The influence of diet on the vitamin B_{12} activity in the serum, urine and faeces of rabbits

By K. INA SIMNETT and G. H. SPRAY

Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford

In this paper the term vitamin B_{12} is used to signify vitamin B_{12} activity measured by microbiological assay with *Lactobacillus leichmannii* as test organism. The concentration of vitamin B_{12} in the serum of the rabbit is remarkably high compared with that of some other species. In man and the rat the highest normal values found in this laboratory have been 0·001 and 0·00088 μg/ml respectively (Spray & Witts, 1958; Booth & Spray, 1960), whereas in normal rabbits we have found levels as high as 0·156 μg/ml. In spite of this difference the levels in tissues are of the same order as those in other species (Kulwich, Struglia & Pearson, 1953; Rosenthal & Cravitz, 1958). Rabbits ingest much more vitamin B_{12} than other species, as a result of coprophagy (Kulwich *et al.* 1953). The high levels of vitamin B_{12} in the serum, and also in the urine, are probably due to the absorption from the gut of large amounts of vitamin B_{12} as a result of the high intake. The mechanism by which this absorption takes place has not been elucidated. Rosenthal (1959), using doses of cyanocobalamin much smaller than the quantities of vitamin B_{12} that rabbits normally ingest, concluded that the mechanism of absorption is essentially the same as in man. However, the possibility cannot be excluded that under physiological conditions comparatively large amounts of vitamin B_{12} are absorbed by another mechanism.

To study further the significance of the level of vitamin B_{12} in the serum of rabbits we have placed them on various diets. It seems that the level is dependent on the diet, and thus it is impossible to establish a normal range for rabbits. We found that the nature of the diet, particularly its cobalt content, also influenced the faecal excretion of vitamin B_{12}, and that differences in diet were reflected in the vitamin B_{12} levels of both serum and urine.

EXPERIMENTAL

Animals and diet. Flemish rabbits of both sexes, whenever possible with litter-mates in each experimental series, were housed in separate galvanized iron cages with metal grid floors. Three diets were used, all without restriction, namely: (1) commercial rabbit pellets and hay, (2) oats and hay, or (3) oats and hay supplemented with Co. The Co was given as $CoCl_2$ in solution in the drinking water.

Estimation of vitamin B_{12} in serum. Vitamin B_{12} was assayed microbiologically with *Lb. leichmannii* as test organism, by the method described by Spray (1955). Blood (4–5 ml) was obtained from a marginal ear-vein dilated with a little xylene and was allowed to clot in centrifuge tubes. The clots were broken up with glass rods, the

35-2

samples were centrifuged, and the serum was removed and stored frozen until required for assay. Extracts were prepared from 0·5 ml portions of serum, as described for human serum (Spray, 1955). The extracts were diluted with water and assayed at levels corresponding to 1 ml of a 1 in 1000 and 1 ml of a 1 in 500 dilution of serum in the total volume of 5 ml assay medium. Measured portions of several extracts were treated as described for rat serum (Booth & Spray, 1960) to determine whether any of the vitamin B_{12} was stable to alkali.

Estimation of vitamin B_{12} in urine and faeces. Some of the rabbits were placed in metabolism cages at various times during the experiments. Urine and faeces were separated, and the total amounts of each were collected for periods of 24 h. To study the effect of coprophagy on the excretion of vitamin B_{12}, the rabbits were fitted with Polythene collars 10 in. in diameter to prevent coprophagy. The total weight of faeces and the volume of urine were recorded. Extracts of urine were prepared in the same way as those from serum, and samples corresponding to 1 ml of a 1 in 40 and 1 ml of a 1 in 20 dilution of urine were used for assay. Faeces were homogenized in a known volume of water with a mechanical blender. A portion (1–2 g) of each homogenate was mixed with 0·4 ml 0·1 % (w/v) NaCN solution and 1 ml 0·4 N-acetate buffer, pH 4·5, and made up to 20 ml with water. The mixture was then treated in the same way as that from serum, and the extract was diluted with water for addition to the assay medium.

Vitamin B_{12} and Co content of diets. The vitamin B_{12} in commercial rabbit pellets was determined by the same method as for faeces. It was assumed that oats and hay did not contain any vitamin B_{12}. The Co contents of representative samples of oats and hay were measured by neutron-activation analysis.

Determination of haemoglobin and examination of blood films. Blood (0·02 ml), obtained at the same times as the samples for determination of vitamin B_{12}, was mixed with 6 ml 0·009 N-NH$_4$OH solution. The colour intensity of the resulting solution of oxyhaemoglobin was measured in a Hilger Biochem Absorptiometer with a 550 mμ filter, and the concentration of haemoglobin in the blood was computed from a standard curve. A small amount of blood was smeared on to a microscope slide and allowed to dry in air. The film was stained with Wright's blood stain and examined microscopically.

Chromatography and bioautography. The methods described by Ford & Holdsworth (1953) and Ford, Holdsworth & Kon (1955) were used, with one modification. It was found that the 2,3,5-triphenyltetrazolium chloride could be added to the medium described by Spray (1955) for the microbiological assay of vitamin B_{12} with *Lb. leich-mannii* after the medium had been autoclaved with agar. Ford & Holdsworth (1953) found it necessary to sterilize their medium by filtration to avoid reduction of the tetrazolium chloride by substances formed during autoclaving. Under our conditions some slight pink coloration developed, but it was insufficient to interfere with reading the bioautographs.

Extracts of serum, urine and faeces were prepared as described above. Each extract was mixed with 0·5 g activated charcoal and shaken every 5 min for 30 min. The charcoal was removed by centrifugation, the supernatant solution was poured away

and the charcoal was re-suspended in water and centrifuged again. The charcoal was eluted with hot 75 % (v/v) acetone and filtered under pressure. The eluate was evaporated to dryness under reduced pressure at room temperature, and the residue was taken up in about 0·5 ml water. Each unknown solution (0·06 ml) and 0·02 ml (containing 0·001 μg) of standard solutions of cyanocobalamin, Factor A or Factor B, were pipetted on to Whatman no. 1 chromatography paper. The chromatograms were developed for about 18 h at room temperature with a solvent containing *s*-butanol, water, acetic acid and KCN (Ford *et al.* 1955). The papers were then dried in air and cut into strips, which were laid on the appropriate agar medium for bioautography.

<div align="center">RESULTS</div>

<div align="center">*Vitamin B$_{12}$ and cobalt content of diets*</div>

The pellets were found to contain 0·038 μg vitamin B$_{12}$/g, of which 0·026 μg was alkali-stable. The Co contents of representative samples were 0·04 μg/g for oats and 0·18 μg/g for hay. The rabbits on supplementary Co received between 25 and 40 μg Co/day.

<div align="center">*Vitamin B$_{12}$ in serum*</div>

Recovery of added cyanocobalamin from serum, and reproducibility of the results. When cyanocobalamin equivalent to 0·02 or 0·04 μg/ml serum was added to samples of serum from seven different rabbits, recoveries of between 80 and 137 % were obtained. The mean recovery from twenty-two determinations was 99·8 %. The mean of eighteen determinations of vitamin B$_{12}$ in a sample of pooled rabbit serum assayed at intervals was 0·015 μg/ml, with a range of 0·009–0·018 μg/ml.

Stability of the vitamin B$_{12}$ to alkali. The extracts from four samples of serum were treated with alkali. The alkali-stable activity was found to be 2 % or less of the total and was therefore neglected.

Preliminary experiments. Initially, in an attempt to establish a normal range of serum vitamin B$_{12}$ levels in rabbits, four weanling rabbits (β, δ, C and T) receiving pellets and hay were studied at fortnightly intervals. Some increase with age was found in the levels (Fig. 1). It was then found that the pellets contained some alkali-labile vitamin B$_{12}$, which was assumed to represent either cyanocobalamin or analogues. To eliminate any possible effect that absorption of these factors from the diet might have on the level of vitamin B$_{12}$ in the serum, the diet was changed to oats and hay. A sharp fall in the serum content of vitamin B$_{12}$ followed. When two of the rabbits were given pellets again a prompt increase occurred, whereas the levels in the other two, though tending to increase, did not rise to the same extent as those in the animals given the pellets.

Comparison of diet of pellets and hay with one of oats and hay. Four weanling litter-mates (D and J males, M and I females) were studied. One of each sex received pellets and hay, and the others oats and hay. The pair on pellets and hay showed prompt and sustained increases in serum vitamin B$_{12}$ level, whereas in the others the levels fell for the first 6–8 weeks and then showed some increase, but not to the same extent as in the first pair (Fig. 2).

Effect of supplementary Co. For reasons discussed below, rabbits D and I were given

558 K. INA SIMNETT AND G. H. SPRAY 1961

supplementary Co later in the experiment. They showed increases in the levels of vitamin B_{12} in the serum. Two rabbits (α and Ω), which were older than those so far studied, were given different diets for three successive periods of 10 weeks. It was
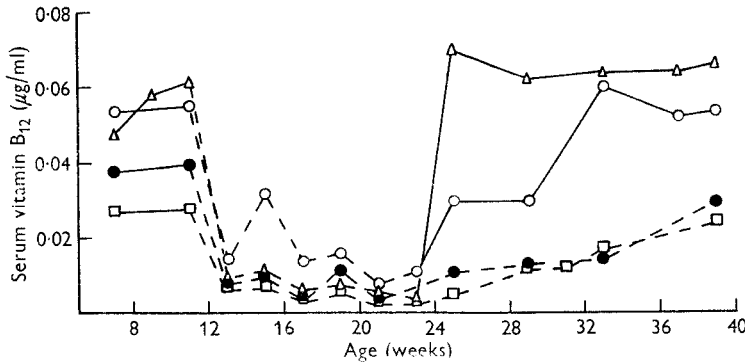

Fig. 1. Effect of diet on level of vitamin B_{12} in the serum of rabbits during growth. ●, rabbit T♂; □, rabbit C♀ (litter-mates). ○, rabbit β♀; △, rabbit δ♀ (litter-mates). ——, diet of commercial rabbit pellets and hay; - - - - - -, diet of oats and hay.
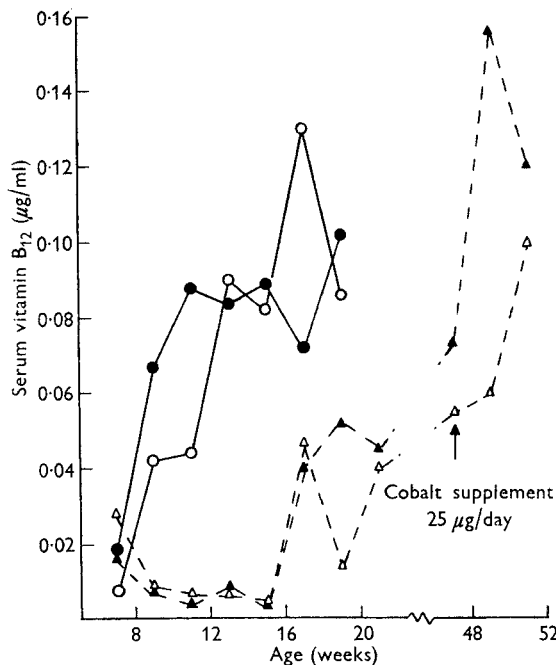

Fig. 2. Effect of diet on level of vitamin B_{12} in the serum of rabbits during growth. ●, rabbit, J♂; ▲, rabbit D♂; ○, rabbit M♀; △, rabbit I♀ (all litter-mates). ——, diet of commercial rabbit pellets and hay; - - - - -, diet of oats and hay.

found (Fig. 3) that on pellets and hay the level of vitamin B_{12} in the serum increased, but fell as soon as the diet was changed to oats and hay. Since the amount of alkali-labile activity (presumed to represent cyanocobalamin or analogues) in the pellets was

https://doi.org/10.1079/BJN19610068 Published online by Cambridge University Press

relatively small, it was thought that the effects might be due to a greater Co content in the pellets. Cobalt chloride equivalent to 40 μg Co/day was therefore added to the diet of oats and hay. A sharp increase in the serum level of vitamin B$_{12}$ followed.
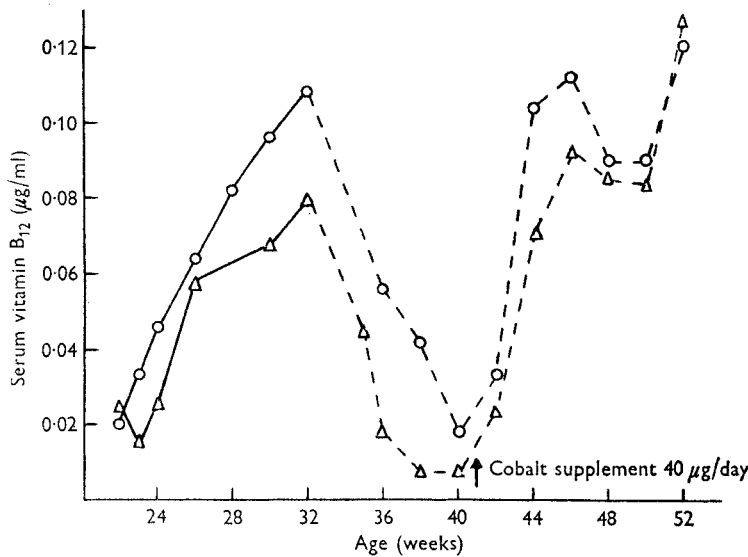

Fig. 3. Effect of diet on level of vitamin B$_{12}$ in the serum of adult rabbits. O, rabbit Ω♀; $\triangle$, rabbit α♀ (litter-mates). ———, diet of commercial rabbit pellets and hay; - - - - - -, diet of oats and hay.
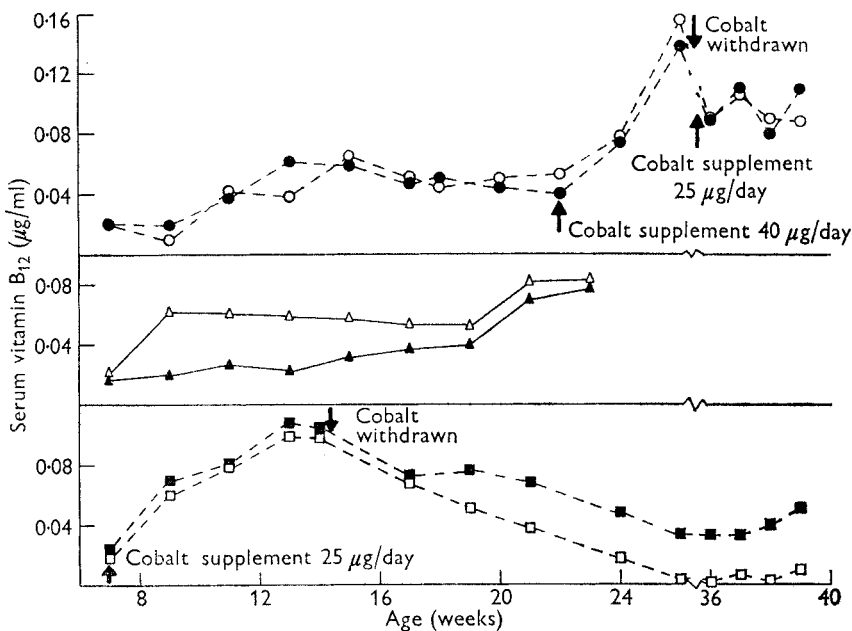

Fig. 4. Effect of diet on level of vitamin B$_{12}$ in the serum of rabbits during growth. ●, rabbit Y♂; O, rabbit P♀; ▲, rabbit S♂; $\triangle$, rabbit K♀; ■, rabbit N♂; □, rabbit Z♀ (all litter-mates). ———, diet of commercial rabbit pellets and hay; - - - - - -, diet of oats and hay.

Three pairs of weanling rabbits (litter-mates, each pair being a male and a female—S and K, N and Z, Y and P) were given different diets, as detailed in Fig. 4. There was a much sharper increase in the serum level of vitamin B_{12} in the animals receiving supplementary Co than in the corresponding pair not receiving it. Withholding of the Co supplement led to a fall in the levels; correspondingly, addition of Co to the diet of the other pair on oats and hay led to a sharp increase. The supplement of $40 \mu g$ Co/day was withheld from rabbits Y and P at the age of 30 weeks, and at the age of 35 weeks they were given $25 \mu g$ Co/day. The levels of vitamin B_{12} in the serum were lower than those with the larger Co supplement. Determinations of haemoglobin in the blood of these animals did not reveal any polycythaemic effect of the Co. Rabbits S and K on pellets and hay showed a slow increase in the levels of vitamin B_{12} in the serum.

Vitamin B_{12} in faeces and urine

Rabbits pass hard faeces during the day and soft faeces at night. The soft faeces contain more vitamin B_{12} than the hard faeces, and the animals probably satisfy their vitamin B_{12} requirements by eating the soft faeces (Kulwich *et al.* 1953). If the amount of vitamin B_{12} synthesized by the intestinal flora depends on the nature of the diet and particularly on its Co content, rabbits on different diets might be expected to receive varying amounts of vitamin B_{12} as a result of coprophagy. This variation might be the cause of the differing levels of vitamin B_{12} found in the serum of rabbits on different diets.

Recovery of added cyanocobalamin from faeces. When cyanocobalamin was added to homogenates of rabbit faeces, recoveries of between 60 and 130 % were obtained. The mean recovery from eleven estimations was 99 %.

Vitamin B_{12} in the faeces of rabbits on different diets. When coprophagy was prevented, the total faecal excretion of vitamin B_{12} could be measured. Under these conditions both of the young rabbits on a diet of pellets and hay excreted more vitamin B_{12} than their litter-mates on oats and hay at corresponding ages (Table 1). The young rabbits on oats and hay with supplementary Co also excreted more vitamin B_{12} than their litter-mates on oats and hay alone, except at 8 weeks of age. The discrepancy at this age may have been due to the use of badly designed collars, which caused some discomfort, for rabbits N and Z. They ate little food, and passed few faeces, at this time. Improved collars were used in all other experiments.

Rabbits Y and P received supplementary Co when they were fully-grown. Except in one instance, their excretion of vitamin B_{12} was greater than that of rabbits N and Z which were then receiving oats and hay alone. More vitamin B_{12} was excreted by adult rabbits than by young animals on the same diet. In distinguishing between young and adult rabbits, we have assumed that the animals are mature at about 6 months of age.

The faecal excretion of vitamin B_{12} by the same rabbits when mature and when coprophagy was allowed is shown in Table 2. Most of the values were lower than those obtained when coprophagy was prevented. In those animals for which comparative figures are available at the same age, all excreted less faeces and less vitamin B_{12}, irrespective of diet, when coprophagy was allowed. Tables 1 and 2 include figures for

the litter-mate rabbits referred to in Fig. 4, and also some for two older litter-mates (D and I, Fig. 2).

Recovery of added cyanocobalamin from urine. When cyanocobalamin was added to rabbit urine, recoveries of between 81 and 132 % were obtained. The mean recovery from ten estimations was 101 %.

Table 1. *Effect of diet on faecal excretion of vitamin B$_{12}$ by young and adult rabbits (coprophagy prevented)*

(S, K, Y, P, N and Z were litter-mates from one litter, D and I were litter-mates from an older litter)

		Pellets and hay				Oats and hay				Oats and hay with supplementary cobalt	
Rabbit	Age (weeks)	Wet weight faeces (g/24 h)	Total vitamin B$_{12}$ (μg/24 h)	Rabbit	Age (weeks)	Wet weight faeces (g/24 h)	Total vitamin B$_{12}$ (μg/24 h)	Rabbit	Age (weeks)	Wet weight faeces (g/24 h)	Total vitamin B$_{12}$ (μg/24 h)
						Young rabbits					
S♂	8	107	192	Y♂	8	77	53	N♂	8	28	37
	10	76	144		10	49	82		10	51	187
	16	96	165		16	117	78		14	50	182
K♀	8	104	111	P♀	8	67	70	Z♀	8	36	87
	10	104	211		10	77	82		10	59	170
	16	97	165		16	103	67		14	115	115
						Adult rabbits					
				N♂	25	167	153		—	—	—
					37	132	128	Y♂	36	106	285
					39	65	127		37	86	290
				Z♀	37	69	66	P♀	37	104	386
					39	51	240		38	111	198
								D♂	48	129	245
									49	129	177
								I♀	48	155	686
									49	110	370

Table 2. *Effect of diet on faecal excretion of vitamin B$_{12}$ by adult rabbits (coprophagy allowed)*

		Oats and hay				Oats and hay with supplementary cobalt	
Rabbit	Age (weeks)	Wet weight faeces (g/24 h)	Total vitamin B$_{12}$ (μg/24 h)	Rabbit	Age (weeks)	Wet weight faeces (g/24 h)	Total vitamin B$_{12}$ (μg/24 h)
N♂	25	99	36	Y♂	25	70	135
	36	58	45		26	70	49
	37	52	35		36	95	122
					37	42	74
Z♀	25	69	43	P♀	25	82	270
	27	113	84		26	53	51
	37	57	24		37	56	139
	39	48	107		38	61	134
				D♂	48	85	62
					49	43	40
				I♀	48	82	127
					49	100	80

Vitamin B_{12} in the urine of rabbits on different diets. Rabbits on oats and hay excreted less vitamin B_{12} in their urine than those on a similar diet supplemented with Co (Table 3). Prevention of coprophagy might have been expected to decrease urinary excretion of vitamin B_{12}, but no consistent effect attributable to coprophagy was found.

Table 3. *Urinary excretion of vitamin B_{12} ($\mu g/24 h$) by adult rabbits on different diets*

(N, Z, Y and P aged 36–39 weeks, D and I aged 46–49 weeks)

| | Oats and hay | | | Oats and hay with supplementary cobalt | |
| | Total vitamin B_{12} | | | Total vitamin B_{12} | |
Rabbit	Coprophagy prevented	Coprophagy allowed	Rabbit	Coprophagy prevented	Coprophagy allowed
N♂	0·20	0·20	Y♂	1·6	1·5
	0·32	0·20		6·2	3·7
Z♀	0·17	0·05	P♀	1·7	1·7
	0·61	0·05		4·0	14
			D♂	0·60	0·93
				0·40	0·13
			I♀	7·7	5·5
				4·0	3·7
Mean	0·32	0·12	Mean	3·3	3·9

Growth rates, haemoglobin concentration and erythrocyte morphology

In an attempt to assess the adequacy of our diets for growth and blood formation, the rabbits were weighed, the concentration of haemoglobin in the blood was measured, and a film was prepared, each time blood was taken. The growth rates of the young rabbits on oats and hay were similar to those of the animals receiving pellets and hay. The concentration of haemoglobin in each rabbit fluctuated considerably, but the values in the animals on oats and hay did tend to be slightly lower than those in their littermates on pellets. Supplementary Co had no noticeable effect on haemoglobin levels. However, all the results were within the normal range of $13\cdot0 \pm 1\cdot5$ g/100 ml quoted for rabbits by Wintrobe (1956). No abnormality was seen in the morphology of the erythrocytes of any of the animals.

Chromatography and bioautography

Extracts of serum, faeces, and urine of rabbits were studied by paper chromatography and bioautography with either *Lb. leichmannii* or *Escherichia coli* 113–3 as test organism, to show what factors contribute to the vitamin B_{12} in these materials. *E. coli* 113–3 gave clearer and more reproducible results and had the additional advantage of responding to Factor B as well as to cyanocobalamin and Factor A.

The results are summarized in Table 4. No relevant differences were found between the results with the different test organisms or between materials collected from animals on different diets; all results are therefore presented together. The R_F values of the standards varied from experiment to experiment, but in any given experiment the R_F of a particular locus from serum, faeces or urine always coincided with the value for a standard and so could be attributed to the substance of that standard.

Loci corresponding to cyanocobalamin were detected consistently in serum, faeces and urine. Factor A was also detected consistently in faeces, but in serum small loci, only just detectable but corresponding in R_F to Factor A, were found only twice in eleven experiments. Similar small loci were found in two out of three experiments on urine. Loci corresponding to Factor B were found in three out of six experiments on faeces. All experiments on faeces and urine were carried out with *E. coli* as test organism.

Table 4. R_F *values of cyanocobalamin and analogues compared with those of factors in the serum, faeces and urine of rabbits*

	No. of determinations	R_F	
		Mean	Range
Standards			
Cyanocobalamin	15	0·26	0·21–0·33
Factor A	4	0·12	0·07–0·16
Factor B	4	0·35	0·29–0·38
Extracts			
Serum (eleven experiments)	11	0·25	0·22–0·33
	2	0·13	0·12–0·14
Faeces (six experiments)	6	0·24	0·19–0·34
	6	0·13	0·07–0·20
	3	0·37	0·29–0·51
Urine (three experiments)	3	0·24	0·23–0·25
	2	0·13	—

DISCUSSION

Our results show that the level of vitamin B$_{12}$ in the serum of rabbits, although much higher than in other species, is subject to wide variations caused by diet. Any attempt to establish a normal range of serum vitamin B$_{12}$ values for rabbits seems therefore to be useless. The principal dietary factor controlling the level of vitamin B$_{12}$ in the serum may be the amount of Co. Adding a small amount of CoCl$_2$, equivalent to 25–40 μg Co/day, to a diet of oats and hay caused marked increases in the vitamin B$_{12}$ level of the serum. Withdrawal of Co led to a fall in the levels. We did not determine the amount of Co in the commercial rabbit pellets, but from the rises in the serum vitamin B$_{12}$ levels of the rabbits receiving them it seems probable that they contained more than the oats and hay. Irrespective of the diet, the level of vitamin B$_{12}$ in the serum tended to increase with the age of the rabbits.

Adding Co to the diet also caused increased intestinal synthesis of vitamin B$_{12}$ as measured by the excretion of vitamin B$_{12}$ in the faeces. A rough assessment of the amount of vitamin B$_{12}$ obtained by coprophagy can be made by comparing the faecal excretions when coprophagy was allowed and when it was prevented. On average, adult rabbits on a diet of oats and hay obtained about 90 μg vitamin B$_{12}$/day by coprophagy, compared with about 200 μg/day on a diet of oats and hay with supplementary Co. We did not determine what proportion of it was absorbed from the gut, and no studies of absorption by the rabbit with doses of this magnitude have been reported. Rosenthal (1959), using doses of 0·05–0·15 μg cyanocobalamin labelled with radio-

active Co per kg body-weight, showed only limited absorption: these doses correspond on a body-weight basis to doses of $3-9\,\mu g$ in a man of 60 kg. According to Doscherholmen & Hagen (1957), small doses such as these are absorbed in man by a mechanism depending on intrinsic factor. The quantities of vitamin B_{12} shown by us to be ingested by rabbits, even if 50 % of it or less is cyanocobalamin (see below), represent the equivalent of doses of $500-1500\,\mu g$ or more in man. Such doses are absorbed in man by a pharmacological mechanism (Doscherholmen & Hagen, 1957), but further studies are needed to determine whether a similar mechanism operates under physiological conditions in the rabbit. There was a marked increase in the urinary excretion of vitamin B_{12} on the Co-supplemented diet, presumably a reflection of the amount of vitamin B_{12} absorbed. Prevention of coprophagy for 24 h did not have any consistent effect on urinary excretion. However, as the animals were only collared for this short period and as there is a delay between the ingestion of cyanocobalamin and its appearance in the blood (Rosenthal, 1959), this finding is not unexpected.

If rabbits could be fed on a diet sufficiently low in Co, it seems possible from our results that deficiency of vitamin B_{12} might be induced. Under our conditions we have obtained decreases in the level of vitamin B_{12} in the serum to $0.002\,\mu g/ml$ without any signs of deficiency as shown by determination of haemoglobin and examination of blood films. This level, however, is still high compared with those in other species. Thompson & Ellis (1947) reared rabbits on a diet of Co-deficient grain and milk and could not produce Co deficiency. There have been no reports of Co deficiency in rabbits on pasture producing Co deficiency in sheep. This situation may indicate either that the requirement of the rabbit for Co is less per unit of body-weight than that of the sheep, or that the rabbit has a more efficient mechanism than the sheep for converting Co into vitamin B_{12}. The high faecal, serum and urinary levels of vitamin B_{12} in the rabbit may thus be a reflection of the activity of the intestinal flora and the habit of coprophagy, rather than of an increased Co or vitamin B_{12} requirement compared with those of other species. This view is supported by the observation that the concentration of liver vitamin B_{12} in rabbits is of the same order as in other species (Kulwich et al. 1953; Rosenthal & Cravitz, 1958). The level of vitamin B_{12} in the serum of the rabbit is thus probably not a reflection of the levels in the tissues, in agreement with observations on the rat (Booth & Spray, 1960).

Our chromatographic results show that the figures for the total vitamin B_{12} in rabbit faeces, measured by assay with *Lb. leichmannii*, represent the sum of the activities of Factor A and cyanocobalamin. The loci corresponding to Factor A on the bioautographs were larger than those corresponding to cyanocobalamin. Since the colour densities and areas of the loci are proportional to the logarithms of the concentrations of the substance applied to the chromatogram (Ford & Holdsworth, 1953), rabbit faeces appear to contain more Factor A than cyanocobalamin. Only minute quantities of Factor A were demonstrable in serum and urine, which shows the high selectivity of the mechanism for absorption of cyanocobalamin from the gastro-intestinal tract of the rabbit. It seems also that figures for vitamin B_{12} in the serum and urine of rabbits represent, within the error of the method, the true level of cyanocobalamin.

The growth rates, haemoglobin levels and normal appearances of the blood films

of our rabbits on a diet of oats and hay suggest that this diet provides all the requirements of the rabbit for growth and blood formation. All our animals appeared to be in good health, irrespective of diet. It is generally recommended that for adequate nutrition rabbits in captivity should be given commercial pellets that include some material of animal origin, and for this reason we used pellets in our preliminary experiments. We did not attempt to determine the adequacy of the diet of oats and hay for reproduction, or to apply any other assessments of nutrition.

SUMMARY

1. Vitamin B_{12} in the serum, urine and faeces of rabbits on different diets was measured by microbiological assay with *Lactobacillus leichmannii* as test organism.

2. The level of vitamin B_{12} in the serum of rabbits can be as much as 150 times higher than the highest values found in man or the rat. In rabbits on a diet of commercial rabbit pellets and hay the level increased with time, but fell sharply when the diet was changed to oats and hay. When cobalt chloride was given with oats and hay, the level increased, and it decreased when the cobalt chloride was withdrawn.

3. Prevention of coprophagy led to large increases in the faecal excretion of vitamin B_{12}, both in the presence and absence of supplementary cobalt.

4. Supplementary Co increased the faecal and urinary excretion of vitamin B_{12} in rabbits receiving oats and hay, whether coprophagy was prevented or not.

5. Experiments by paper chromatography and bioautography with *Lb. leichmannii* or *Escherichia coli* 113–3 as test organisms indicated that the vitamin B_{12} assayed in the faeces of rabbits represents the sum of the activities of Factor A and cyanocobalamin. In serum and urine the method apparently measures cyanocobalamin almost exclusively.

6. Our results indicate that the amount of vitamin B_{12} in the serum, faeces and urine of rabbits depends on the nature of the diet and particularly on its Co content. The concentration in the serum is probably not a reflection of the stores of the vitamin in the tissues.

REFERENCES

Booth, M. A. & Spray, G. H. (1960). *Brit. J. Haemat.* **6**, 288.
Doscherholmen, A. & Hagen, P. S. (1957). *J. clin. Invest.* **36**, 1551.
Ford, J. E. & Holdsworth, E. S. (1953). *Biochem. J.* **53**, xxii.
Ford, J. E., Holdsworth, E. S. & Kon, S. K. (1955). *Biochem. J.* **59**, 86.
Kulwich, R., Struglia, L. & Pearson, P. B. (1953). *J. Nutr.* **49**, 639.
Rosenthal, H. L. (1959). *Amer. J. Physiol.* **197**, 1048.
Rosenthal, H. L. & Cravitz, L. (1958). *J. Nutr.* **64**, 281.
Spray, G. H. (1955). *Clin. Sci.* **14**, 661.
Spray, G. H. & Witts, L. J. (1958). *Brit. med. J.* i, 295.
Thompson, J. F. & Ellis, G. H. (1947). *J. Nutr.* **34**, 121.
Wintrobe, M. M. (1956). *Clinical Hematology*, 4th ed., p. 1129. Philadelphia: Lea and Febiger.