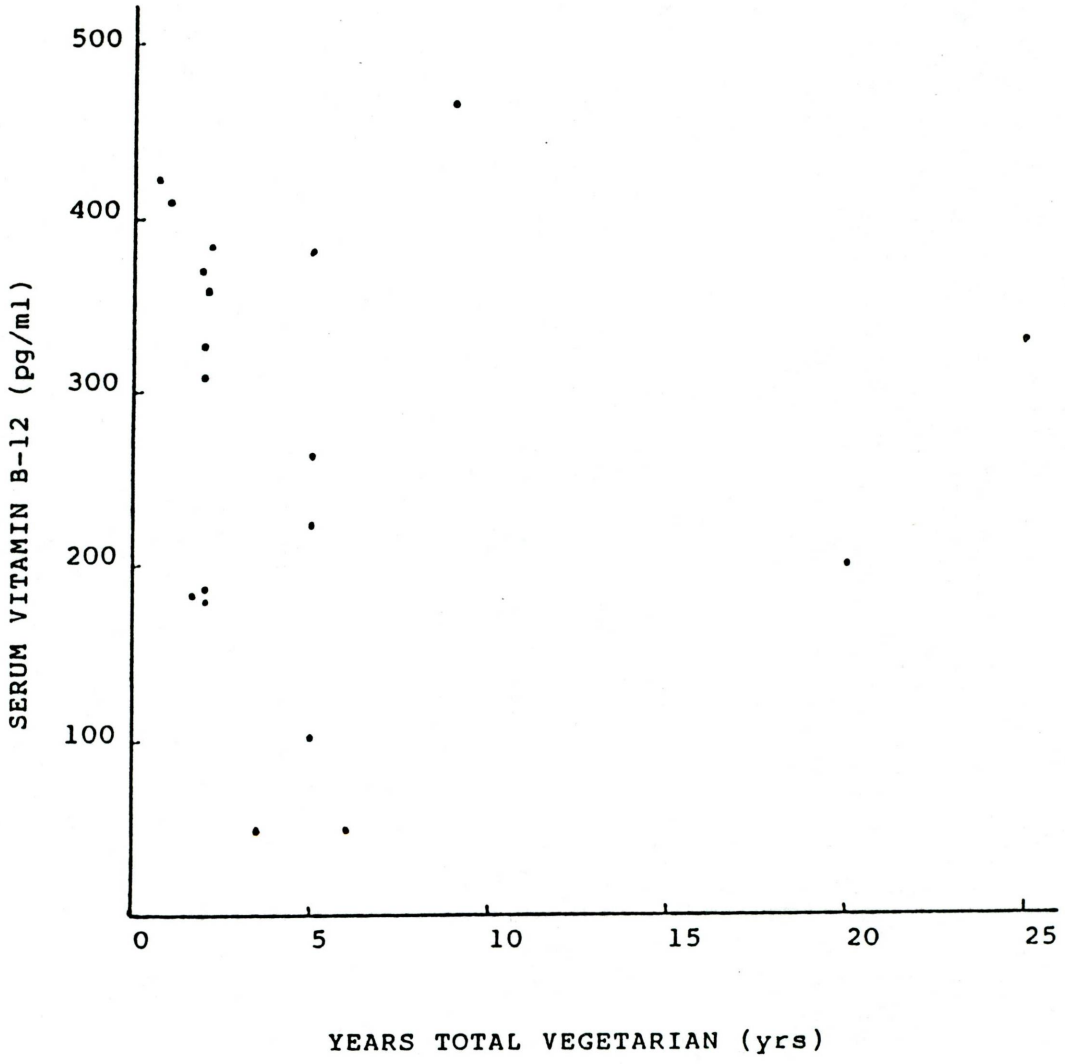

500
400
300
200
100
SERUM VITAMIN B-12 (pg/ml)
0
5
10
15
20
25
YEARS TOTAL VEGETARIAN (yrs)

Figure 3. Urinary methylmalonic acid level in relation to serum vitamin B-12 levels.

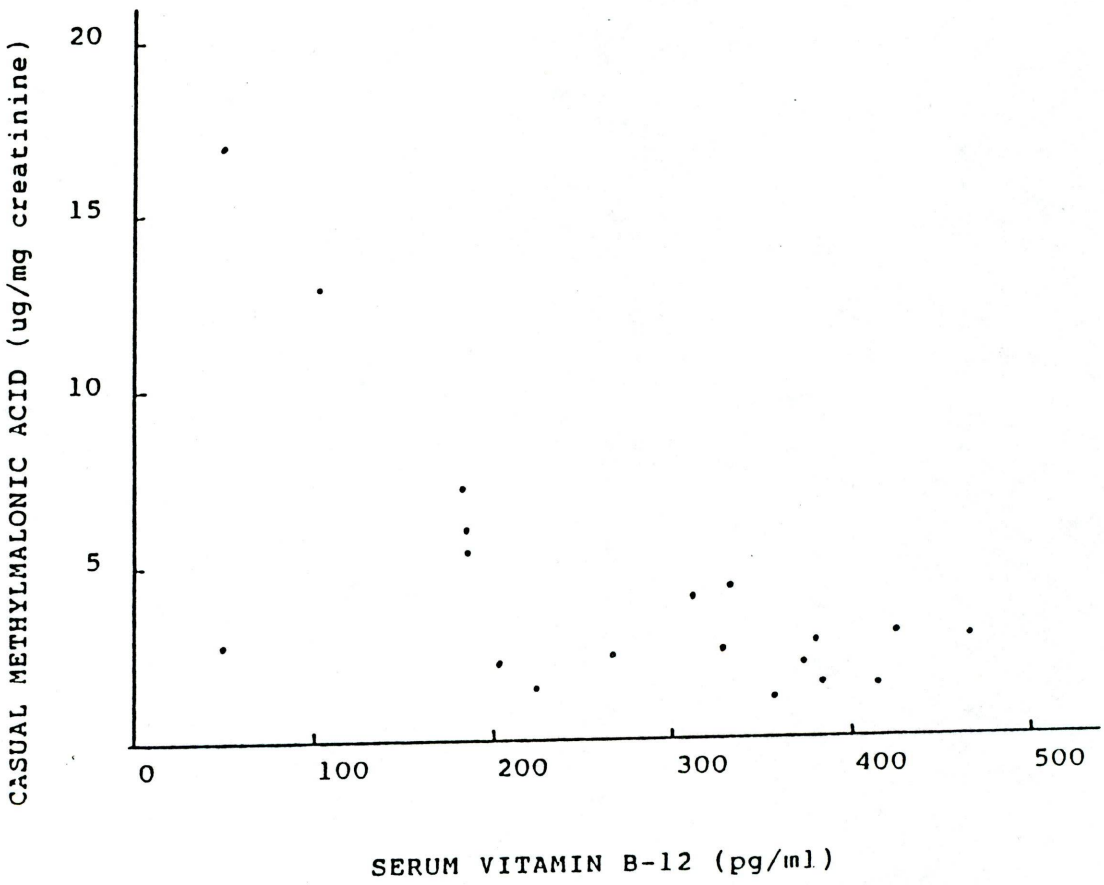

CASUAL METHYLMALONIC ACID (ug/mg creatinine)
20
15
10
5
0
100
200
300
400
500
SERUM VITAMIN B-12 (pg/ml)

TABLE 3

BLOOD VALUES FOR SUBJECTS WITH NORMAL SERUM VITAMIN B-12 LEVELS

#	SEX	HGB	HCT	RBC	MCV	MCH
		gm/dl	%	millions/mm^3	u3	uug
		14±2#	42±5#	4.8±0.6#	82-101~	27-34~
		16±2^	47±5^	5.4±0.7^		
2	F	14.0	44.1	5.11	86	27.4
3	M	14.4	44.6	4.76	93	30.3
4	F	12.6	39.0	4.49	86	28.0
5	M	16.2	50.6	5.49	92	29.5
9	F	16.3*	50.7*	5.44*	93	29.9
10	M	15.0	43.3	4.92	87	30.5
11	M	17.0	49.5	5.51	89	30.9
12	F	13.7	40.8	4.65	87	29.5
13	F	13.9	41.0	4.42	92	31.4
14	F	14.0	41.4	4.28	96	32.7
15	F	13.7	45.1	4.60	98	29.8
16	M	13.9*	42.7	4.17*	102*	33.4
17	M	16.3	49.7	5.37	92	30.4

*Abnormal value ^Normal values for males #Normal values for females ~Normal values for both males and females HGB=Hemoglobin HCT=Hematocrit RBC=Red Blood Cell MCV=Mean Corpuscular Volume MCH=Mean Corpuscular Hemoglobin

BLOOD VALUES FOR SUBJECTS WITH LOW SERUM VITAMIN B-12 LEVELS

#	SEX	HGB	HCT	RBC	MCV	MCH
		gm/dl	%	millions/mm^3	u3	uug
		14±2#	42±5#	4.8±0.6#	82-101~	27-34~
		16±2^	47±5^	5.4±0.7^		
1	F	13.6	40.3	3.67*	109*	37.2*
6	M	15.8	50.2	5.01	100	31.6
7	F	13.6	40.7	4.30	94	31.5
8	M	14.2	42.8	4.35*	98	32.7
18	M	13.1*	38.6*	3.69*	104*	35.4*
19	F	12.8	38.9	4.13*	94	31.1

*Abnormal value ^Normal values for males #Normal values for females ~Normal values for both males and females HGB=Hemoglobin HCT=Hematocrit RBC=Red Blood Cell MCV=Mean Corpuscular Volume MCH=Mean Corpuscular Hemoglobin

TABLE 4

DIETARY ANALYSIS

Female

	KCAL	PRO	FAT	FAT %	CHO	Vit B-12	FOLACIN	FIBER Crude
	kcal	gms	gms	kcals	gms	ug	ug	gms
1	2245	57.9	16.1	6.5	535	0.0	293	25.4
4	1955	71.2	34.0	15.6	373	1.4	348	14.9
7	1411	39.2	44.6	28.4	232	0.1	266	10.5
9	1593	43.5	54.5	30.8	255	1.3	364	10.8
12	1277	36.5	33.1	23.3	231	1.0	346	9.6
13	1029	28.1	23.1	20.2	193	0.0	251	7.0
14	1199	42.2	31.6	23.7	206	0.0	356	8.4
15	1389	38.4	35.0	22.7	247	0.0	153	8.3
19	2556	72.7	82.0	28.9	417	0.0	591	19.6

Dietary Analysis done on The Professional Dietitian TM* (*Constellation Research)

Male

	KCAL	PRO	FAT	FAT %	CHO	Vit B-12	FOLACIN	FIBER Crude
	kcal	gms	gms	kcals	gms	ug	ug	gms
3	1793	47.4	45.4	22.8	329	0.1	273	12.2
6	1337	37.0	25.9	17.5	259	0.4	250	9.1
8	2380	57.9	109.2	41.3	327	0.1	364	16.5
10	2649	73.4	78.2	26.6	457	1.4	519	17.9
16	2141	60.9	64.3	27.0	358	0.1	307	13.8
17	2322	65.4	60.0	23.3	417	0.2	620	16.1
18	2438	84.0	63.1	23.3	413	0.0	461	5.6

Dietary Analysis done on The Professional Dietitian TM* (*Constellation Research)

three diet records were never returned. Therefore dietary intake is reported on 16 subjects.

The mean reported daily intake of calories (kcals) for the seven male subjects was 2151 kcals (range 1337-2649 kcals) and for the nine females was 1628 kcals (range 1029-2556 kcals).

The mean daily intake of protein was 60.9 grams/day (gms/day) (range 37.0-84.0 gms/day) for the seven males and 47.8 gms/day (range 28.1-72.7 gms/day) for the nine females.

The mean percent of calories as fat in the 16 subjects was 23.9 % (range 6.5-41.3 %).

The mean reported daily intake of vitamin B-12 was 0.4 ug/day (range 0.0-1.4 ug/day); of folic acid was 360 ug/day (range 153-620 ug/day); and of crude fiber was 12.9 grams (gms) (range 7.0-25.4 gms). Sources of vitamin B-12 found in soy milk, tofu milk, and fortified cereals are presented in Tables 5-7.

TABLE 5

SELECTED BRANDS OF SOY MILK AND TOFU MILK

Name	Company	Vitamin B-12 Amount	% USRDA
C'est Natural Farms Instant Soy Oat Beverage	Response Multimarketers	8 fl oz	25%
Edensoy	Eden Food Inc	8.45 fl oz	0%
Luscious Tofu Nondairy Better than Milk	Solvex Natural Foods	8 fl oz	10%
Nutritious Soyamel	Worthington Foods	8 fl oz	20%
Solait Powdered Soy Beverage	Millers Farm Food Co.	8 fl oz	0%
SoyMoo	Health Valley Foods	8.45 fl oz	0%
Soyagen Soy Beverage Powder	Loma Linda Foods	8 fl oz	25%
Tofu Carob Drink Mix	Magic Mill	8 fl oz	15%
Tofu White Drink Mix	Magic Mill	8 fl oz	15%
Vitasoy	Vitasoy (USA) Inc	6 fl oz	0%

Weimar Institute General Store, Weimar, California

TABLE 6

CEREALS FORTIFIED WITH VITAMIN B-12

Alpha Bits	Golden Grahams	Raisin Bran
Bran 100%	Grape-Nuts	Raisin, Rice & Rye
Bran Chex	Grape-Nut Flakes	Rice Chex
Bran Flakes	Honey Bran	Sugar Frosted Flakes
Cap'n Crunch	Honeycomb	Sugar Frosted Rice
Cheerios	Honeynut Cheerios	Sugar Puffs
Cocoa Pebbles	King Vitamin	Sugar Sparkled Flakes
Cookie Crisp	Kits	Super Sugar Crisp
Corn Bran	Lucky Charms	Tasteeos
Corn Chex	Most	Team
Corn Flakes	Nutrigrain Barley	Toasty O's
Crispy Rice	Nutrigrain Corn	Total
Crispy Wheat & Raisins	Nutrigrain Rye	Trix
C.W. Post	Nutrigrain Wheat	Waffelos
Fortified Oat Flakes	Product 19	Wheat Chex
Fruit & Fiber	Quaker 100%	Wheat & Raisin Chex
Fruity Pebbles	Quisp	Wheaties

Bowes & Church Food Values of Foods Commonly Used 14th edition

TABLE 7

OTHER CEREALS FORTIFIED WITH VITAMIN B-12

All Bran Fruit & Almonds	Ghost Busters	Raisin Square
Almond Delight	Honey Buc & Wheat Crisp	Smores Crunch
Apple Raisin Crisp	Honey Graham Oh's	Smurf-Berry Crp
Crunch Berries	Instant Breakfast	Sun Flakes
Dairy Crisp	Just Right	Super Golden Crp
Fiber One	New Horizon Trail Mix	Swt Wht Puffs
Fruitful Bran	Raisin Fruit Wheats	Toasted Oat Cer

9-10-86 Stater Brothers, Loma Linda, California.

DISCUSSION

Six of the 19 subjects (32%) in this study had blood levels below normal (< 200 pg/ml). Five of these six had elevated UMMA excretion. Figure 2 shows serum vitamin B-12 levels in relation to the number of years on a total vegetarian diet. It is of interest to note that all the subjects who had been total vegetarians for 2 years or less (n=7) had serum vitamin B-12 levels above 200 pg/ml, whereas 30% of the subjects who had been total vegetarians for 3 years or less (n=10) had serum vitamin B-12 levels below 200 pg/ml. This may indicate a susceptibility in some persons to develop a vitamin B-12 deficiency more rapidly than usually anticipated.

We might have expected subject 12 who had been a total vegetarian for 25 years and did not supplement with vitamin B-12 to have a serum vitamin B-12 level much lower than was found (295 pg/ml). She is however, an international traveler and would be subjected to all types of diets. In contrast, subject 2, who had been a total vegetarian for 20 years, stated that she took an occasional multi-vitamin supplement fortified with vitamin B-12 (Life Line). We might have anticipated that her serum vitamin B-12 level would have been higher than was found (202 pg/ml) due to the occasional vitamin B-12 supplementation. However, since Kondo and Binder (35) found that 20-90% of the total vitamin B-12 present in three popular multivitamin-mineral pills were actually vitamin B-12 analogues, the vitamin B-12 supplements could have contained more vitamin B-12 analogues and contributed to the subject's lower than expected serum vitamin B-12 level.

The complex molecule of active vitamin B-12 consists of four basic parts; 1) a corrin, 2) an aminopropanol, 3) a sugar, 4) and a nucleotide. Because there are many possible variations to this complex molecule, vitamin B-12 or cobalamin has many analogues. There are five known "human-active" forms of vitamin B-12; 1)

hydroxocobalamin, 2) aquocobalamin, 3) 5'-deoxyadenosylcobalamin, 4) methylcobalamin, 5) and cyanocobalamin (9,36). The nonactive vitamin B-12 analogues have been shown to block the absorption of active vitamin B-12 (37).

Five of 19 subjects (26%) had elevated UMMA values for casual or 24-hour urine samples indicating a possible vitamin B-12 deficiency. All of these subjects had low serum vitamin B-12 levels. Therefore, 5 out of the 6 subjects (83%) who had below normal serum vitamin B-12 levels also had elevated UMMA levels (Figure 3). As discussed previously, it is known that "30% of serum MMA is excreted in the urine and 70% is metabolized to unknown products via undefined pathways (27)." It is possible that the subject with low serum vitamin B-12 levels but normal UMMA values, may metabolize more MMA via the undefined pathways and thus have normal UMMA in spite of low serum vitamin B-12.

Although there are scattered abnormal values in Table 3, two particular subjects in the group with low serum vitamin B-12 levels stand out. Hemoglobin (Hgb) and hematocrit (Hct) values of subject 1 were within normal limits but the red blood cell (RBC) count was low and the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were slightly elevated. Subject 18 had low values for Hgb, Hct, and RBC count and elevated values for MCV and MCH. Both these subjects were probably in the beginning stages of developing macrocytic anemia.

Since vitamin B-12 is synthesized only by microorganisms (bacteria, fungi, and algae) (9,10), it is not present in ordinary plant foods and is almost exclusively found in foods of animal origin (7,8). It was once thought that fermented soy bean products such as tempeh (3,9,29,37-42) shoyu (3,38,43) miso (3,38,39,41) and natto (41,44) were good sources of vitamin B-12. Spirulina (9,25,37,41) yeasts (3,9,41) and sea vegetables (23,29,41) were also thought to contain the bacteria which produced the active vitamin B-12. This confusion arose because the standard US

Pharmacopoeia method for determining vitamin B-12 assays both active vitamin B-12 as well as inactive analogues of the vitamin (9,35,37).

It is now doubtful whether these products contain more than 20% of active vitamin B-12 (38,45-47). However, Specker et al (29) reported that arama, wakame, and kombu, collectively known as sea vegetables could potentially be a good source of vitamin B-12. Values of vitamin B-12 content in these sea vegetables were measured using the method of Richardson et al (48). One patient used "sea vegetables and fermented foods" to improve vitamin B-12 status and within two months the UMMA concentration had decreased (29). Another exception appears to be yeasts which have been grown on a vitamin B-12 enriched medium which is included or "mixed in" with the consumed yeast. This appears to be a source of vitamin B-12 but it comes from the medium and not the yeast (9).

Another source of the vitamin may be vitamin B-12 fortified soy milk (7) (Table 5). Other foods such as meat analogues (38), and breakfast cereals (Tables 6 and 7) may be fortified with vitamin B-12. There are no investigations in the current literature that report testing these fortified foods to see whether the vitamin B-12 present is active vitamin B-12 or analogues of the vitamin. Further research is needed to assess the vitamin B-12 availability in these fortified foods .

The mean daily intake of vitamin B-12 for the 16 total vegetarian subjects was 0.4 ug/day. Sources of vitamin B-12 in their diets included fortified cereals, meat analogues, tofu milk, and soy milk. Throssell (22) who also collected a 7-day diet history from total vegetarians found an mean daily intake of 0.6 ug/day of vitamin B-12. Sources of vitamin B-12 in that study included fortified cereals, soy milk, or an occasional multi-vitamin supplement. Abdulla et al (23) estimated the daily vitamin B-12 intake of their subjects to be 0.3-0.4 ug/day using the duplicate portion sampling technique. The minimum daily requirement for vitamin B-12 was estimated to be

between 0.3-0.65 ug/day (49). More recently 1-2 ug/day of cobalamin was suggested to be adequate to maintain vitamin B-12 nutriture (50). The RDA for vitamin B-12 was lowered from 3 ug/day to 2 ug/day in the 10th edition of the RDA's. The mean intake in the present study is only 20% of the RDA for vitamin B-12. Six subject reported zero intakes of the vitamin. Of these three had low serum vitamin B-12 levels and elevated UMMA excretion. The remaining three subjects reporting no vitamin B-12 intake had normal and UMMA values. It appears that most of the subjects consumed enough vitamin B-12 or had adequate vitamin B-12 stores to prevent anemia or physical symptoms of a vitamin B-12 deficiency from occurring in the time period they had been total vegetarians. The length of time it takes for vitamin B-12 body stores to become depleted have been reported to be from 5-20 years (1,4). However, the relatively rapid decrease in serum vitamin B-12 levels, occurring in some subjects in less than three years after adopting a total vegetarian diet, and the frequently associated elevated UMMA levels suggests the need to assure a source of the vitamin.

The mean daily intake of calories for the males was 2151 kcals and for the females was 1628 kcals. Mean energy intake of the 4 male subjects between the ages of 25-50 was 2286 kcals, only 79% of the RDA for that age group. Mean energy intake for the 3 male subjects over 51 years was 1972 kcals, 86% of the RDA.

The 6 female subjects between the ages of 19-50 had a mean energy intake of 1798 kcals which is 82% of the RDA for their age group. The females over 51 years were consuming only 68% of the RDA for energy.

This low-energy consumption pattern in both male and female total vegetarian subjects has been reported by a number of other investigators (1,51). Dwyer reviewed possible reasons for the low energy intake (1). The low energy intake appears to be the result of the reliance on high bulk foods.

In studies done on rats (52-54) and humans (55) it has been shown that as the intake of protein in the diet decreases, the requirement of vitamin B-12 also decreases. In addition it was also found that use of egg albumin as the protein source in rat diets caused a greater depletion in vitamin B-12 than if a soybean protein was used (53).

The mean daily intake of protein for the male subjects was 60.9 gms/day, 119% of the RDA for protein (10). The female subjects consumed 112% of the RDA for protein. Although these vegetarians consumed more than the RDA for protein, the level of intake is below average U.S. intake of protein (1,10). This lower intake of protein may be of benefit so far as vitamin B-12 nutriture is concerned.

There are three major influences on protein utilization to consider when assessing the adequacy of protein intake: 1) protein calorie ratios, 2) protein quantity, 3) and protein quality (1). As reviewed by Dwyer (1) the protein quantity and quality for subjects living in America should not be a problem if a wide variety of plant foods are eaten. Energy intake is an important factor to consider because if calorie needs are not met from other energy sources the protein will be broken down to meet energy needs.

The mean daily intake of fat as a percentage of calories was 23.9%. Current recommendations state that the fat content should not exceed 30% of total calories and that 10% or less of total calories should come from saturated fats (10). Total vegetarian diets are characteristically low in fat (1).

In experiments with mice (56) and rats (57,58) it was shown that a diet decreased in fat may aggravate a vitamin B-12 deficiency. The growth retardation of mice due to a vitamin B-12 deficiency was greater on a diet lower in fat than in mice on a diet higher in fat (56). However, it appears that the type of fat may also be a factor. The fats with a higher" ketogenic potency" such as saturated fats cause a greater drop in

vitamin B-12 levels than unsaturated fats (53). It is not known what if any impact on B-12 status was due to the low fat content of the diets in the present study.

Although it is known that bacteria in the colon can manufacture vitamin B-12 it is not thought that the colon mucosa will absorb it and therefore this synthesis appears to be of little value to the human body (9,37).

A study done by Albert, Mathan, and Baker (59) found that vitamin B-12 may be synthesized by Pseudomonas and Klebsiella organisms in the small bowel and could contribute significantly to the vitamin B-12 nutriture assuming that intrinsic factor was also present. However, the study used microbiological assays that may have measured both active vitamin B-12 and inactive vitamin B-12 analogues and before any conclusion can be drawn more research must be done (9). Thus, it is not known to what extent intestinal vitamin B-12 synthesis contributes to maintaining normal levels of the vitamin.

Studies in rats show that the kind of dietary fiber consumed with a vitamin B-12 deficient diet can have an effect on vitamin B-12 nutriture (9,60,61). Highly fermentable dietary fibers including pectin, guar gum or xylan resulted in a significant increase in excretion of UMMA compared to controls, indicating a probable vitamin B-12 deficiency. Pectin induced the most pronounced effect. Whether these highly fermentable fibers somehow increased the production of propionate in the cecum of the rat which in turn increased the production of UMMA (Figure 1), or if the fiber physically interfered with vitamin B-12 absorption needs to be explored. Further research must be done in this area for a better understanding of the relationships between type of dietary fiber and intestinal bacterial synthesis of vitamin B-12 (9,60,61).

The mean daily intake of crude fiber in the present study was 12.9 gms. Although the computer dietary analysis also provided the total grams of dietary fiber this

information is of little use since there were many missing values. Unfortunately this is a weakness of computer software programs that use USDA Handbooks as a data source. These results would be more meaningful if the missing fiber values were currently available and could be added to the data to get a closer estimate of both crude and dietary fiber.

The mean daily intake of folic acid was 399 ug/day in the males and 330 ug/day in the females compared to the RDA 200 ug and 180 ug respectively (10). Folic acid intake is typically high in total vegetarians due to the very high consumption of fruit and vegetables (1). Increased intake of folic acid is thought to mask a vitamin B-12 deficiency because the megaloblastic anemia characteristic of the vitamin B-12 deficiency is prevented by the high folate intake (13,17,19,44). However neurological damage resulting from a vitamin B-12 deficiency is not prevented by the increased folate intake and may continue to progress (1).

Our bodies have a very efficient enterohepatic circulation of vitamin B-12 as long as absorption is not impaired. The bile releases from 1 to 10 ug of vitamin B-12 per day. Much of the active vitamin B-12 is recovered and used again. This could explain why some total vegetarians do not develop a vitamin B-12 deficiency for many years (16). However, consumption of an increased amount of dietary fiber, particularly pectin could possibly block the absorption of vitamin B-12 and lead to a decrease in enterohepatic circulation of the vitamin. A vitamin B-12 deficiency may appear sooner in persons consuming a high dietary fiber intake with minimal vitamin B-12. Also if dietary fermentable fibers increase the bacterial production of propionate which in turn increases the production of UMMA, the requirement of vitamin B-12 may also increase. Again, more research needs to be done in this area.

A variety of factors typical of a total vegetarian diet may impact on vitamin B-12 nutriture. These include some that may increase the need for this vitamin, such as

increased dietary fiber and low fat intake and some that may decrease the need for this vitamin such as lower protein intake. More research is needed to determine the relative importance of these various factors. An underlying concern is to prevent the neurologic damage that may be masked by high folate levels and progress to an irreversible state.

SUMMARY

The purpose of this research project was to evaluate vitamin B-12 status in subjects who had followed a total vegetarian diet for more than six months. Daily intake of vitamin B-12, total calories, protein, % calories as fat, folic acid, and crude fiber were determined from 7-day diet records. Serum vitamin B-12 levels and UMMA excretion were measured.

The mean daily intake of vitamin B-12 for the 16 subjects who completed their diet record was 0.4 ug/day. Sources included fortified cereals, meat analogues, tofu milk, and soy milk. The reported dietary intake of six subjects contained no vitamin B-12. Of these, three had below normal serum vitamin B-12 values as well as elevated UMMA excretion. Serum vitamin B-12 and UMMA excretion were within normal ranges in the other three subjects with no reported vitamin B-12 intake.

Six of the 19 serum vitamin B-12 levels were below normal. Of these, three reported no vitamin B-12 intake. UMMA was elevated in the casual and/or 24-hour urine specimens of 5 of the 19 subjects indicating a possible vitamin B-12 deficiency. Three of the five reported no intake of vitamin B-12 and one reported 0.1 ug/day. The remaining subject with elevated UMMA reported 0.4 ug/day of vitamin B-12. Finally, all of the subjects with elevated UMMA had below normal serum B-12 levels and 5 out of the 6 subjects who had below normal serum vitamin B-12 levels also had elevated UMMA levels.

In summary, almost a third of the total vegetarians in this study had below normal serum vitamin B-12 levels. Of the group that had low serum vitamin B-12 levels, 80% also had elevated UMMA excretion indicating a possible biochemical defect due to inadequate vitamin B-12. In addition, 30% of subjects following a total vegetarian diet for 3 years or longer had below normal serum vitamin B-12 values

indicating a relatively rapid decline in vitamin B-12 status. It would therefore be prudent to strongly recommend that this population supplement their diet with a reliable vitamin B-12 source on a regular basis to prevent the potentially serious consequences of a deficiency.

APPENDIX A. GENERAL FORM LETTER SENT TO TOTAL VEGETARIAN SUBJECTS

Date: May 10, 1989
To: Weimar Staff and Students
From: Mrs. Shari Patchett, R.D., and Milton Crane, M.D.
Re: Your vitamin B-12 level

In 1986 and 1987 Dr. Crane found that about half of the staff members who were on the Weimar type diet had blood levels of B-12 which were below the normal limits of 180 pg/ml. Some of the staff have learned of this and have started taking B-12 tablets. Others may not know of this and are not taking B-12. Some people wonder whether or not the low serum B-12 indicates a true vitamin deficiency or not. There is a way that we may determine whether a person has enough B-12 when their blood level is below normal. Let us explain in further below.

Germs and yeast are the only known sources of B-12. When we only use total vegetarian food, and when we keep the food very clean, we may not get enough vitamin B-12. The fruit bat does not get a B-12 deficiency when it eats fruit in the wild, but it does get a B-12 deficiency when it is put in a cage and fed the same fruit that has been washed. When the soil is lacking in sufficient cobalt, even the germs cannot make enough B-12. When a person does not get enough B-12 in their food, the stores of B-12 in the liver and other cells are gradually depleted over the subsequent year or so. The body cells then begin to suffer from lack of B-12. The bone marrow cannot make good red blood cells or white blood cells. The intestines cannot make good cells to line the gut, and the absorption of foods in not done properly. The nerve cells begin to suffer, and we get numbness and tingling and then nerve damage. When the Cells do not get enough B-12, the body chemistry cannot work right. One of the chemicals that is made after several months of lack of B-12 is methylmalonic acid. This can be measured on a casual urine sample.

There is some discussion to the effect that the low B-12 in total vegetarians does no mean that the body lacks vitamin B-12. We, along with Dr. U.D. Register, a well-known SDA nutritionist at LLU, would like to measure the methylmalonic acid excretion rate in total vegetarians who have as low serum B-12 level in the blood. To do this we will need the cooperation of the staff at Weimar Institute who would like to help answer this question, Does a low Serum B-12 level mean that the body is deficient in B-12.

If you have been a total vegetarian for a year or more, eating food as is served in the cafeteria at Weimar Institute, we would appreciate it if you would fill out the enclosed questionnaire and then allow us to obtain a blood sample for B-12. If you serum B-12 level is reported 180 pg/ml or lower, we would like to obtain a casual urine sample for methylmalonic acid, and a seven-day dietary history. Mrs. Shari Patchett will be performing the study, collect the urine sample from you, and help you make out the dietary history form. You will receive a small honorarium for your part in this study and the thanks of the scientific team. The laboratory tests will be free of cost to you.

We thank you

Note: Please fill out the questionnaire on the back and return it to Dr. Crane within the next week. Shari will contact you later about the further plans.

APPENDIX B. THE VITAMIN B-12 SCREENING QUESTIONNAIRE SENT TO THE TOTAL VEGETARIANS

Vitamin B-12 Questionnaire

Please answer the following questions for the evaluation of your Vitamin B-12 intake. Your answers will be kept confidential.

Name: _____ Date:_____

Address: _____ Zip:_____

Phone Number: (W) _____ (H) _____

Date of Birth: _____ Weight: _____ Height: _____

1) If you drink a glass of milk or eat its equivalent in yogurt or cheese more than twice a week, circle yes, and return this form to Dr. Crane. Yes No

If you have been off **ALL** flesh foods, eggs, and milk products (skim milk, cheese, cream, yogurt, ect.) for the last **YEAR OR MORE** please fill out the following questions.

2) How many months/years have you been off flesh foods? _____

3) How many months/years has it been since you have eaten less than one egg per week? _____

4) How many months/years has it been since you have eaten less than 1 serving (a glass) of milk or milk products per week? _____

5) How often are you likely to attend church potlucks?
   a) Weekly
   b) 1-2 times/month
   c) less than 1 time/month

6) Do you try to avoid all milk products and eggs at potlucks? Yes No

7) How often do you get away from Weimar and eat with others who may serve milk products or eggs?
   a) Weekly
   b) 1-2 times/month
   c) less than 1 time/month

8) Do you try to avoid all milk products and eggs on such trips? Yes No

9) Do you take any Vitamin B-12 supplements? Yes No

10) Do you take any Multiple Vitamin Supplements? Yes No

11) How often do you take the above supplements? _____

12) Please name the brand of the supplements. _____

f you are not on B-12 supplements, please do not start one until after we get a blood and urine sample. If you have a special concern about this study, please call Shari or Dr. Crane.

Thank you!

APPENDIX C. TOTAL VEGETARIAN'S INFORMED CONSENT SHEET

Informed Consent
A Study on Vitamin B-12
Weimar Institute/Loma Linda University

I have been told that the purpose of this study is to explore the question, Does a low serum B-12 level mean that the body is deficient in B-12.

I have been told that a sample of blood will be drawn for a serum B-12 assay and a complete blood count (CBC). I have been informed that these laboratory tests are free of charge to me.

I have been told how to collect a casual urine sample by Dr. U.D. Register, Dr. M. Crane, Shari Patchett or a member of the staff.

I also understand that I will be asked to take a 7-day diet history.

I have been told there will be no risk to me. I have been told that there may be no particular benefits to me personally, but the benefits to humanity will be that the results of the study will contribute to a greater understanding of the relationship of low serum B-12 level and the body's concurrent B-12 status.

I have been told my name will not in any way be associated with the data determined form analysis performed on the samples given.

I have been told that there is no cost to me. I have also been told I can withdraw my consent to be part of this study at any time without prejudice to my subsequent treatment.

I have read the contents of this consent form and have listened to the verbal explanation given by the investigators. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. I may call Shari Patchett if I have additional questions or concerns.

I have been told I will be given a copy of this consent form upon request.

Date: _____

_____
Signature of Participant

_____
Signature of Witness

APPENDIX D. INSTRUCTION SHEET FOR 24-HOUR URINE COLLECTION

Instructions for 24-hour urine sample

1) The morning of the collection discard first void.

2) Collect the following voids throughout the day and night.

3) Keep the urine refrigerated.

4) Collect the first void of the following morning.

If you have any questions or problems, please give me a call. Thank you for your cooperation!

Shari H. Patchett

APPENDIX E. INSTRUCTION SHEET FOR 7 DAY DIET RECORD

Dear Participant,

Thank you for your cooperation and support of this project! I appreciate your willingness to help me out. The following is some written instructions of how to keep your 7-day food record.

1) I will need a 7-day food record diary. That is, if you start your food diary on Monday morning, you will be finished with the food record on the following Sunday evening.

2) Try to eat as normal as possible during this week.

3) If you have access to a scale, please weigh yourself the first thing every morning wearing only light clothing. Record your weight in the upper right hand corner of the day sheet.

4) Record the time the food is consumed.

5) Record the approximate amount of food consumed.

Example: 1 Cup = 1 C
1 Tablespoon = 1 T
1 Teaspoon = 1 t

6) Record everything that goes into your mouth including food, water, seasonings, juice, pop, medications, or vitamins.

7) Use one line for each food item. Try to identify specific foods if possible.

Example: 3 cups tossed salad
1 c iceberg lettuce
1 c spinach
1/8 c chopped carrots
1/8 c chopped celery
1/8 c chopped cucumber
1/8 c bell pepper
1/8 c chick peas
2 T chopped radishes
2 T bread crumbs
1 T Wish Bone Italian Dressing

8) If the food that you are eating is a mixture of foods such as a casserole or nut patty, please identify to the best of you ability what foods are present in the combined food. If you eat in the Weimar Institute cafeteria, please identify the name of the casserole or nut patty. If you eat a recipe form the Weimar Cookbook, again please name the food.

Example: 1 Taco
1 corn Tortillas
1/2 c pinto beans cooked
pinch of cumin
pinch of salt
pinch of chili power
1/4 c chopped lettuce
1/8 chopped olives
1/2 medium tomato

9) I would suggest that you record the foods you are going to eat before you actually consume them so that you do not leave out or overlook any food items.

10) If you have any questions or problems please feel free to call me. Thank you!

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