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The effect of vegetarianism and antibiotics upon proteins and vitamin B_{12} in the blood

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In a previous study of the plasma-protein pattern of a hundred normal Indian students, the authors pointed out that there was an abnormal variation in the level of albumin and a significant elevation of the globulin fraction (Satoskar & Lewis, 1954*a*). Furthermore, this rise in globulin was associated with high intake of vegetable proteins. In subsequent studies it was shown that the administration of 50 mg daily of various antibiotics over a period of 2 months led to a considerable rise in the level of albumin and a concomitant fall in the level of globulin, particularly the γ fraction (Satoskar & Lewis, 1954*b*, 1955). Studies in children, where a proportionately smaller amount of antibiotic was given, also indicated that the administration of broad-spectrum antibiotics could lead to a rise in serum albumin and a fall in globulin, when the latter was higher than normal to start with (Lewis, Bhagat, Wagle, Kulkarni & Satoskar, 1956). The present investigation was undertaken to determine if there is a difference in the blood vitamin B_{12} level in vegetarians and individuals on a mixed diet, and further to study the effect of small doses of antibiotics upon the blood vitamin B_{12} level and to correlate this effect with the changes in plasma proteins.

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EXPERIMENTAL

Ten normal medical students, including five vegetarians and five non-vegetarians, volunteered for the study. The vegetarians did not eat fish, fowl, meat or eggs; they took some 6 oz. whole milk daily, three of them were taking about 4 oz. curds daily and all had buttermilk occasionally. The non-vegetarians ate a mixed diet that included eggs and meat, fowl or fish daily. The volume of packed red blood cells, the plasma proteins, and the blood vitamin B_{12} level of the two groups of students were measured at the beginning of the experiment. Then the vegetarian group was given 50 mg aureomycin (chlorotetracycline) daily for 2 months.

Blood was taken for measurement of plasma protein and vitamin B_{12} after 1 month and again after 2 months of antibiotic administration. After each of the three blood samplings the subjects were injected intramuscularly with 50 μg vitamin B_{12}, and vitamin B_{12} was determined in the plasma 24 h later. Total protein was measured by determination of specific gravity and the protein fractions were measured by electrophoretic separation, as described in detail by Satoskar & Lewis (1954*b*).

For estimation of vitamin B_{12} level a 3 ml. sample of blood was taken. The A.O.A.C. method (Krieger, 1954) was employed, after preliminary enzymic hydrolysis of the blood, to release the bound form of the vitamin, and subsequent autoclaving to get a clear filtrate as suggested by Couch, Olcese, Witten & Colby (1950). The bacterial growth was measured by estimating turbidity with a spectrophotometer.

RESULTS

Table 1 shows the results obtained in the two groups of students, those on a mixed diet and those on a vegetarian diet. There was almost no difference in the volume of packed red blood cells (haematocrit), and the level was near the normal limits for young males resident in non-tropical areas.

Although there was little difference in the total protein level between the two groups the vegetarians showed a lower level of albumin and a higher level of all the globulin fractions. All of these changes were statistically significant except for γ-globulin, in which the individual variations were so great that a larger series of observations would be required to show a statistically significant difference (Satoskar & Lewis, 1954*a*).

The difference in blood vitamin B_{12} level was very marked, and even after the administration of 50 μg the level in the vegetarians did not reach the fasting level of the students who took a mixed diet. The rise in level after administration of vitamin B_{12} was 32 mμg/100 ml. in the non-vegetarians and 23 mμg in the vegetarians. The highest vitamin B_{12} level in the vegetarians was 17 mμg/100 ml. and the lowest in the meat-eating group was 29, so that there was no overlap in the initial values.

Table 2 shows the effect of aureomycin on the volume of packed red blood cells, the various protein fractions and the blood vitamin B_{12} level. There was no appreciable change in the haematocrit value.

There was a marked rise in the plasma albumin level and within 1 month the vegetarians had levels higher than the meat eaters, and after 2 months the average level was even greater. In one individual, B.D.S., the level at 2 months was no higher than

Table 1. *Plasma-protein pattern and blood vitamin B_{12} in non-vegetarian and in vegetarian Indian students at the beginning of the experiment*

Group	Subject	Haemato-crit (%)	Albumin (g/100 ml.)	α-Globulin one (g/100 ml.)	α-Globulin two (g/100 ml.)	β-Globulin (g/100 ml.)	γ-Globulin (g/100 ml.)	Vitamin B_{12} (mμg/100 ml.) Initial	Vitamin B_{12} (mμg/100 ml.) After injection of 50 μg vitamin B_{12}
Non-vegetarians	J.A.D.	48	3·95	0·12	0·36	0·43	1·24	52	103
	F.E.U.	55	4·74	0·08	0·31	0·50	1·47	32	44
	T.A.K.	50	4·88	0·30	0·40	0·56	1·26	53	89
	D.K.D.	48	4·31	0·13	0·31	0·62	1·93	29	62
	A.A.H.	48	4·66	0·12	0·41	0·54	1·07	39	67
	Mean	50	4·50	0·15	0·36	0·53	1·39	41	73
	Standard deviation	3	0·35	0·09	0·05	0·07	0·33	11	23
	Standard error of the mean	1	0·16	0·04	0·02	0·03	0·15	5	10
Vegetarians	B.D.S.	50	4·18	0·28	0·65	0·74	1·45	10	42
	R.R.	47	4·05	0·29	0·57	0·71	1·38	9	38
	R.G.D.	48	4·08	0·37	0·77	1·11	1·87	12	30
	R.D.G.	52	3·77	0·33	0·67	1·05	1·68	13	23
	B.S.K.	50	3·50	0·20	0·40	0·80	2·10	17	44
	Mean	49	3·92	0·29	0·61	0·88	1·70	12	35
	Standard deviation	2	0·28	0·06	0·14	0·19	0·30	3	9
	Standard error of the mean	1	0·12	0·03	0·06	0·08	0·13	1	4
Difference between means		−1	−0·58	0·14	0·25	0·35	0·31	−29	−38
2 × standard error		3	0·40	0·10	0·13	0·18	0·40	10	22

Table 2. *Effect of a daily dose of 50 mg aureomycin for 1 and for 2 months on the plasma-protein pattern and blood vitamin B_{12} of Indian vegetarian students*

Time	Subject	Haemato-crit (%)	Albumin (g/100 ml.)	α-Globulin one (g/100 ml.)	α-Globulin two (g/100 ml.)	β-Globulin (g/100 ml.)	γ-Globulin (g/100 ml.)	Vitamin B_{12} (mμg/100 ml.) Initial	Vitamin B_{12} (mμg/100 ml.) After injection of 50 μg vitamin B_{12}
Before dosing	B.D.S.	50	4·18	0·28	0·65	0·74	1·45	10	42
	R.R.	47	4·05	0·29	0·57	0·71	1·38	9	38
	R.G.D.	48	4·08	0·37	0·77	1·11	1·87	12	30
	R.D.G.	52	3·77	0·33	0·67	1·05	1·68	13	23
	B.S.K.	50	3·50	0·20	0·40	0·80	2·10	17	44
	Mean	49	3·92	0·29	0·61	0·88	1·70	12	35
	Standard deviation	2	0·28	0·06	0·14	0·19	0·30	3	9
	Standard error	1	0·12	0·03	0·06	0·08	0·13	1	4
After 1 month	B.D.S.	49	5·22	0·13	0·15	0·44	0·76	46	—
	R.R.	47	4·80	0·17	0·34	0·59	1·40	56	119
	R.G.D.	50	5·10	0·24	0·39	0·69	1·08	21	41
	R.D.G.	52	4·95	0·15	0·60	0·67	1·13	28	—
	B.S.K.	47	3·71	0·28	0·66	0·81	1·54	17	—
	Mean	49	4·76	0·19	0·43	0·64	1·18	34	80
	Standard deviation	2	0·60	0·06	0·21	0·14	0·30	17	55
	Standard error	1	0·27	0·03	0·09	0·06	0·14	7	25
After 2 months	B.D.S.	49	5·05	0·14	0·28	0·80	1·33	28	64
	R.R.	48	5·00	0·16	0·40	0·60	1·34	19	28
	R.G.D.	48	5·71	0·17	0·56	0·84	1·12	33	78
	R.D.G.	53	5·61	0·16	0·33	0·52	0·88	13	78
	B.S.K.	50	4·93	0·11	0·27	0·47	1·62	15	20
	Mean	50	5·26	0·15	0·37	0·65	1·26	22	54
	Standard deviation	2	0·38	0·02	0·13	0·17	0·28	8	28
	Standard error	1	0·17	0·01	0·06	0·08	0·13	4	12

after 1 month on the antibiotic, but all the others showed a further rise in the 2nd month.

There was a marked fall in the various globulin fractions, and the difference was statistically significant for all the fractions. It is interesting to note that the fall in β- and γ-globulin was maximal at the end of 1 month, although there seemed to be a further slight drop in the α fractions during the 2nd month, and one of the students, R.D.G., did show a fall in γ-globulin in the 2nd month.

The mean values for vitamin B_{12} showed a marked rise during the 1st month with a marked drop during the 2nd. Nevertheless, after 2 months the level was still significantly higher. In two of the individuals, R.D.G. and B.S.K., the final value was no higher than at the beginning. In fact subject B.S.K. never showed a higher level than before the administration of aureomycin. The blood vitamin B_{12} levels after the administration of 50μg intramuscularly showed the same trend as the fasting levels.

DISCUSSION

These results clearly indicate that a lower than normal vitamin B_{12} level, as well as a rather constantly elevated globulin and a variably depressed albumin level, of the plasma are related to the vegetarian diet. On the other hand, it is only proper to point out that, despite the lower than normal vitamin B_{12} level, there was no significant difference in the volume of packed red blood cells, which was in the range of normal. This finding would suggest that the lack of vitamin B_{12} in the vegetarians did not result in any clinical evidence of deficiency of this vitamin despite the fact that the level in the vegetarians was so much lower than in the meat eaters.

Table 3. *Blood vitamin B_{12} level in vegetarians and non-vegetarians compared with 'normals' and with patients with nutritional macrocytic anaemia*

	Vitamin B_{12} level		
Group	Mean value (mμg/100 ml.)	Range (mμg/100 ml.)	Reference
Non-vegetarians	41	29–53	This paper
Normal subjects	30	10–60	Das Gupta, Chatterjee, Ghosh & Banerjee (1955*a*)
Subjects with nutritional macrocytic anaemia, after treatment	16	10–23	Das Gupta *et al.* (1955*b*)
Vegetarians	12	9–17	This paper
Subjects with nutritional macrocytic anaemia, before treatment	7·5	2–15	Das Gupta *et al.* (1955*b*)

Our figures are compared in Table 3 with those obtained elsewhere in India where vegetarians and non-vegetarians were not separated. It may be seen that according to these figures the students on a mixed diet had a level of vitamin B_{12} higher than that of 'normals', whereas those on a vegetarian diet had a level lower than that of the 'normals'. Furthermore, the value for the vegetarians was between the pre- and post-treatment values for individuals with nutritional macrocytic anaemia.

The lower than normal albumin levels and higher than normal globulin levels of the vegetarian individuals may be of greater significance because of the much greater osmotic activity of albumin as compared with globulin. One may speculate that the

high globulin levels of the vegetarian are a compensatory measure for the lower than normal albumin levels, or the result of eating proteins with a propensity for forming globulin, or the result of a reaction to a different intestinal flora. The plasma-protein pattern of the vegetarian predisposes to the occurrence of oedema should the individual be exposed to certain stresses.

Turning to the results obtained from the administration of small doses of antibiotic we find a more varied picture. The rise in vitamin B_{12} level seen in the 1st month almost disappeared during the 2nd month. Furthermore, not all the students showed a rise. On the other hand, the rise in albumin was quite regular and required the full 2 months for the maximal change except in one student.

From these observations nothing decisive can be said concerning the relation between the rise in vitamin B_{12} and the increased albumin:globulin ratio. However, there was no close correlation between the rise in vitamin B_{12} and the rise in albumin either in the means or in the individual values. Furthermore, we have also found rather higher than usual blood vitamin B_{12} levels in children with very low albumin levels, and the syndrome of kwashiorkor (Dhopeshwarkar & Lewis, unpublished). In addition, the administration of vitamin B_{12} did not have any effect on the plasma-protein pattern of similar children (Lewis *et al.* 1956) or of normal medical students (Satoskar & Lewis, 1954*b*).

It appears likely that vegetarianism produced both a lower than normal blood vitamin B_{12} level and independently a lower than normal plasma albumin with a compensatory increase in the plasma globulin.

SUMMARY

1. Plasma protein and blood vitamin B_{12} were determined in five Indian medical students on a vegetarian diet, which included milk and milk products but no other food of animal origin, and in five non-vegetarian students whose diet included eggs and fish, fowl or meat daily. The vegetarians were then given 50 mg aureomycin daily for 2 months, and the determinations were repeated after 1 and 2 months.

2. The albumin content of the plasma was below normal and the globulin content above normal in the vegetarian students, who also had a lower blood content of vitamin B_{12} than the non-vegetarians.

3. The administration of aureomycin to the vegetarians produced a rise in plasma albumin and a fall in all the globulin fractions. Values for blood vitamin B_{12} increased significantly.

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