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Effect of Lacto-Ovo and Total Vegetarian Diets on Serum, Urinary and Fecal B_{12} Levels

Cathleen D. Throssell

Abstract

EFFECT OF LACTO-OVO AND TOTAL VEGETARIAN DIETS

ON SERUM, URINARY AND FECAL B_{12} LEVELS

by Cathleen D. Throssell

Serum, urinary and fecal levels of ten lacto-ovo-vegetarians and nine total vegetarians were assayed using Ochromonas malhamensis. Intakes of vitamin B_{12}, protein, fat, carbohydrates, fiber and calories were calculated using 7-day diet histories and questionnaires. Correlations between nutrients consumed, years on diet, serum, urinary and fecal B_{12} levels were also calculated.

Mean serum B_{12} levels of the lacto-ovo-vegetarians and total vegetarians were 657 pg/ml and 381 pg/ml respectively. There was no significant difference between the two groups. The average daily intake of vitamin B_{12} was 2.0 µg for lacto-ovo-vegetarians and 0.6 µg for the total vegetarians.

When nutrients were expressed as percent of calories, the total vegetarians consumed a significantly smaller percent of calories as protein, but there was no significant difference in the percent of calories consumed as fat or carbohydrate or in the percentage of required calories consumed. When nutrient intake was expressed as grams per day, the total vegetarians consumed significantly fewer grams of protein and fat and significantly more grams of fiber than the lacto-ovo-vegetarians. Both groups consumed fewer calories than required, but the total vegetarians consumed significantly fewer calories than the lacto-ovo-vegetarians.

There was no significant difference in the amount of urine excreted by the two groups, but the total vegetarians excreted a significantly greater amount of feces. This is probably a result of the greater fiber intake by the total vegetarians. There was no significant difference in the amount of B_{12} excreted in the feces or urine of the two groups.

A positive correlation was found between the percentage of calories as protein in the diet and the level of B_{12} in the serum. This may be a result of an increased synthesis of the transcobalamin proteins. There were no other significant correlations between serum B_{12} and dietary components, but in the lacto-ovo-vegetarians there was a positive correlation between serum B_{12} and fecal B_{12}.

A positive correlation was also found between serum B_{12} and the period of time on a total vegetarian diet; serum B_{12} increased as the number of years on the diet increased.

This study concludes that the amount of vitamin B_{12} consumed by a total vegetarian can be adequate to maintain serum B_{12} unless the individual has extra needs (infants or the elderly). The need for dietary B_{12} can be met with a balanced diet containing adequate protein and B_{12}-fortified plant foods.

LOMA LINDA UNIVERSITY

Graduate School

EFFECT OF LACTO-OVO AND TOTAL VEGETARIAN DIETS ON SERUM, URINARY AND FECAL B_{12} LEVELS

by

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

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LITERATURE REVIEW

A diet of plant foods has long been by necessity a way of life for much of the world's population, and even in more affluent nations a vegetarian diet has been adopted for a variety of social, moral and physical reasons.

In recent years, political and medical science have brought the public to an awareness of crucial food issues: will there be enough food to support life on this earth, and what foods will improve the quality of that life? With the threat of a world food shortage, attention has been turned to the efficiency of food production and the increasing necessity of using plant proteins to replace animal proteins. Animal food sources are also increasingly implicated in modern diseases for reasons such as their lack of fiber and high content of saturated fats.

The adequacy of a diet free from animal products is thus of primary interest. There no longer seems to be any doubt that a vegetarian diet which includes dairy products and eggs contains adequate nutrition for a high quality of human function. The 1974 statement on vegetarian diets by the National Research Council[78] concludes that man's nutrient requirements can be met by the vegetarian diet, but warns those who exclude all animal products from their diet--milk, eggs and cheese as well as meat. This type of diet is called the total vegetarian diet to distinguish it from the lacto-ovo-vegetarian diet which includes dairy products and eggs. The warning is made because foods containing animal protein usually also contain vitamin B_{12}, but this vitamin is very scarce, if not absent, in plant foods.

History

Many questions about vitamin B_{12} have not yet been resolved, partially because the field of vitamin B_{12} research is still relatively young. Even though Minot and Murphy discovered in 1926 that eating liver cured pernicious anemia,[52] it was not until 1948 that the crystalline liver factor was isolated and given the name vitamin B_{12}.[64,73] In 1955, Hodgkin determined the structure of vitamin B_{12},[41] and in 1973 the vitamin was synthesized--25 years after its isolation.[50]

Chemistry

Vitamin B_{12} is a red crystalline substance which is freely soluble in water and alcohol, but insoluble in acetone, chloroform or ether. It is labile in strong acid, alkali and light.[71,44] This complex vitamin has

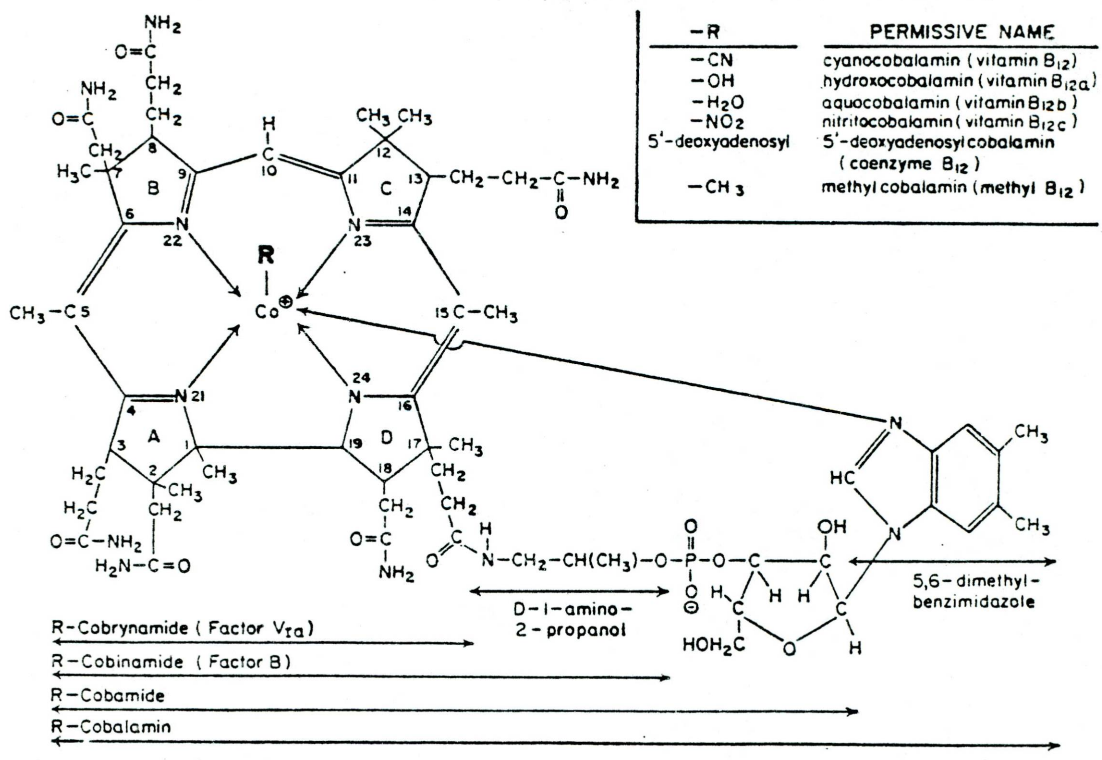

Figure 1. Structure of vitamin B_{12}

a molecular weight of 1357[44] and consists of two principle parts: a porphyrin-like ring structure with a cobalt atom in its center, and a nucleotide containing phosphorylated ribose (see Figure 1).[50,6] The presence of the cobalt atom gave rise to the generic name cobalamin.[35] In the stable extraction form there is a cyano group attached to the cobalt atom,[75] however cyano-cobalamin is partially oxidized and is not metabolically active until it is reduced. The cyano group can be replaced with hydroxo or methyl groups. These forms, along with coenzyme B_{12} (5'-deoxyadenosyl-cobalamin), are active in man.[35]

Absorption

Vitamin B_{12} is the largest and most complex of the B vitamins and for its absorption the presence of a still larger molecule, intrinsic factor (IF), is required (M.W. in the range of 50,000). The process of absorption is still somewhat obscure and there is the possibility that even if B_{12} is present in the diet the body will not be able to utilize it.

The vitamin is released from animal protein in cooking or by the stomach acids.[1] The parietal cells secrete the intrinsic factor, a glycoprotein which binds to the free vitamin B_{12} in order to navigate the vitamin to the ileum. The IF-B_{12} complex, protected by pancreatic secretions,[11] arrives at the receptor sites on the microvilli of the ileal mucosal cells.[14] The physical adsorption of the complex is calcium-dependent[37] and is aided by a pH of 6 or above. Whether the entire IF-B_{12} complex enters the cell[82] or whether B_{12} enters alone has not been determined, but no IF is found in the portal vein.[82,27,37,48,35] 1.5 to 3.0 µg of B_{12} can be absorbed at one time by this intrinsic factor method, but if the dose is greater than 50 μg, some (about 1%) is absorbed through simple diffusion.[72,35] Any B_{12} not absorbed enters the feces, and is joined by large amounts produced by intestinal microflora.

Once in the mucosal cell, B_{12} combines with cell protein and is slowly transported through the cell for passage into the portal vein where it attaches to a glycoprotein carrier, transcobalamin II (β globulin).[37] Passage into the plasma takes two to three hours, with peak levels within eight to twelve hours.[16] Almost all the absorbed vitamin stays within the body as the kidney can only excrete unbound B_{12}. However, if insufficient glycoprotein carriers are available, the absorbed but unbound vitamin will be excreted in the urine.[72] Bound B_{12} is circulated through the bloodstream to its sites for metabolic activity.

Whereas methylcobalamin is the main serum transport form, coenzyme B_{12} is the main storage form.[35,72] Transcobalamin I (α globulin) is the binding protein responsible for storage.[35] Body stores range from one to ten milligrams[22,60] of which 50-90% is in the liver.[34] Total body reserves seem to be reflected closely by levels in the blood serum. After an injected dose the serum level rises, but falls within hours to its former equilibrium value, therefore B_{12} status can be assessed by a simple serum assay.[72] Adequate serum levels range from 140-900 pg/ml, and less than 80 pg/ml could be indicative of a B_{12} deficiency.[80] There are variations of 80 pg/ml daily and 160 pg/ml monthly.[68] There are no differences between the sexes, but serum levels are somewhat lower in the aged.[10,68] The plasma level represents only 0.1% of the total body content.[68]

Excretion

Any loss of vitamin B_{12} occurs by excretion rather than catabolism.[35] About 34% of the ingested dose is excreted in the feces,[44] and is added to the B_{12} synthesized by intestinal bacteria. Shinton reports an average fecal excretion of 3-6 µg/day.[68] However, using the radioassay with samples from total vegetarian subjects, Lee reports fecal B_{12} ranging from 8-19 µg/day.[45] These subjects were on a high fiber diet, and their fiber intake was found to be positively correlated with their fecal B_{12}. However, adaptation of the radioassay method to fecal B_{12} assay by Lee has not been correlated with known microassays.

In the bile, up to 40 µg[68,56,63,62] are excreted daily, but most of the vitamin is reabsorbed in the ileum. Any that is not reabsorbed would be included in the fecal excretion. This enterohepatic circulation is an efficient method of conserving B_{12} and accounts for the length of time required to deplete body stores.

Unbound vitamin B_{12} is excreted by the kidneys at an average rate of 0-250 mµg daily.[5,25,32,54,61,68] Jathar found the amount of B_{12} excreted was lower in lacto-vegetarians (37 mµg/d) than in non-vegetarians (69 mµg/d), however the vegetarians' absorption was just as efficient--suggesting adaptation to a low B_{12} diet.[42] Lee, using the radioassay with total vegetarian subjects, found urinary B_{12} levels ranging from 160-1490 mµg/d.[45] No hypothesis for such high levels was proffered, but may be due to radioassay technique.

Woodliff and Armstrong[85] found that in normal patients high plasma levels correlated with high urinary excretion and low fecal excretion.

In malabsorptive patients a low plasma level correlated with low urinary excretion and high fecal excretion.[85] Whole body excretion rates are 0.1 to 0.2% daily.[9,22,39]

Metabolism

Vitamin B_{12} functions as a coenzyme in cellular reactions and as a methyl donor, thereby influencing the synthesis of nucleic acids and the metabolism of the major food elements. The synthesis of thymidylate, necessary in the formulation of DNA and RNA, is dependent upon vitamin B_{12}.[38] In B_{12} deficiency, therefore, cells do not divide properly, and can be first detected in the rapidly multiplying tissues such as the production of red blood cells by the bone marrow. In a deficiency state only a few immature large cells are produced, resulting in megaloblastic anemia.[8] Another area of high cell turnover is the digestive tract where disturbance leads to a sore tongue, indigestion and bowel disorders.[35]

Vitamin B_{12} maintains the integrity of the central nervous system by its role in myelin synthesis.[24] B_{12} deficiency will result in spinal cord deterioration producing symptoms such as back soreness, numbness in hands and feet, and loss of balance. Confusion, overt psychosis and even death can eventually follow.[35]

Vitamin B_{12} is involved in protein synthesis through its role in the methylation of homocysteine to synthesize methionine.[71] It is involved in both fat and carbohydrate metabolism through its coenzyme role in converting methylmalonyl CoA to succinyl CoA which can enter the Kreb's cycle.[49] In B_{12} deficiency urinary excretion of methylmalonate is increased and highly-branched chain and odd-numbered fatty acids build up in the blood thus providing a diagnostic assay.[2] Vitamin B_{12} also functions to keep sulfhydral groups in the reduced state necessary for carbohydrate metabolism.[49]

Apart from a lack of vitamin B_{12} in the diet there are other causes of B_{12} deficiency. Deficiency may be caused by inadequate absorption due to failure of the stomach to adequately secrete intrinsic factor. Gastric or small intestinal disorders, drugs, or competitive parasites or bacteria may adversely affect absorption. There may also be inadequate utilization of vitamin B_{12} in protein malnutrition or calcium deficiency. Children, pregnant women and hyperthyroid cases have an increased requirement for vitamin B_{12}. In cases of inadequate binding protein there may be increased excretion of B_{12}.[36]

Sources of Vitamin B_{12}

All vitamin B_{12} available to man comes from non-plant sources--ultimately from synthesis of microorganisms in soil, water and animal intestines. Vitamin B_{12} synthesized in the human intestine is generally considered not to be available for absorption,[26,44] but ruminants such as cows and sheep readily absorb the vitamin because it is produced in the fore-stomach prior to digestion; thus their tissues are a rich source of the vitamin.[34] Higher plants cannot manufacture vitamin B_{12}, but certain species of algae appear to have the ability and some seaweeds contain a little due to the microorganisms living on their fronds.[72] When vitamin B_{12} is found in higher plants it seems to be due to a contaminant (soil or mold),[72] confined to root nodules of legumes[35,68] or occasionally in stems and leaves if roots are in a strong B_{12} solution such as concentrated manure.[72] Any vitamin B_{12} from such plant sources would be insufficient for man's needs. Rich sources of cobalamin are lamb and beef organ meats and bivalves.[35] Moderate amounts are found in muscle meats and seafood, and only small amounts in dairy products.[68] One cup of milk or one large egg contains 1.0 μg of B_{12},[65] and the Recommended Daily Allowance for adults is 3.0 μg. It is, therefore, not difficult for the lacto-ovo-vegetarian to meet the requirement and non-vegetarians often consume up to 85 μg daily.[68]

Studies on Dietary Vitamin B_{12}

It appears that total vegetarians have no dietary source of vitamin B_{12}. The question, however, seems to be not why some people on this form of diet develop B_{12} deficiency, but why many subjects do not.

Studies of the total vegetarian diet over the past 20 years in the United States and Great Britain are in agreement that no protein deficiency is detected where there is adequate intake of calories from unrefined foods. Combinations of legumes, nuts, and cereals provide adequate essential amino acids in the diet.[12] Hardinge, Crooks and Stare concluded that the total vegetarian diet generously exceeds twice the minimal requirement of essential amino acids.[30] The significant condition that develops in some total vegetarians is a vitamin B_{12} deficiency. In spite of the low dietary intake of vitamin B_{12}, it has been observed that fewer than half of total vegetarians studied developed low B_{12} serum levels.[18] Even those with lower than recommended serum levels often go for years without presenting symptoms of deficiency.

The normal body store of the vitamin in omnivores is estimated at

2-5 mg; sufficient to maintain adequate serum levels for up to five years.[20,22,60] The obligatory daily rate of loss is about 0.1% of the body pool, so a minimum daily adult requirement of vitamin B_{12} is approximately 0.1-1.0 µg,[20,35,76] although the RDA is 3.0 µg, from which 1.0-1.5 µg is absorbed.[28]

The range of serum B_{12} levels of normal healthy persons lies between 140 and 900 pg/ml, an average for omnivores being about 350 pg/ml, for lacto-ovo-vegetarians about 250 pg/ml,[20] and for total vegetarians approximately 220 pg/ml (averages from all studies in this review). Values below 80 pg/ml indicate vitamin B_{12} deficiency, and between 80-140 pg/ml there may or may not be deficiency signs and symptoms.[20]

Some of the symptoms of vitamin B_{12} deficiency are megaloblastic anemia, menstrual disturbances in young women, nervous symptoms, numbness of hands and feet, a sore tongue, and pains and stiffness of the back and spine.

In 1954, Hardinge and Stare[31] conducted the first major study of the total vegetarian diet. In a group of 26 subjects, each of whom had been on this diet for more than five years, they found no deficiency symptoms.

Wokes[84] studied 149 British total vegetarians and compared the results with Dutch and American studies.[15,31] The British group had serum B_{12} levels ranging from 45-193 pg/ml with a mean of 111 pg/ml. Fifty percent of the total vegetarians had serum levels below 120 pg/ml. Wokes found oral symptoms in 27% of the subjects and back pains in 20%. This compared with 12% and 6% respectively for the Dutch population. No clinical symptoms were found in the Americans. Since a diet poor in protein can affect the serum B_{12} levels, the total protein intakes of the total vegetarians were taken into consideration. The Americans obtained 10.4% of their dietary calories from protein, the Dutch 9% and the British about 7%. Wokes thought that the low protein intake for the British might help to explain their lower serum B_{12} level and increased number of deficiency symptoms. He also hypothesized that the more years spent on the total vegetarian diet, the greater incidence of deficiency symptoms, and the lower the serum level of B_{12}. The range of serum levels obtained, he felt, was due to differences of the supply of B_{12} from the subjects intestinal flora.

Smith[70] studied 12 total vegetarians, only three of whom showed clinical deficiency symptoms (subacute combined degeneration of the cord). The symptoms present in Wokes' study[84] were absent from this group. The mean serum B_{12} was 128 pg/ml with a range of 48-220 pg/ml. Ten of the subjects had been on the diet for more than 12 years. Smith discussed a theory that the lack of symptoms in nine of the subjects was due to the major protion of their low serum B_{12} being in the hydroxo form, but this theory may have been unnecessary as only one of the subjects had a level (48 pg/ml) below the minimum required 80 pg/ml. Smith also found EEG's of eight of the subjects to be abnormal, but Ellis[20] states that this could not be due to lowered serum B_{12} levels since large doses of the vitamin failed to correct the EEG's. Smith found low iron levels, but no hypochromic anemia, for which he had no explanation. The lowered iron levels he blamed on the considerable quantity of phytic acid in the wholemeal bread of the total vegetarian diet. The absence of macrocytic anemia normally expected from low serum B_{12} levels was explained by the fact that these subjects consumed an unusually large quantity of folic acid.

Ellis and Mumford[20] found a mean serum level of 253 pg/ml in their total vegetarian subjects with a range of 50-650 pg/ml. They also found a high serum folate level of 14.6 ng/ml in the total vegetarians compared with a low level of 4.9 in the controls (normal levels are 6-20 ng/ml). Increased levels of folate may be a result of the high intake of fruits and vegetables.

Ellis and Wokes[21] studied 31 total vegetarians, many of whom used supplements of vitamin B_{12}. Their mean serum B_{12} was 166 pg/ml; 39% of the subjects had levels below 140 pg/ml. This study recommended that total vegetarians should supplement their diet with vitamin B_{12} preparations.

Ellis and Montegriffo[19] studied 26 total vegetarians who had a mean serum B_{12} level of 236 pg/ml, with a range of 30-650 pg/ml. Some of these were consuming B_{12} supplements. Nine subjects had serum levels below 140 pg/ml with no deficiency symptoms (three of these had levels less than 80 pg/ml). Three subjects had been on the total vegetarian diet for 17 years, taking no vitamin B_{12} supplements, and had serum levels of 450, 375 and 150 pg/ml. Another subject had been on the diet for 13 years, taking no supplements, and had a level of 275 pg/ml. These levels are exceptionally high and compare with serum levels in the non-vegetarian controls (441 pg/ml). The total vegetarian subjects again had a much higher serum folate level than the controls. Three of the subjects had very low serum B_{12} levels (30-60 pg/ml) but showed no signs of anemia, and Ellis theorizes that the high serum folate levels prevent the development of megaloblastic anemia. Ellis also theorizes that the four total vegetarians who had been on the diet for 13 years and longer were possibly absorbing intestinal synthesized vitamin B_{12}. Another explanation he offered, was that they maintained a natural enterohepatic circulation of vitamin B_{12} and economized their small body store, although it seems unlikely that this could maintain a serum B_{12} for as long as 13 years.

Lee[45] compared serum B_{12} levels of total vegetarians, lacto-ovo-vegetarians and non-vegetarians. The 18 total vegetarians had a mean level of 440 pg/ml (ranging from 100-1020 pg/ml), the 10 lacto-ovo-vegetarians had a mean of 680 pg/ml, and the 11 non-vegetarians had a mean of 1159 pg/ml. The diet of these total vegetarians was different in that it consisted largely of raw foods. These serum levels are much higher than those in previous studies. This could be due in part to the use of the radioassay method of determining vitamin B_{12} rather than the microbiological assay widely used previously. The radioassay is highly specific and not influenced by antibiotic or sulfa drugs. Lee found, contrary to Wokes,[84] that serum B_{12} was not related to the length of time the subject had been on the total vegetarian diet. Lee also ran correlation studies comparing serum levels of B12 with carbohydrate intake, fat intake, total unsaturated fatty acids, fiber, protein intake, amount of vitamin B_{12} found in feces, urine, or feces and urine. Protein intake was the only factor to show significant correlation with serum B_{12}

levels. An increased intake of protein increased the amount of B_{12} in the serum. The amount of vitamin B_{12} found in the feces was not influenced by the amount of protein, carbohydrate, total fat, or total unsaturated fatty acids. However, more fiber intake produced more vitamin B_{12} in the feces. The amount of vitamin B_{12} excreted in the urine was not influenced by any of the nutrient intakes.

Serveral case reports[18,40,83] have been published of vitamin B_{12} deficiencies in certain individuals who used no animal foods. In these individuals, symptoms were usually reversed by injections of vitamin B_{12} and a change to a lacto-ovo-vegetarian diet. It seems evident that a lack of B_{12} caused the deficiency symptoms but it is not known why these individuals developed deficiency symptoms where many others on the same diet did not.

<u>Lack of Deficiency Symptoms in Total Vegetarians</u>

Several theories have been proposed as to why total vegetarians do not develop deficiency symptoms. Length of time on the diet is one factor; if it is less than five years the body may still be using its store of vitamin B_{12}.[20] However, there are many reported cases of total vegetarians who have been on the diet for much longer; their reserves are used up, the serum B_{12} level falls to about 100 pg/ml and stabilizes at this near danger line, yet they appear to have excellent health.[19,72] It is a puzzle to determine where they get enough B_{12} to maintain even this low level. There is, of course, the enterohepatic circulation, where B_{12} released in the bile is reabsorbed in the intestine: this may be operating more efficiently. Indeed, Hepner[33] has found the enterohepatic circulation of cholic acid to be more efficient in vegetarians than in non-vegetarians; daily fractional turnover rate of cholic acid was significantly smaller in the vegetarians. Jathar's[42] finding that vegetarians have a lower urinary B_{12} excretion while having an equally efficient absorption also suggests an adaptation that improves B_{12} conservation.

It is also possible that pure vegetarians are absorbing intestinally synthesized vitamin B_{12}. It has been suggested that there is an invasion of the normally sterile upper intestine by B_{12}-producing microorganisms, perhaps stimulated by a high fiber diet.[45] Or, it is possible that an ability has been acquired for absorption from the lower intestine.[86] There is support for the theory that meat eating has caused the bacterium E. coli to move lower into the digestive tract beyond the point of absorption.[46] It may be that a return to a total vegetarian diet pattern would encourage the upward movement of E. coli.

Sometimes vitamin B_{12} deficiency symptoms are slow in appearing. The reason for this is the close relationship between B_{12} and folacin. A diet high in folic acid, as is often the case with vegetarians, can retard the development of macrocytic anemia. However, the neurological damage continues unnoticed until it is very severe.[35]

The fact that some total vegetarians quickly develop deficiency symptoms is not necessarily attributable to their diet; B_{12} deficiency is at least as prevalent among meat eaters for a variety of malabsorptive reasons.[66] In total vegetarians without absorption disorders, deficiency of B_{12} has been mostly in those who have switched, perhaps too abruptly, from a diet based on animal products.[86] It has been observed, but not documented, that children brought up from birth on a total vegetarian diet are able to absorb B_{12} from bacterial production in the colon.[86] It may be that a metabolic alteration due to meat eating has made it impossible for a small number of people to change to a pure vegetarian diet later in life without showing signs of B_{12} deficiency.

Diet Factors and Vitamin B_{12} Levels

Since vitamin B_{12} is so closely involved with protein, carbohydrate and fat metabolism it would be expected that dietary components, besides dietary B_{12} would have an effect on body levels of the vitamin. Studies have shown that rats on a high protein diet develop a B_{12} deficiency that can be fatal.[17,69] This indicates that B_{12} is involved in the metabolism of proteins so that an increase in protein increases the demand for B_{12}.

Some studies have suggested that a low protein diet results in a decreased synthesis of the transcobalamin proteins, resulting in the release of free B_{12} into the serum and its ultimate excretion.[47,59] Lee[45] reported that in pure vegetarians with low protein diets (19-53 grams per day) that serum B_{12} was positively correlated with dietary protein. However, Jathar[43] in studies with protein-starved rats, found considerably higher concentrations of B_{12} in the serum and several other organs and tissues than with controls; this despite the fact that serum protein concentrations were lowered. This prompted the researchers to speculate that the B_{12} binding material was non-protein and increased during early protein depletion. Absorption of B_{12} was also improved in the protein-depleted rats. Studies of protein malnourished children also found a higher serum B_{12} activity than for a control group.[57] It may be that if protein depletion is severe enough to cause liver damage, the storage of B_{12} is interfered with, resulting in an accumulation of the vitamin in the serum.

In a study of total vegetarians on a high-fiber diet (14 g/day) Lee[45] found a positive correlation between the amount of fiber in the diet, and the amount of fecal B_{12}. Plant fiber is resistant to digestion by human enzymes, yet much is metabolized by intestinal microflora. Some of these microflora produce B_{12}, and the increased fiber may stimulate their growth. Sutton and Elliot[77] reported that, in sheep, B_{12} production in the intestine increased as the percentage of roughage in their diet increased. Ruminants can take advantage of this increased production of B_{12} and absorb the vitamin but it is generally believed that humans cannot. Lee found no correlation between increased fiber intake and serum B_{12} concentration in total vegetarians.

Cullen and Oace,[13] in work with rats, found that cellulose and pectin increased fecal B_{12} excretion, but also the urinary excretion of methylmalonic acid (MMA). MMA excretion indicates a deficiency of a B_{12} containing coenzyme responsible for the conversion of methylmalonyl CoA to succinyl CoA. Their conclusion was that fiber may interfere with the enterohepatic reabsorption of vitamin B_{12}, thus creating a deficiency state in the host. Also interesting was the fact that pectin caused far greater MMA excretion than cellulose, thus indicating the importance of differentiating between fibers in dietary studies.

PURPOSE

It is the purpose of this research to study the effect of dietary components on the storage and excretion of vitamin B_{12} in the body. An attempt will be made to (1) determine whether vegetarians without a dietary source of vitamin B_{12} have body levels of the vitamin significantly different from lacto-ovo-vegetarians; (2) ascertain correlations between the following factors: years on the total vegetarian diet, carbohydrate, fat, protein or fiber intake, serum B_{12}, urinary B_{12} and fecal B_{12}; (3) compare serum assay results with previous literature on total vegetarians; (4) and compare urine and fecal B_{12} levels assayed microbiologically with a previous radioassay of urine and fecal B_{12}. (see Appendix B).

METHODS AND MATERIALS

Design

Vitamin B_{12} was assayed microbiologically in serum, urine and fecal samples of ten lacto-ovo-vegetarians and nine total vegetarians. Dietary analysis was carried out using a 7-day diet history and a questionnaire. Correlations were then determined between dietary and assay results.

Subjects

The ten lacto-ovo-vegetarian subjects were all from the Loma Linda University area. Their ages ranged from 16 to 49. There were six females and four males. Of the nine total vegetarians, seven were from one extended family and two ate only raw unprocessed foods. Their ages ranged from 3 to 64. Six were females and three were males. The length of time on the total vegetarian diet ranged from 3 to 14 years. The four children (ages 3-12) had been on the diet all their life, and had consumed soymilk formula as infants.

All subjects completed a questionnaire which determined how often they consumed dairy products, eggs or B_{12}-fortified foods (Appendix A).

Diet Analysis

A 7-day diet history was completed by all 19 subjects. Computer analysis of these diets for intake of protein, fat, carbohydrate, calories and fiber was completed using the Agriculture Handbook No. 8[79] and Agriculture Handbook No. 456.[3] The required calories per day for each subject were calculated according to age and weight using the standards set for the 1971 National Nutrition Survey (derived from WHO, ICNND and NRC recommendations).[55] Dietary carbohydrate, fat and protein were also determined as percentage of calories consumed using an average of the conversion values in Agriculture Handbook No. 8.[79]

Serum, Urine, and Fecal Collection and Preparation[6,29,45]

All glassware used throughout the procedure was acid washed and rinsed with distilled water up to 12 times, because a B_{12} contaminant as small as 0.01 mμg can give a definite growth response in the assay.[51]

From each subject, 10 ml of blood was drawn into Vacutainers containing no anticoagulant. The blood was permitted to clot at room temperature then separated by centrifugation. Two ml of the serum were diluted with 8.0 ml of aconitic acid buffer (transaconitic acid, 5.0 g; water, 1000 ml; KOH to pH 4.5). For use with the serum, 0.5 mg/ml sodium metabisulfite was added to the buffer; a total of 2.0 mg sodium metabisulfite for every milliliter of fluid to be analyzed. Serum samples were then frozen until needed for assay, at which time they were capped loosely and autoclaved for 30 minutes to destroy any antibiotic effect and to release the B_{12} from its protein carriers. Tubes were then centrifuged and 0.5 and 1.0 ml of the supernatant were added to assay tubes.

Each subject was given two one-liter bottles, containing a toluene preservative, for a 24-hour urine collection. After the total volume of each sample was measured, 15 ml were transferred to small beakers for freezer storage. To prepare for assay, 1.0 ml of sample was pipetted into a centrifuge tube and diluted with 1.0 ml of aconitic acid buffer containing 0.1 mg/ml sodium metabisulfite. Samples were loosely covered and autoclaved 30 minutes. Each tube was then diluted with 8.0 ml of sterile water and centrifuged. 1.0 and 0.5 ml of the supernatant were added to assay tubes.

A camp stool with plastic bags was provided to each subject for the 3-day fecal collection, and samples were frozen immediately after each collection. The total weight of each 3-day sample was recorded and the sample was homogenized with sterile water in milliliters equal to five times the weight of the sample in grams. One milliliter of each was then diluted 50 times with sterile water and stored in a freezer. For assay, 1.0 ml of aconitic acid buffer containing 0.1 mg/ml sodium metabisulfite was added to each milliliter of fecal sample. Samples were then loosely covered, autoclaved 30 minutes, centrifuged and 0.5 and 1.0 ml were added to assay tubes.

Ochromonas malhamensis Assay[6,29,74]

The quantity of vitamin B_{12} in human tissue is so small that it can only be measured microbiologically or by radioassay. Many microorganisms require vitamin B_{12} for growth but most investigators agree that Ochromonas malhamensis is the most specific of the vitamin B_{12} assay organisms in its requirement for cobalamins. Only clinically active forms of vitamin B_{12} satisfy the growth factor requirement of this protozoan. The concentration of vitamin B_{12} in a sample will be proportional to the amount of growth of the organism, which can be assessed turbidimetrically. O. malhamensis is economic, sensitive-- detects 1.0 pg of B_{12} per milliliter-- and is relatively free from stimulation in biologic fluids and tissues.[4,7,23,53,81]

Culture and Inoculum

Ochromonas malhamensis ATCC 11532 was maintained in single strength Difco Bacto-B_{12} Ochromonas Medium to which was added 0.2 mμg B_{12}/ml and autoclaved according to the Difco Manual.[51] The organism was incubated in 10 ml amounts in a cabinet beneath a tungsten lamp and 0.5 ml transferred at 4-6 day intervals to new media.

For inoculum, the 4-6 day old culture was centrifuged, supernatant discarded and organisms washed with 10 ml single strength assay medium three times to avoid any trace of B_{12} from the culture media. Organisms were resuspended in 10 ml single strength medium then diluted 1:2 with an additional 10 ml single strength medium. Two drops of this solution were used to inoculate assay tubes, and care was taken to keep the organism uniformly suspended during inoculation.

Assay Standards

U.S.P. Cyanocobalamin Reference Standard was dissolved in 25% ethyl alcohol to a dilution of 10.0 μg B_{12}/ml and stored at 2-4° C. Each time an assay was run a standard curve was prepared in duplicate. To each tube was added a dilution of the B_{12} standard, 1.0 ml of five times strength assay medium* and sterile water to a total volume of 5.0 ml. The final B_{12} concentration of the tubes in pg/ml was 0.3, 0.6, 1.0, 3.0, 6.0, 10, 30, 60, 100 and 300. Two blanks were also set up containing 4.0 ml sterile water and 1.0 ml five times strength assay

*Contained 2.0 mg NaCn/100 ml.[6] The cyanide ensures a more complete extraction of B_{12}.(74)

medium.

To the 0.5 and 1.0 ml of sample in each tube was added sterile water to 4.0 ml total and 1.0 ml five times strength assay medium. Each sample dilution was prepared in triplicate.

All tubes were loosely capped and sterilized for 10 minutes at 15 pounds pressure (121^{o} C). After tubes cooled, all were inoculated with two drops of the inoculum as described, recapped and incubated in the dark on a slant at 28^{o} C for 72 hours.

A Gilford spectrophotometer was used to measure the growth turbidimetrically. A setting of 525 mμ was used for all readings. The inoculated water blank was used to zero the spectrophotometer, then readings were taken for the standard curve and the samples. The B_{12} content of each sample was determined by comparison with the standard curve.

RESULTS AND DISCUSSION

Diet Analysis

An examination of the diet questionnaires and the 7-day diet histories revealed that the lacto-ovo-vegetarians ate fewer animal products and B_{12} fortified foods than is expected in an average population. This may be due in part to an increased diet awareness resulting from their association with a Department of Nutrition. The lacto-ovo-vegetarians consumed a serving (1.0 µg) of B_{12}-containing foods approximately 14 times a week. Their average daily intake of 2.0 µg of B_{12} is lower than the RDA (3.0 µg) but higher than the minimum daily requirement (1.0 µg). The total vegetarians averaged a B_{12} intake of 4.0 µg per week, or 0.6 µg a day. This level of intake fails to meet the minimum daily requirement. Two of the total vegetarian subjects (#18, 19) ate only unprocessed raw foods and the others received their vitamin B_{12} from soy-milk or an occasional multi-vitamin supplement. Both groups of subjects, therefore, consumed relatively small amounts of vitamin B_{12} when compared with an average daily intake of 16 µg for non-vegetarians.

In Table I the sex and age of each subject is listed with the serum level and the period of time on the total vegetarian diet. Age and sex bear little correlation with serum level, except when absorption decreases in old age. One subject, a 64-year-old male, had a serum B_{12} level of 133 pg/ml which is below the normal range (140-900 pg/ml); it may be that his ability to absorb nutrients was decreasing. The period of time on a total vegetarian diet can influence the serum B_{12} as noted in the literature review; the correlation between years on the diet and serum B_{12} will

TABLE I

SERUM B_{12} LEVELS OF LACTO-OVO-VEGETARIANS AND TOTAL VEGETARIANS IN RELATION TO AGE, SEX AND YEARS ON DIET

SUBJECT	SERUM B_{12} pg/ml	SEX/AGE	YEARS ON DIET
LACTO-OVO-VEGETARIANS			
1	675	F 26	
2	925	M 32	
3	225	M 30	
4	475	M 25	
5	300	M 24	
6	460	F 21	
7	260	F 49	
8	875	F 16	
9	1600	F 25	
10	775	F 24	
MEAN:	657		
S.D.:	417		
TOTAL VEGETARIANS			
11	360	M 36	14
12	775	F 36	14
13	750	F 12	12
14	270	F 9	9
15	400	F 7	7
16	185	F 3	3
17	133	M 64	6
18	320	F 28	4
19	235	M 34	7.5
MEAN:	381		
S.D.:	232		

be discussed below. The four children who were raised on a total vegetarian diet (subjects 13-16) all have adequate serum B_{12} levels (185-750 pg/ml).

The results of an analysis of the 7-day diet histories are shown in Tables II and III. Table II expresses the daily intake of nutrients as a percent of calories. For the lacto-ovo-vegetarian the mean values of nutrients consumed were 12 percent of calories as protein, 57 percent as carbohydrate, and 31 percent of calories as fat. Eight of the ten subjects were consuming calories below their required levels; average caloric consumption was at 86% of that required.

For the total vegetarians, the mean values were 10% of calories as protein, 63% as carbohydrate and 28% as fat. None of the subjects were consuming their required calories; an average of 73% of required calories were consumed. The total vegetarians consumed a significantly smaller percent of calories as protein ($p<0.05$), but there was no significant difference in the amount of calories consumed as fat or carbohydrate or in the percent of calories required.

However, when nutrient intake was expressed as total grams per day (Table III) significant differences between the total and lacto-ovo-vegetarians were revealed. The lacto-ovo-vegetarians consumed 62 grams of protein, 272 grams of carbohydrate, 68 grams of fat, 8 grams of fiber and 1858 calories per day. The total vegetarians consumed 43 grams of protein, 232 grams of carbohydrate, 48 grams of fat, 11 grams of fiber and 1424 calories per day. The total vegetarians consumed significantly fewer calories, grams of protein and grams of fat, and significantly greater amount of fiber than the lacto-ovo-vegetarians ($p<0.05$). This is consis-

TABLE II

NUTRIENT INTAKE IN PERCENT OF TOTAL CALORIES PER DAY IN RELATION TO SERUM B_{12} OF LACTO-OVO-VEGETARIANS AND TOTAL VEGETARIANS

SUBJECT	SERUM B_{12} pg/ml	PROTEIN % Cal	CHO % Cal	FAT % Cal	CALORIES % of required
LACTO-OVO-VEGETARIANS					
1	675	12	57	30	126
2	925	10	57	32	91
3	225	9	65	27	59
4	475	14	38	45	48
5	300	10	62	26	76
6	460	11	62	27	88
7	260	12	61	29	88
8	875	11	48	38	62
9	1600	14	63	22	92
10	775	13	53	35	125
MEAN:	657	12	57	31	86
S.D.:	417	2	8	7	26
TOTAL VEGETARIANS					
11	360	10	68	25	79
12	775	10	63	28	82
13	750	11	65	25	56
14	270	11	65	25	55
15	400	10	64	27	86
16	185	10	68	23	73
17	133	12	64	25	91
18	320	8	54	38	94
19	235	7	55	38	41
MEAN:	381	10	63	28	73
S.D.:	232	2	5	6	18

TABLE III

NUTRIENT INTAKE IN GRAMS PER DAY IN RELATION TO SERUM B_{12} OF LACTO-OVO-VEGETARIANS AND TOTAL VEGETARIANS

SUBJECT	SERUM B_{12} pg/ml	PROTEIN gm	CHO gm	FAT gm	FIBER gm	CALORIES
LACTO-OVO-VEGETARIANS						
1	675	76	322	79	10	2208
2	925	78	377	97	10	2593
3	225	51	339	64	9	2038
4	475	59	145	79	4	1474
5	300	62	326	63	5	2044
6	460	55	277	56	4	1752
7	260	52	234	51	12	1504
8	875	42	166	60	4	1336
9	1600	62	252	41	7	1565
10	775	79	278	85	12	2062
MEAN:	657	62	272	68	8	1858
S.D.:	417	13	75	17	3	396
TOTAL VEGETARIANS						
11	360	49	295	50	14	1700
12	775	50	264	55	12	1645
13	750	44	240	43	11	1435
14	270	44	220	39	9	1325
15	400	41	225	44	10	1373
16	185	24	141	22	6	806
17	133	67	313	57	14	1910
18	320	44	241	74	17	1646
19	235	23	145	44	9	974
MEAN:	381	43	232	48	11	1424
S.D.:	232	13	59	14	3	355

tent with studies reviewed by Ellis[20] where total vegetarians were found to have a lower intake of calories, protein and fat than lacto-ovo-vegetarians.

Two of the total vegetarian subjects (#16,19) were eating a very low protein diet (24 and 23 g/day, respectively) and also had serum B_{12} levels that were low (185 and 235 pg/ml, respectively). This indicates a correlation between protein intake and serum B_{12} which will be calculated below.

Serum Vitamin B_{12} Levels

The serum B_{12} levels of lacto-ovo-vegetarians and total vegetarians were compared (Table IV). The lacto-ovo-vegetarians had a mean serum B_{12} level of 657 pg/ml, with a range of 225-1600 pg/ml. The nine total vegetarians had a mean value of 381 pg/ml ranging from 133-775 pg/ml. The difference between the serum levels of the two groups was found to be not significant ($p<0.05$). The total vegetarians were able to maintain a serum B_{12} not significantly different from that of the lacto-ovo-vegetarians despite the fact that their intake of B_{12} was one-third that of the lacto-ovo-vegetarians.

All serum B_{12} levels were within the normal range (140-900 pg/ml) except subject #9 with a level of 1600 pg/ml. This subject was also consuming a greater amount of B_{12} in the diet than the other subjects: 22 µg/week or 3.0 µg/day compared with an overall mean of 9 µg/week or 1.3 µg/day.

The mean serum levels of subjects in this study were higher than generally reported in past studies (250 pg/ml for lacto-ovo-vegetarians

TABLE IV

SERUM, URINARY AND FECAL B_{12} LEVELS OF LACTO-OVO AND TOTAL VEGETARIANS

SUBJECT	SERUM B_{12} pg/ml	URINARY B_{12} mμg/day	FECAL B_{12} μg/day
LACTO-OVO-VEGETARIANS			
1	675	32	1.3
2	925	34	4.5
3	225	40	1.8
4	475	81	2.6
5	300	68	0.2
6	460	123	8.9
7	260	78	0.8
8	875	37	5.0
9	1600	86	8.4
10	775	64	4.7
MEAN:	657	64	3.8
S.D.:	417	29	3.0
TOTAL VEGETARIANS			
11	360	82	5.4
12	775	44	6.3
13	750	34	3.1
14	270	75	2.6
15	400	38	0.6
16	185	14	0.9
17	133	90	5.1
18	320	65	4.0
19	235	93	8.3
MEAN:	381	59	4.0
S.D.:	232	28	2.5

and 220 pg/ml for total vegetarians). This may be due to the Ochromonas assay, the recent fortification of many foods with B_{12}, or a difference in dietary balance of the subjects. The serum levels of this study are, however, comparable to those reported by Lee,[45] whose total vegetarians had a mean serum B_{12} of 440 pg/ml and lacto-ovo-vegetarians had a mean of 680 pg/ml (radioassay method).

Urine and Fecal Analyses

The amount of vitamin B_{12} excreted through the urine and feces is shown in Table IV. For the lacto-ovo-vegetarians, the average value of B_{12} excreted in the urine was 64 mμg/day and in the feces was 3.8 μg/day. In the total vegetarians, the mean urine B_{12} was 59 mμg/day and 4.0 μg/day in the feces. There was no significant difference between the two dietary groups in the amount of B_{12} excreted ($p<0.05$).

The amount of urinary B_{12} excreted is consistent with the literature for microassays (0-250 mμg/day, p. 9). Fecal B_{12} excretion is also consistent with microassay literature (3-6 μg/day, p. 9).

Amounts of urine and feces excreted per day by the subjects are shown in Table V. In the lacto-ovo-vegetarian group the mean volume of urine excreted per day was 1033 ml and for feces was 123 grams. In the total vegetarian group the mean volume of urine was 1023 ml/day and for feces was 253 g/day. The daily urinary excretion was not significantly different for the two groups, but the amount of feces excreted by the total vegetarians was significantly greater than that of the lacto-ovo-vegetarians ($p<0.05$). The fact that the total vegetarians had a significantly greater intake of fiber as well as a significantly greater fecal

TABLE V

AMOUNT OF FECES AND URINE EXCRETED PER DAY BY LACTO-OVO-VEGETARIANS AND TOTAL VEGETARIANS

SUBJECT	FECES g/day	URINE ml/day
LACTO-OVO-VEGETARIANS		
1	76	738
2	263	620
3	178	590
4	85	950
5	68	1698
6	119	2060
7	108	1560
8	33	270
9	146	860
10	150	980
MEAN:	123	1033
S.D.:	66	563
TOTAL VEGETARIANS		
11	414	1020
12	201	890
13	511	680
14	146	1000
15	251	500
16	128	125
17	283	2140
18	235	1900
19	363	1970
MEAN:	253	1023
S.D.:	127	708

excretion is consistent with the literature. A high fiber intake effects a high water-binding capacity of the stool, resulting in an increased stool weight.

Interrelationships Between Variables

The correlation coefficients between variables were obtained. As there were 19 observations for each analysis, the absolute value of the correlation coefficient must be equal to or greater than 0.456 in order to be significant at a level of 0.05. For the group of ten lacto-ovo-vegetarians, the correlation coefficient must be greater than 0.632; for the group of nine total vegetarians it must be greater than 0.666 (p 0.05).

The correlation coefficients of variables in this study are shown in Table VI. A significant correlation of 0.48 was found between the percentage of calories as protein in the diet, and the level of B_{12} in the serum. This is consistent with Lee's[45] finding with a group of total vegetarians on a low protein diet. In moderately low protein diets, an increase in protein intake has been found to increase the serum B_{12} levels of the subjects. This may be a result of an increased synthesis of the transcobalamin proteins.[47,59]

There were no other significant correlations between serum B_{12} and dietary components, however a positive correlation between serum B_{12} and fecal B_{12} may be indicated (r=0.42). When the group of ten lacto-ovo-vegetarians was analyzed separately the correlation between serum B_{12} and fecal B_{12} increased to a significant 0.65 (p 0.05). Fecal excretion of B_{12} may result when dietary intake is very high, or when the enterohepatic system does not reabsorb all the B_{12} excreted in the bile. At higher serum B_{12} levels reabsorption need not operate as efficiently, thereby increasing the loss of B_{12} through the feces. Fecal

TABLE VI

SIMPLE CORRELATION COEFFICIENTS FOR DIETARY FACTORS AND YEARS ON DIET WITH SERUM, URINARY AND FECAL B_{12}

VARIABLES	COMPLETE GROUP (#1-19)	LACTO-OVO-VEGETARIANS (#1-10)	TOTAL VEGETARIANS (#11-19)
SERUM B_{12} and:			
PROTEIN[1]	0.48*	0.48	0.11
CARBOHYDRATE[1]	-0.17	-0.04	0.10
FAT[1]	-0.02	-0.15	-0.10
FIBER	-0.18	-0.02	0.11
FECAL B_{12}	0.42	0.65*	0.07
URINARY B_{12}	-0.11	-0.05	-0.42
YEARS ON DIET			0.71*
FECAL B_{12} and:			
PROTEIN[1]	-0.01	0.28	-0.23
CARBOHYDRATE[1]	-0.01	0.07	-0.35
FAT[1]	0.02	-0.17	0.36
FIBER	0.03	-0.31	0.28
URINARY B_{12} and:			
PROTEIN[1]	0.16	0.38	0.01
CARBOHYDRATE[1]	-0.01	0.11	-0.22
FAT[1]	-0.01	-0.19	0.19
FIBER	0.01	-0.37	0.33

[1]Nutrients expressed as percent of calories

*Significant correlation

B_{12} also results from B_{12} synthesized by microflora.

An interesting correlation was found when the period of time on the total vegetarian diet was compared with serum B_{12} levels. There was a significant correlation ($r=0.71$) between the number of years the subject had been a total vegetarian, and the level of B_{12} in the serum ($p<0.05$). In other words, the subjects who had been on the diet the longest also had the highest levels of serum B_{12}. All but two of the subjects had been on the diet for longer than five years and would not be expected to be utilizing body stores. One 36-year-old female subject who had been a total vegetarian for 14 years had a high serum B_{12} level of 775 pg/ml. Another 12-year-old female subject who had been a total vegetarian all her life had a serum B12 of 750 pg/ml (Table I). Since these subjects were consuming extremely small amounts of dietary B_{12} it may be that they were experiencing an increased ability to adjust and maintain serum levels on a low B_{12} intake, such as increased conservation through the enterohepatic circulation.

The correlations found above appear to be significant. However, it should be noted that the chance of obtaining significant results increases as the number of tests performed increases. It would be advisable to repeat tests on fewer variables with a larger sample size.

The interesting outcome of this study is that there was little difference in serum B_{12} whether the subjects' diet included animal products or not. All but one subject, who was elderly, had adequate serum B_{12} levels.

Now, more than ever, people are considering the question of whether they can survive on a total plant diet and thus maximize the world food supply and minimize health risks associated with an intake of animal products.

The total vegetarian diet has long been approved except for its lack of a vitamin B_{12} source. However, there has recently been a proliferation of B_{12}-fortified plant products, and vitamin B_{12} serum levels may now be maintained by a bowl of cereal, a glass of soymilk, or an occasional multivitamin supplement. Indeed, subjects in this study maintained serum levels on an average intake of only 4.0 μg of B_{12} per week. The children in the study, raised on soymilk formula, had adequate serum levels, but it appears that the elderly subject would be advised to increase B_{12} intake above the minimum. All persons should take care to eat a well-balanced diet, especially with regard to adequate protein intake, in order to maintain serum B_{12}.

The indication of this study is that there need no longer be a dependence on animal products to obtain vitamin B_{12}. Adequate B_{12} levels may be maintained with a well-balanced diet including B_{12}-fortified plant foods.

SUMMARY AND CONCLUSIONS

Serum, urinary and fecal levels of ten lacto-ovo-vegetarians and nine total vegetarians were assayed using *Ochromonas malhamensis*. Intakes of vitamin B_{12}, protein, fat, carbohydrates, fiber and calories were calculated using 7-day diet histories and questionnaires. Correlations between nutrients consumed and serum, urinary and fecal B_{12} levels were also calculated.

Mean serum B_{12} levels of the lacto-ovo-vegetarians and total vegetarians were 657 pg/ml and 381 pg/ml respectively. There was no significant difference between the two groups. The average daily intake of vitamin B_{12} was 2.0 µg for lacto-ovo-vegetarians and 0.6 µg for the total vegetarians.

When nutrients were expressed as percent of calories, the total vegetarians consumed a significantly smaller percent of calories as protein, but there was no significant difference in the percent of calories consumed as fat or carbohydrate or in the percentage of required calories consumed. When nutrient intake was expressed as grams per day, the total vegetarians consumed significantly fewer grams of protein and fat and significantly more grams of fiber than the lacto-ovo-vegetarians. Both groups consumed fewer calories than required, but the total vegetarians consumed significantly fewer calories than the lacto-ovo-vegetarians.

There was no significant difference in the amount of urine excreted by the two groups, but the total vegetarians excreted a significantly greater amount of feces. This is probably a result of the greater fiber intake by the total vegetarians. There was no significant difference in the amount of B_{12} excreted in the feces or urine of the two groups.

A positive correlation was found between the percentage of calories as protein in the diet and the level of B_{12} in the serum. This may be a result of an increased synthesis of the transcobalamin proteins. There were no other significant correlations between serum B_{12} and dietary components, but in the lacto-ovo-vegetarians there was a positive correlation between serum B_{12} and fecal B_{12}.

A positive correlation was also found between serum B_{12} and the period of time on a total vegetarian diet; serum B_{12} increased as the number of years on the diet increased.

Mean serum levels were higher than most of those reported in the literature. This may be due to the Ochromonas assay, the recent fortification of many foods with B_{12}, or a difference in dietary balance of the subjects. Serum B_{12} levels were, however, similar to those reported by Lee who used the highly specific radioassay method.

Urinary and fecal B_{12} levels corresponded to past literature but were lower than those reported by Lee[45] using the radioassay method. There are indications that the radioassay method is not accurate for urine and fecal assay without further adaptation (see Appendix B).

This study concludes that the amount of vitamin B_{12} consumed by a total vegetarian can be adequate to maintain serum B_{12} unless the individual has extra needs (infants or the elderly). The need for dietary B_{12} can be met with a balanced diet containing adequate protein and B_{12}-fortified plant foods.

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APPENDIX A: VITAMIN B_{12} QUESTIONNAIRE

DATE: ______________

NAME: ______________________________ PHONE: __________

ADDRESS: ______________________________________

AGE: ________ SEX: ________ WEIGHT: ________ HEIGHT: ________

/_/ I am a non-vegetarian.

How many times a week do you eat: Meat ________

Dairy products ________

Eggs ________

/_/ I am a total vegetarian.

Years as a total vegetarian ________

Previous years as a lacto-ovo vegetarian ________

Do you occasionally eat milk, eggs or cheese? ________

How often? ______________________________

Do you use the following: Amount

Soymilk ______________ ______________

Multiple vitamin supplements ______________

Vitamin B_{12} supplements ______________

Processed soy products ______________

Breakfast cereals (see attached)

/_/ I am a lacto-ovo vegetarian

Years as a lacto-ovo vegetarian ________

Do you occasionally eat meat? ________ How often? ________

How many times a week do you eat: Eggs ________

Cheese ________

Milk ________

BREAKFAST CEREALS

VITAMIN B_{12} IS PRESENT IN:

Post
Fortified Oat Flakes
Grape Nuts
Family-Style Cereal
Alpha-Bits
Honeycomb
Fruity Pebbles
Cocoa Pebbles
Super Sugar Crisp
40% Bran Flakes
Raisin Bran
Post Toasties
Grape-Nut Flakes

Kellogg's
Raisin Bran
40% Bran Flakes
Product 19

Nabisco
Team
100% Bran

General Mills
BucWheats
Total
Kix
Trix
Lucky Charms
Cocoa Puffs
Cheerios
Wheaties

Quaker
Quisp
Cap'n Crunch varieties

Ralston Purina
Wheat, Rice, Corn, Bran Chex
Grins & Smiles
Freakies

Hot cereals
Wheatena
Maypo

OTHER SOURCES

Torumel
Yeast-Plus
Hoffman's Hi-Proteen Reducing Plan Aids
Soyagen
Soyalac
Fortified Soyamel

APPENDIX B. COMPARISON OF MICROASSAY AND RADIOASSAY FOR VITAMIN B_{12} IN URINARY AND FECAL SAMPLES FROM TOTAL AND LACTO-OVO-VEGETARIAN SUBJECTS

SUBJECT	URINARY B_{12} mug/day		FECAL B_{12} ug/day	
	O. malhamen.	Radioassay	O. malhamen.	Radioassay
1	32	546	1.3	802
2	34	508	4.5	3515
3	40	413	1.8	715
4	81	760	2.6	392
5	68	2377	0.2	269
6	123	8446	8.9	550
7	78	1638	0.8	640
8	37	216	5.0	233
9	86	903	Not obtained	
10	64	745		
11	82	5100		
12	44	1068		
13	34	544		
14	75	940		
15	38	490		
16	14	375		
17	90	2996		
18	65	3610		
19	93	1950		

APPENDIX B (con'd)

Comparisons of the radioassay and microassay methods for determining serum B_{12} have shown that both methods give accurate and comparable results.[1] However, the radioassay method, when used to assay urinary and fecal B_{12} yields results vastly different from accepted microassay results.[2]

In this paper a trial of determining urine and fecal B_{12} with a radioassay technique[3] was attempted. Results are above. All radioassay readings were considerably higher than levels determined by O. malhamensis. Variations in readings on different days and with varying dilutions indicate a complicating factor in urine and fecal assays which interferes with the radioassay. Results found by Lee[2] must be considered in this light. Further investigations on application of the radioassay technique to urine and fecal samples should be conducted before this method is considered to be valid.

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