SERUM VITAMIN B_{12} IN VEGETARIANS

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The vegetables normally consumed by man in general have a negligible amount of vitamin B_{12} (Lewis *et al.*, 1949; Peeler *et al.*, 1951). The vitamin B_{12} content of milk is much lower than that of liver, meat, fish, and egg. Thus an average vegetarian diet may not have enough vitamin B_{12} to ensure optimum human nutrition. The vitamin B_{12} content of serum in vegetarians has been reported to be low (Wokes *et al.*, 1955; Dhopeshwarkar *et al.*, 1956; Chatterjea *et al.*, 1959). In nutritional macrocytic anaemia (N.M.A.) a low concentration of serum vitamin B_{12} has been found in approximately 50% of cases (Das Gupta *et al.*, 1955; Chatterjea, 1958). The main cause of vitamin-B_{12} deficiency in N.M.A. is held to be inadequacy of the diet (Das Gupta *et al.*, 1953; Chatterjea, 1958); the possibility of an intestinal malabsorptive factor has also been raised (Baker, 1958). A significant proportion of the population of India may be categorized as vegetarian, due to social custom, to religious tenets, or to poor economic status. The average red-cell and haemoglobin levels of vegetarian Indians cannot, however, be regarded as significantly different from those of non-vegetarians in India or in other parts of the world (Sokhey *et al.*, 1937, 1938; Das Gupta, 1949).

In view of the above considerations, it was thought worth while to investigate the serum vitamin B_{12} in two groups of subjects—non-vegetarian and vegetarian—as seen in the general population and in those with haematological disorders.

Material and Method

The present communication deals with observations on 232 subjects, consisting of 61 normal persons, 67 with tropical eosinophilia, 39 with N.M.A., 35 with aplastic anaemia, and 30 with chronic myeloid leukaemia. The distribution of non-vegetarians and vegetarians is shown in the Tables.

Subjects belonging to the vegetarian group were taking rice, wheat, vegetables, pulses, and milk, but no fish, meat, or egg. Each one of them had been taking the vegetarian diet for a period varying from 5 to 25 years. The daily consumption of milk was in general small, varying from 6 to 12 oz. (170 to 340 ml.) per head.

The serum vitamin B_{12} was estimated by the method of Ross (1952), using *Euglena gracilis* var. *bacillaris* as the test organism. The total vitamin B_{12} content was expressed in micromicrograms per millilitre.

Results

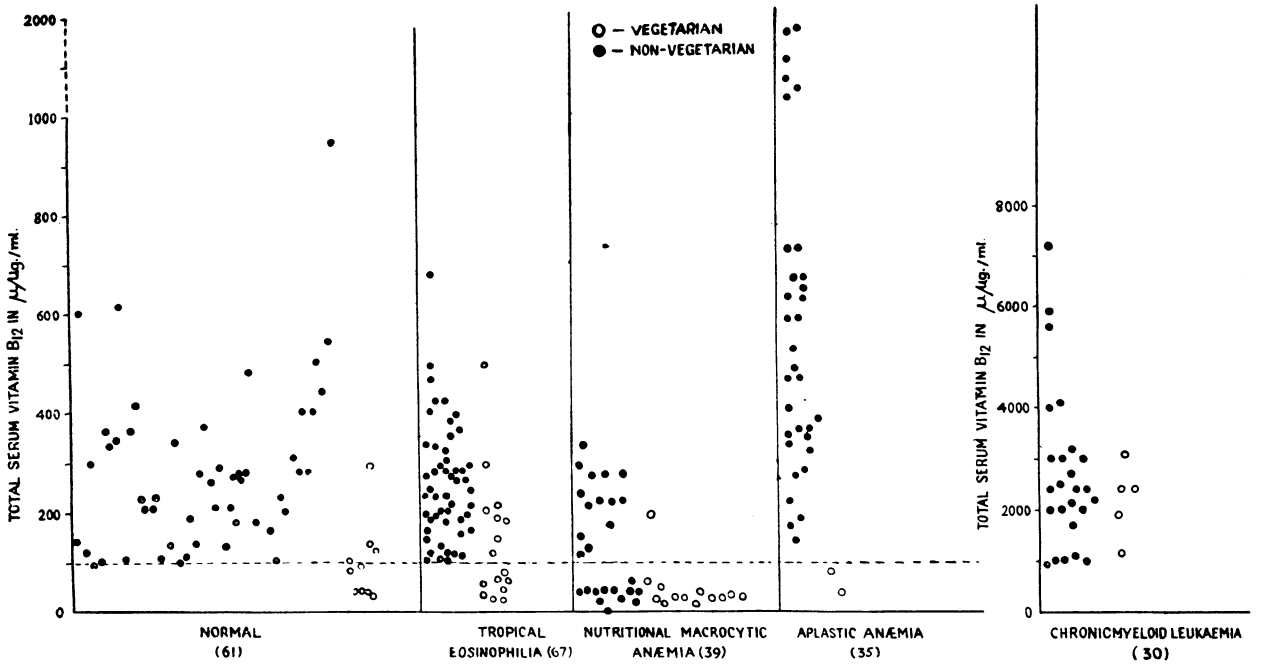
The concentration of total serum vitamin B_{12} in each individual subject belonging to the two groups is represented in the Chart.

The mean value and range of variation of total vitamin B_{12} of serum in the different subjects belonging to the two groups are recorded in Tables I and II.

From the Chart it is seen that six of the normal subjects belonging to the vegetarian group had serum vitamin B_{12} values lower than the lowest value in the non-vegetarian group—that is, 98 $\mu\mu$g./ml. In tropical eosinophilia a low level of serum vitamin B_{12} was found in 8 out of 16 cases. In nutritional macrocytic anaemia,

TABLE I.—*Non-vegetarian*

Subjects	No. of Cases	Total Vitamin B_{12} in $\mu\mu$g./ml. of Serum	
		Range	Mean ±S.E.
Normal	51	98 to 940	281·9 ± 22·7
Tropical eosinophilia	51	100 to 670	254·8 ± 16·1
Nutritional macrocytic anaemia	26	0 to 330	136·9 ± 20·7
Aplastic anaemia	33	144 to 1,800	621·9 ± 76·6
Chronic myeloid leukaemia	25	960 to 7,200	2,754·0 ±317·2



Scattergram showing distribution of serum vitamin B_{12} in vegetarians and non-vegetarians.

TABLE II.—*Vegetarian*

	No. of Cases	Total Vitamin B_{12} in $\mu\mu$g./ml. of Serum		Cases with <98 $\mu\mu$g./ml. Vitamin B_{12}
		Range	Mean ± S.E.	
Normal	10	36 to 290	92·3 ± 24·3	60%
Tropical eosinophilia	16	20 to 490	139·5 ± 30·6	50%
Nutritional macrocytic anaemia	13	20 to 196	47·5 ± 12·7	92·3%
Aplastic anaemia	2	40 and 80	—	Low in both the cases
Chronic myeloid leukaemia	5	1,190 to 3,100	2,219 ±318·8	0

a low serum vitamin B_{12} value was found in all the vegetarians except one. Of the aplastic anaemia patients only two were vegetarians, and in both of them the serum vitamin B_{12} was low, being 40 and 80 $\mu\mu$g./ml. respectively. No differential pattern was seen in chronic myeloid leukaemia, all the subjects showing elevated level.

A comparative study of Tables I and II shows that in the vegetarians the mean values were significantly lower than those in non-vegetarians in all groups except chronic myeloid leukaemia. In the vegetarians the percentage of cases having a serum vitamin B_{12} level lower than 98 $\mu\mu$g./ml. was 92.3% in N.M.A., 60% in normals, and 50% in tropical eosinophilia.

Discussion

Results of the present investigation indicate that the vegetarians have a serum vitamin B_{12} concentration which is significantly lower than that in the non-vegetarians. On the basis of clinical evaluation and analysis of dietary habits, vitamin B_{12} deficiency in the vegetarians appears to be dietary in origin. In a parallel study with ^{60}Co-labelled vitamin B_{12} for investigating the pathogenesis of vitamin B_{12} deficiency in N.M.A., dietary inadequacy appeared to be more important than the factor of intestinal malabsorption (Banerjee *et al.*, 1959).

In normal subjects and the cases of tropical eosinophilia there was no significant anaemia or macrocytosis. The blood picture in vegetarians and non-vegetarians was more or less similar, though their serum vitamin B_{12} levels were different. Some of the vegetarians followed for one or two years did not show any decline of haemoglobin or R.B.C. level in spite of the serum vitamin B_{12} level continuing to be low. These findings are not in complete agreement with those of Wokes *et al.* (1955), who found evidence of anaemia and macrocytosis in a significant proportion of vegans investigated by them. It must, however, be remembered that, owing to the exclusion of milk and milk products from their food, the average diet of a vegan was relatively more deficient in vitamin B_{12} than that of a normal Indian vegetarian. Maintenance of normal haemoglobin and R.B.C. values with a serum vitamin B_{12} value as low at 40 $\mu\mu$g./ml. poses certain questions of fundamental importance. Mollin and Ross (1952) observed that a serum level of 100 $\mu\mu$g./ml. was the critical concentration below which signs of vitamin B_{12} deficiency could be recognized. They confirmed their observation later by stating that early megaloblastic change was noticed in the bone-marrow of pernicious anaemia (P.A.) patients a few days after the serum vitamin B_{12} level had fallen below 100 $\mu\mu$g./ml.; at levels greater than this, the marrow remained normoblastic (Mollin and Ross, 1953).

In the present series the bone-marrow was normoblastic in tropical eosinophilia and aplastic anaemia. Bone-marrow examination was not done in normal subjects. Hence it is not possible to state whether they had any sign of vitamin B_{12} deficiency in the marrow. Repeated examination of peripheral blood did not, however, show any evidence of erythrocytic macrocytosis or granulocytic abnormality. In N.M.A. the bone-marrow was, however, found to be megaloblastic. It must be pointed out that, in N.M.A., megaloblastic bone-marrow was also seen in all the cases with high serum vitamin B_{12} (see Chart), suggesting that in this disease there is no direct correlation between serum vitamin B_{12} and bone-marrow changes. It is well known that N.M.A. as seen in the eastern part of India represents deficiency of folic acid and/or vitamin B_{12} (Das Gupta *et al.*, 1953; Chatterjea, 1958). The lack of correlation between serum vitamin B_{12} and bone-marrow changes in N.M.A. may therefore be explained on the basis of associated folic-acid deficiency, the degree of which cannot be ascertained from the serum vitamin B_{12} level.

In none of the cases with low serum vitamin B_{12}, including those of N.M.A., was there any neurological complications. This observation also was not in complete agreement with that of Wokes *et al.* (1955), who recorded neurological symptoms and signs in some of their subjects. In a few Indians with classical Addisonian pernicious anaemia subacute combined degeneration has, however, been found (Das Gupta and Chatterjea, 1951). In N.M.A. and in malabsorption syndrome neurological complications are conspicuous by their absence, though the serum vitamin B_{12} level in many cases may be as low as in pernicious anaemia. The main difference between P.A. and N.M.A. (or malabsorption syndrome) is with reference to intrinsic factor and neurological complications. In the present series all the vegetarians with low serum vitamin B_{12} had presumably no deficiency of intrinsic factor. Thus the circumstantial evidence becomes very strong in favour of intrinsic factor having some role, direct or indirect, in protecting the nervous system. If vitamin B_{12} *per se* was responsible for the protection of the nervous system, absence or rarity of neurological signs in N.M.A., in malabsorption syndrome, and in the present series of vegetarians cannot be satisfactorily explained.

It is possible that vitamin B_{12} absorbed from a system with an adequate amount of intrinsic factor (as in vegetarians, and in patients with N.M.A. and malabsorption syndrome) may be qualitatively different from vitamin B_{12} absorbed from a system grossly deficient in intrinsic factor (as in P.A.). Concentration for concentration, the former vitamin B_{12} may be more protective to the nervous system than the latter. The efficacy of parenteral vitamin B_{12} therapy in correcting the neurological complication of P.A. may be explained by assuming that the vitamin B_{12} as used in therapeutics is similar to the former but perhaps different from the latter. An alternative explanation would be the suggestion that therapeutically administered vitamin B_{12} is "activated" by extragastric sources of vitamin B_{12} (Callender and Lajtha, 1951). The above facts would tend to indicate that intrinsic factor, in addition to its role as promoter of absorption, may have an activating or maturing influence on vitamin B_{12}. Adequacy of intrinsic factor would ensure not only absorption but also activation or maturation of whatever small amount of vitamin B_{12} may be available to the system due to dietary inadequacy and/or intestinal malabsorption (Chatterjea, 1960).

An elevated level of serum vitamin B_{12} in chronic myeloid leukaemia, first reported by Beard *et al.* (1954), has been conclusively confirmed by a number of investigators. The exact cause of this elevated level is not clear. Lack of any differential pattern in the two groups—vegetarian or non-vegetarian—as revealed in the present study, indicates that in this disease the factor responsible for hypervitaminosis far outweighs the hypovitaminic effect of a vegetarian diet.

Summary

The serum vitamin B_{12} level was studied in 232 subjects (196 non-vegetarians and 46 vegetarians). The subjects belonged to the following five categories: normal, tropical eosinophilia, nutritional macrocytic anaemia, aplastic anaemia, and chronic myeloid leukaemia.

Serum vitamin B_{12} in the vegetarian group was, in general, lower than that in the non-vegetarian group. Chronic myeloid leukaemia was an exception, all the patients in this category showing a raised level as previously reported.

Low serum vitamin B_{12} was not, however, associated with any anaemia or neurological complications. The implications of these findings are discussed.

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The Italian Olympic Medical Committee is conducting a statistical study of competitors who took part in the Olympic Games in order to discover the correlations between medical characteristics and performance. To obtain this comprehensive medical data—in all, 78 medical characteristics for each athlete—for at least 2,000 of the 7,000 competitors in this year's games, the medical sections of various national Olympic Committees (including the German, Italian, and Russian) have agreed to supply the results of medical examinations of their athletes carried out in their own countries. Once this information has been collected it will be recorded on punched-cards which will then be processed by the IBM 305 RAMAC computer.

TREATMENT OF RESPIRATORY INFECTION IN SCHOOLBOYS WITH PHENETHICILLIN

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The administration of penicillin to children has hitherto presented several practical problems. Crystalline penicillin by injection gives satisfactory blood levels, but is painful and very unpopular with young patients. Oral penicillins G and V are far more acceptable, but the blood levels obtained are much lower than those achieved by parenteral administration, and are indeed often insufficient for satisfactory therapeutic effect. When phenethicillin (6-(α-phenoxypropionamido)penicillanic acid) was marketed as "broxil," it was claimed that this preparation would give blood levels at least equal to those after intramuscular injection of corresponding doses of penicillin G. Knudsen and Rolinson (1959) investigated the absorption and excretion of phenethicillin in adults, and their results confirmed this claim. Garrod (1960) compared the antibacterial activity of phenethicillin, penicillin G, and penicillin V. He found that, weight for weight, phenethicillin was more active than penicillin V or G against resistant staphylococci, and very slightly less active against streptococci and pneumococci.

In February, 1960, an outbreak of severe pharyngitis, with some complications, occurred among schoolboys aged 7–13 at Summer Fields Preparatory School, Oxford. The school is residential, and it was essential to deal promptly with the infection, which spread in a few days to 31 boys. Table I shows the incidence of the various types of infection. Most of the boys had severe pharyngitis and cervical lymphadenopathy. The illness ran much the same course both in those infected with β-haemolytic streptococci group A and in those from whom other organisms (*Str. pneumoniae, Str. viridans,* neisseria) were isolated. Fourteen boys had complications. One boy had uncomplicated lobar pneumonia and one had cellulitis of a finger.

TABLE I.—*Clinical Diagnosis and Treatment*

Condition	No.	Dose of Phenethicillin	Duration of Fever (Hours)	Failure
Pharyngitis due to β-haemolytic streptococci	13	12–250 mg. q.d.s. 1–500 ,, ,,	10–24 2–48	— —
As above + otitis media ..	3	250 mg. q.d.s.	24	—
Pharyngitis due to other organisms	2	250 ,, ,,	1–24 1–48	—
As above + otitis media ..	2	250 ,, ,,	48	—
Pharyngitis + bronchitis (2 β-haemolytic streptococci)	5	2–500 mg. q.d.s 3–250 ,, ,,	4–24 1–48	—
Pharyngitis + sinusitis ..	4	3–500 ,, ,, 1–250 ,, ,,	3–48	2, resistant staphs
Lobar pneumonia	1	250 mg. q.d.s.	73, with initial dramatic fall	
Cellulitis of finger	1	250 ,, ,,	24	—

Methods

The boys were all treated with phenethicillin. The standard dose was 250 mg. (one tablet) four-hourly