The effect of a low-cobalt diet in rabbits

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As in our earlier paper (Simnett & Spray, 1961), the term vitamin B_{12} is used here to signify vitamin B_{12} activity measured by microbiological assay with *Lactobacillus leichmannii*. We showed then that changes in the diet, particularly in its cobalt content, induced large changes in the levels of vitamin B_{12} in the serum, urine and faeces of rabbits. This finding suggested that on a diet sufficiently low in Co rabbits might develop deficiency of vitamin B_{12}. Through the kindness of Dr J. W. S. Reith of the Macaulay Institute of Soil Research, we obtained supplies of oat grain and hay grown on Co-deficient soil in Ross and Cromarty, Scotland. Four rabbits were fed on this diet for a year. Although no signs of vitamin B_{12} deficiency appeared, the diet induced marked falls in the levels of vitamin B_{12} in the serum, faeces, liver and kidneys of the animals.

EXPERIMENTAL

Animals and diet. Six Flemish rabbits from the same litter were housed in separate cages for the experiment. Nos. 1, 3 and 5 were males and nos. 2, 4 and 6 were females. They were 24 weeks old and weighed between 760 and 1320 g at the beginning of the experiment. They had been fed since weaning on oats and hay obtained locally. Rabbits nos. 1 and 2, the controls, were kept in galvanized iron cages and were fed on oats and hay purchased locally. The others were kept in aluminium cages and were fed on the oats and hay grown on Co-deficient soil. Food and water were supplied without restriction.

Determination of vitamin B_{12} in liver and kidney. At the end of the experiment the animals were killed, and the livers and kidneys were removed and weighed. Extracts were prepared for determination of vitamin B_{12} by the method used for rat liver (Booth & Spray, 1960).

Other methods. The methods described previously (Simnett & Spray, 1961) were used to measure at intervals throughout the experiment the Co content of representative samples of the oats and hay, to determine the vitamin B_{12} in the serum and faeces and the concentration of haemoglobin in the blood and to study blood films and the growth rates of the animals.

RESULTS

Cobalt content of oats and hay

Representative samples of the oats and hay used for the control animals contained 0·04μg and 0·18μg Co/g respectively. Samples of the oats and hay grown on Co-deficient soil contained 0·01μg and 0·03μg Co/g respectively.

Vitamin B_{12} in serum

The results are shown in Fig. 1. In one control animal the level of vitamin B_{12} fell during the first 8 weeks and then rose gradually to 0·035 μg/ml at the end of the experiment. In the other control animal the level rose to 0·065 μg/ml after 4 weeks; thereafter it fluctuated between 0·064 and 0·081 μg/ml. In contrast, all the rabbits on the diet low in Co showed after 4 weeks falls in the levels to between 0·0042 and 0·0013 μg/ml. No further fall was detected in any animal, the values fluctuating for the rest of the experiment around the low levels established at 4 weeks.

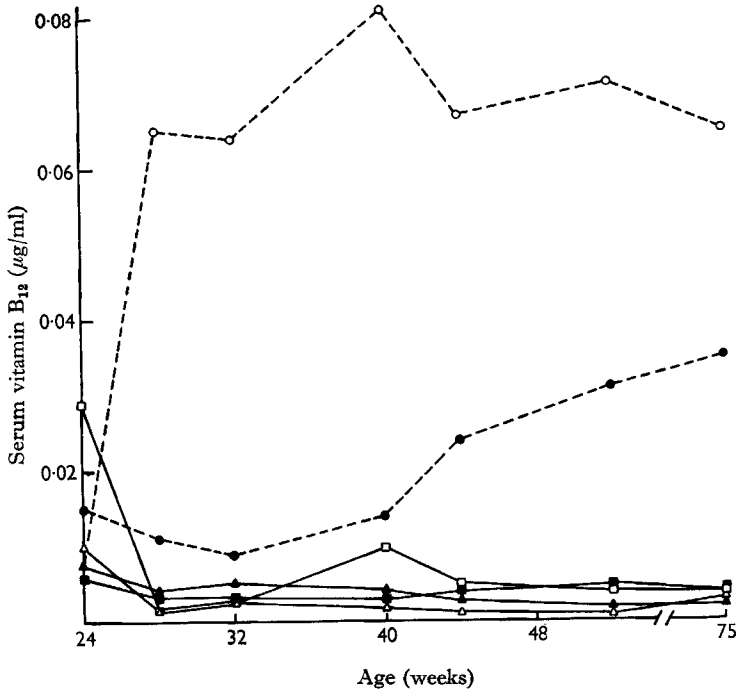

Fig. 1. Vitamin B_{12} in the serum of rabbits receiving diets of normal or low Co content. ●, rabbit 1 ♂; ○, rabbit 2 ♀; ▲, rabbit 3 ♂; △, rabbit 4 ♀; ■, rabbit 5 ♂; □, rabbit 6 ♀. - - - -, diet of oats and hay of normal Co content; ——, diet of oats and hay of low Co content.

Vitamin B_{12} in faeces

Four collections of faeces were made for periods of 24 h from each rabbit at different times during the experiment. During two of the collections from each rabbit the animals were collared to prevent coprophagy. The animals receiving the diet low in Co excreted less vitamin B_{12} than the control animals, whether coprophagy was prevented or allowed (Tables 1 and 2).

Vitamin B_{12} in livers and kidneys

Added cyanocobalamin was recovered quantitatively from rabbit liver and kidney by the method used (Simnett & Spray, 1962).

After 1 year on the diets, both the livers and the kidneys of the rabbits that had been

fed on the diet low in Co contained less vitamin B_{12}, in terms of concentration and of total quantity, than those of the control animals (Table 3).

Haemoglobin concentration, erythrocyte morphology and growth rates

The haemoglobin concentration in each animal fluctuated during the experiment without showing any trend attributable to the differences in diet. Only one value, 9·8 g/100 ml in rabbit no. 6 at the end of the experiment, was outside the normal range of 13·0 ± 1·5 g/100 ml quoted for rabbits by Wintrobe (1961). This animal appeared to be in good health. No abnormalities in erythrocyte morphology were

Table 1. *Faecal excretion of vitamin B_{12} by rabbits on diets of normal or low Co content (coprophagy prevented)*

Rabbit no.	Age (weeks)	Wet weight of faeces (g/24 h)	Total vitamin B_{12} excreted (μg/24 h)
	Diet of normal Co content		
1	36	120	130
	45	102	128
2	36	84	122
	45	68	104
	Diet of low Co content		
3	36	139	43
	44	69	41
4	36	69	53
	44	104	39
5	36	77	36
	44	93	55
6	36	59	62
	44	73	39

Table 2. *Faecal excretion of vitamin B_{12} by rabbits on diets of normal or low Co content (coprophagy allowed)*

Rabbit no.	Age (weeks)	Wet weight of faeces (g/24 h)	Total vitamin B_{12} excreted (μg/24 h)
	Diet of normal Co content		
1	37	70	67
	46	48	35
2	37	77	45
	46	93	43
	Diet of low Co content		
3	37	59	16
	47	63	20
4	37	82	24
	47	43	14
5	37	45	13
	47	51	12
6	37	43	20
	47	39	12

found in the film prepared when this blood sample was taken nor in any films prepared at other times from this or from any other animal. The bone marrow of rabbit no. 6 appeared normal when the animal was killed.

No gross differences in growth rates were found between the rabbits on the different diets. The mean increases in weight during the first 20 weeks of the experiment, by which time most of the animals were fully grown, were 2570 g for the two control rabbits and 2190 g for the four on the diet low in Co. The number of animals is too small for general conclusions to be drawn from this difference.

Table 3. *Vitamin B_{12} in the livers and kidneys of rabbits after 51 weeks on diets of normal or low Co content*

	Liver			Kidneys		
Rabbit no.	Weight (g)	Vitamin B_{12} concentration (μg/g)	Total vitamin B_{12} (μg)	Weight (g)	Vitamin B_{12} concentration (μg/g)	Total vitamin B_{12} (μg)
		Diet of normal Co content				
1	83	0·76	63	16	0·48	7·7
2	79	0·78	62	18	0·80	14
		Diet of low Co content				
3	71	0·40	29	12	0·24	2·9
4	77	0·065	5·0	17	0·16	2·7
5	57	0·38	22	12	0·32	3·8
6	68	0·63	42	15	0·32	4·8

DISCUSSION

Our results provide further evidence that the Co content of the diet of rabbits influences the level of vitamin B_{12} in the serum and faeces. There was no evidence of Co deficiency in any of the animals on the diet low in Co, but in all these animals the levels of vitamin B_{12} in the serum, faeces, liver and kidneys were much below those for the animals receiving the diet containing more Co. To produce Co deficiency in rabbits it would probably be necessary to devise a diet of Co content much lower than can be obtained in any natural diet grown on soil with a low Co content.

The results for the serum of the control rabbits were similar to those found in the earlier study for animals on a similar diet. Strict comparison is impossible because the animals in the experiments now reported were fed on oats and hay from weaning. Previously all the rabbits received a diet which included commercial rabbit pellets until it was changed to oats and hay for the experiments, but with oats and hay the serum vitamin B_{12} usually fell for between 2 and 12 weeks and then tended to increase. The faecal excretion of vitamin B_{12} by the control rabbits was similar to that of adult rabbits on a diet of normal oats and hay in the earlier work, whether coprophagy was prevented or allowed.

According to Marston (1959), to fulfil the physiological requirements of sheep, the fodder must contain more than 0·08μg Co/g. This is several times higher than the Co content of the Co-deficient oats and hay given to our rabbits, yet these did not show

any signs of Co deficiency; Marston (1959) states that rabbits thrive in areas where sheep develop Co deficiency.

In sheep the rumen produces 600–1000μg vitamin B_{12}/day when there is no limit to the Co in the diet. This can fall to 40–50μg/day when the intake of Co is low. Little more than 3% of these quantities is absorbed from the intestine (Marston, 1959). If in rabbits the difference between the faecal output of vitamin B_{12} when coprophagy is prevented and when it is allowed represents the amount of vitamin B_{12} available for absorption, our rabbits on the normal diet had an average of 74μg vitamin B_{12}/day available for absorption, compared with 30μg/day for those on the low-Co diet. Although less than 50% of these quantities is cyanocobalamin (Simnett & Spray, 1961), it appears that Co is converted into vitamin B_{12} more efficiently in the rabbit caecum than in the sheep rumen, taking into account the difference in size of the animals. Measurements of the faecal excretion of radioactivity after oral administration of cyanocobalamin labelled with radioactive Co (Simnett & Spray, unpublished results) have shown that about 16% is absorbed from doses comparable to those our rabbits obtained by coprophagy. Thus rabbits probably synthesize vitamin B_{12} more effectively and absorb it more efficiently than sheep, which enables them to survive in conditions under which sheep develop deficiency.

SUMMARY

1. Vitamin B_{12} in the serum, faeces, liver and kidneys of six rabbits on different diets was measured by microbiological assay with *Lactobacillus leichmannii*.

2. In two rabbits on a diet of oats and hay of normal Co content the level of vitamin B_{12} in the serum increased with time. In four rabbits receiving oats and hay grown on Co-deficient soil the level fell after 4 weeks and remained low for a year.

3. The rabbits on the low-Co diet excreted less vitamin B_{12} in the faeces than those on the normal-Co diet, whether coprophagy was prevented or not.

4. The concentration and the total quantity of vitamin B_{12} in the livers and kidneys of the rabbits on the low-Co diet was lower than in those of rabbits on the normal diet.

5. No evidence of vitamin B_{12} deficiency was found in the rabbits on the low-Co diet.

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