Production, absorption, distribution and excretion of vitamin B_{12} in sheep

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1. The efficiency of production and utilization of vitamin B_{12} was studied with sheep given a cobalt-deficient diet with and without supplementary Co (1 mg/d). Vitamin B_{12} to lignin ratios in rumen contents were used to estimate minimum rates of production and these were related to faecal and urinary excretion. Tissue distribution and excretion of vitamin B_{12} were studied with [^{58}Co]cyanocobalamin and 5′-deoxyadenosyl[^{60}Co]cobalamin.

2. Labelled Co was rapidly sequestered by particulate material in the rumen and was largely excreted in the faeces. Most of the vitamin B_{12} in whole rumen contents was contained in micro-organisms, but was released on incubation at pH 2. Added cyanocobalamin was partly degraded in the rumen.

3. The vitamin B_{12} to lignin ratio in rumen contents began to decline 1–3 d after cessation of a daily Co drench. Estimated ruminal production of vitamin B_{12} on full feed was not less than 400–700 μg/d with supplementary Co and 50–110 μg/d from the Co (0·01–0·05 μg/g dry weight) in the basal diet. Production of vitamin B_{12} appeared to be limited by food intake with or without additional Co.

4. At full feed the efficiency of production of vitamin B_{12} from Co in the basal diet was about 13 % while that from added Co was about 3 %. Part of the vitamin B_{12} produced in the rumen was degraded before reaching the faeces and about 5 % was absorbed. The minimum total requirements of sheep for vitamin B_{12} are assessed at about 11 μg/d.

5. Injected 5′-deoxyadenosylcobalamin was better retained than injected cyanocobalamin, faecal excretion exceeded urinary excretion with both. Labelled cobalamin was selectively retained by liver (particularly by the mitochondria), kidneys and the walls of parts of the alimentary tract. Vitamin B_{12} was secreted into the duodenum and reabsorbed in the ileum, but little secretion occurred above the duodenum and little absorption below the small intestine.

Production of vitamin B_{12} in rumen contents of sheep depends on the presence of cobalt (Hale, Pope, Phillips & Bohstedt, 1950; Hoekstra, Pope & Phillips, 1952; Hine & Dawbarn, 1954; Kercher & Smith, 1956) and this is reflected in faecal excretion (Hale *et al.* 1950; Dawbarn, Hine & Hughes, 1952; Dawbarn & Hine, 1955). Little is known, however, either of the quantities produced or of the efficiency of absorption.

Kercher & Smith (1955) estimated that retention of oral cyanocobalamin in sheep was about 3 % that of injected cyanocobalamin, but large doses of injected cyanocobalamin may not be well retained. Thus, with sheep on a Co-deficient diet, 1 mg Co/d orally led to greater liver storage of vitamin B_{12} than did 25 μg cyanocobalamin/d by injection (Marston, 1970); it led to little urinary excretion but, on the other hand, 30–36 % of injected cyanocobalamin (50 μg/d) was excreted in the urine (Dawbarn & Hine, 1955).

Smith & Loosli (1957) estimated that sheep given a Co-deficient diet required an additional 10 μg injected cyanocobalamin/d, but the amount provided by the diet was unknown. Marston (1970) in long-term experiments has estimated the minimum prophylactic dose of injected cyanocobalamin to be about 6 μg/d for sheep on a diet

containing 0·03 μg Co/g (dry). The absolute requirements of sheep for vitamin B_{12} may be estimated from this dose if both the efficiency of production from the Co in the diet and the efficiency of absorption are known.

The quantities of vitamin B_{12} in rumen contents of sheep given Co-deficient diets are not negligible, and it is not necessarily true that the efficiency of absorption is the same as that when additional Co is supplied or the same as that when crystalline cyanocobalamin is given orally. The work now described was aimed at estimating these rates and efficiencies and in addition at determining the tissue distribution and excretory pattern of injected cobalamins. The total requirements of sheep for vitamin B_{12} are assessed at about 11 μg/d and, although the arguments leading to this estimate are not entirely rigorous, the results permit a reasonable description of the production, absorption and excretion of vitamin B_{12} in the sheep.

EXPERIMENTAL

Animals and diet. Australian Merino ewes, 2–6 years of age, were fed on a Co-deficient diet in cages permitting collection of excreta (Smith & Marston, 1970). A daily ration was given of 1000 g wheaten hay-chaff (0·01–0·05 μg Co/d (dry)), 50 g washed gluten (< 0·02 μg Co/g (dry)) and 10 g sodium chloride; daily food intakes were recorded and body-weights were measured each week. The ration contained 85–90% dry matter but unless otherwise stated food intakes are quoted on a moist-weight basis.

All animals received weekly drenches supplying 70 mg copper (as sulphate) and 25 ml cod-liver oil. Animals treated with Co were drenched daily at the time of feeding with 1 mg Co (as chloride) in 20 ml water. Animals given vitamin B_{12} orally received a daily drench of 500 μg cyanocobalamin in 20 ml water. Animals treated with vitamin B_{12} parenterally were injected daily with 50 μg cyanocobalamin in 0·5 ml water into the gluteus maximus muscle. Injections of [^{60}Co]cyanocobalamin (subcutaneously on the shoulder) or 5′-deoxyadenosyl[^{58}Co]cobalamin (into the jugular vein) were given at a rate equivalent to 25 μg cyanocobalamin/d in 2 ml isotonic saline.

Samples of rumen contents. These were obtained via fistulas (Jarrett, 1948) with a stainless-steel sampler mounted on a flexible handle. The hollow cylindrical sampler (10 cm × 2·2 cm diameter) contained a gate (7·5 cm × 1·8 cm) closed by a second hollow cylinder closely fitting inside the first and containing a similar gate. A lever on the handle permitted rotation of the inner cylinder through 180° to open or close the gate. The closed sampler was inserted into the rumen, the gate was opened for 1 or 2 s, then closed and the sample (15–22 g) recovered. All samples analysed comprised composites of ten such withdrawals taken from random points in the rumen. Dry-matter measurements of replicate single withdrawals from individual sheep showed the coefficient of variation of such composite samples to be about 3%. Except where otherwise stated, samples of rumen contents were taken 4 h after feeding.

Chemical estimations. Co in fodder and rumen contents was measured in samples of 15–25 g (dry) by the method of Marston & Dewey (1940). Lignin was measured by the method of Norman & Jenkins (1934*a*, *b*). The dried and ground samples of fodder or

of 50% (v/v) ethanol-insoluble dry matter from rumen contents were refluxed in 5% (w/v) H_2SO_4 for 1 h to remove protein and hydrolysable polysaccharides, then again dried before leaving for 16 h at 18° in 72% (w/w) H_2SO_4. The results were corrected for ash content. Nitrogen was estimated by the Kjeldahl procedure of McKenzie & Wallace (1954). Dry matter in tissues was determined after heating 0·3–0·5 g samples in a drying oven for 18 h at 105°.

Assay and nomenclature of vitamin B_{12}. Vitamin B_{12} activity was determined microbiologically by four procedures (*Ochromonas malhamensis*, *Lactobacillus leichmanii*, *Escherichia coli* (tube assay) and *E. coli* (plate assay)) as described by Hine & Dawbarn (1954). All methods were standardized with cyanocobalamin. Results are expressed as vitamin B_{12} activity for the particular organism except that vitamin B_{12} activity for *Ochromonas* in any material or for *L. leichmanii* in liver (Hine & Dawbarn, 1954) are assumed to represent only cobalamins and are referred to as vitamin B_{12}.

Measurement of radioactivity. Radioactivity due to ^{58}Co or ^{60}Co was determined with an Ekco scintillation counter type N550 with an Ekco scaler type N530C (Ekco Electronics Ltd, Southend-on-Sea, Essex). Samples of faeces, alimentary tract contents and those tissues difficult to homogenize were made soluble by wet digestion in boiling nitric acid, sulphuric acid and perchloric acid, made to volume with water and the samples were counted. Soft tissues were homogenized in water for 3 min in a Servall Omnimixer (Ivan Sorvall Inc., Norwalk, Conn., USA) and samples of the homogenates counted. Urine, whole blood and bile were counted direct. Samples containing ^{58}Co were counted with 15% efficiency using a probe-type crystal for annular counting. Samples (other than fluids) of 10–20 g were made to 50 ml, and 10 ml counted. Samples containing ^{60}Co were counted with 62% efficiency using a well-type crystal. Samples (other than fluids) of 2–4 g were made to 20 ml, and 5 ml counted. All samples were counted for 10^3 s. Measured activity of ^{58}Co was corrected for radioactive decay during the experiment, but with ^{60}Co the correction was negligible and was omitted.

Materials. Crystalline cyanocobalamin was obtained from Glaxo–Allenburys (Aust.) Pty Ltd, Melbourne, and was standardized spectrophotometrically at 361 nm (Barker, Smyth, Weissbach, Munch-Petersen, Toohey, Ladd, Volcani & Wilson, 1960). ^{60}Co as cobaltous chloride and [^{58}Co]cyanocobalamin were obtained from the Radiochemical Centre, Amersham. The latter was diluted with unlabelled cyanocobalamin to a specific activity of 26 nCi/μg. 5′-Deoxyadenosyl[^{60}Co]cobalamin and also the unlabelled compound were obtained by the courtesy of Dr D. Perlman (E. R. Squibb & Sons, New Brunswick, NJ, USA). The radioactive coenzyme was chromatographed on Dowex 50 as described by Barker, Smyth, Weissbach, Toohey, Ladd & Volcani (1960). The final aqueous solution, purged of phenol, was standardized spectrophotometrically at 375 nm (Barker, Smyth, Weissbach, Toohey *et al.* 1960) and diluted with the unlabelled compound, similarly standardized to a specific activity of 72 nCi/μg. All samples of the coenzyme were carefully protected from light.

RESULTS

Faecal and urinary excretion of ^{60}Co. A 6-year-old ewe (no. 110) was fed on the Co-deficient diet supplemented with Co for 7 months, after which Co treatment was discontinued. The final supplement of 1 mg Co contained 0·2 mCi ^{60}Co and was introduced direct into the rumen through a fistula. Faecal and urinary excretion of ^{60}Co for the following 14 d are shown in Fig. 1. Food intake over this period averaged 1016 g/d.

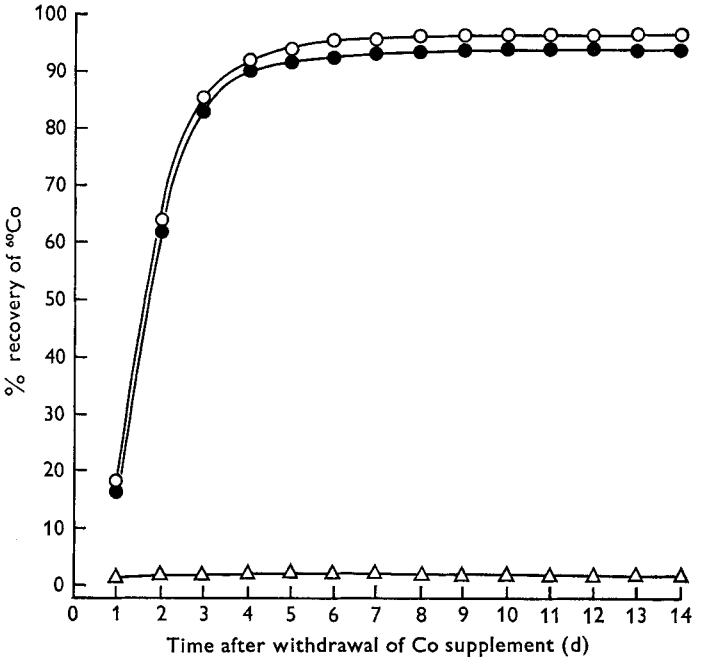

Fig. 1. Excretion of ^{60}Co by sheep after administration of 0·2 mCi ^{60}Co in 1 mg cobalt. ○, total; ●, in faeces; △, in urine.

Most (93%) of the administered ^{60}Co was excreted in the faeces within 5 d. In the 14 d period 95·1% appeared in the faeces and only 2·5% in the urine. The results are in general agreement with those of Comar (1948) for cattle, and of Monroe, Sauberlich, Comar & Hood (1952) for wether lambs.

Distribution of vitamin B_{12} *activity in rumen contents*. Table 1 shows the results of two experiments in which vitamin B_{12} distribution was measured. In the first experiment (sample A) an animal on dry summer pasture containing adequate Co (0·15 μg Co/g (dry)) was killed and a portion of whole rumen contents strained under gravity through cheese-cloth. A little over 40% of the weight of the sample passed freely through, and this and the residual wet fibrous matter were assayed with *L. leichmanii* for vitamin B_{12} activity, as was the initial sample. The fluid fraction contained only 4% of the vitamin B_{12} activity, and, even on the assumption that all the residual water in the wet residue contained vitamin B_{12} activity at the same level, less than 10%

Table 1. *Distribution of vitamin B_{12} activity in whole rumen contents of sheep*

(Sample A was from a sheep on pasture containing about 0·15 μg cobalt/g (dry); sample B was from an animal maintained on the Co-deficient diet supplemented with 1 mg *Co*/d)

			Distribution of vitamin B_{12} activity					
			Ochromonas			*L. leichmanii*		
Original sample	Treatment	Description of separated fractions	Whole rumen contents (μg/100 g)	In separated fractions (%)	Total recovery in all fractions (%)	Whole rumen contents (μg/100 g)	In separated fractions (%)	Total recovery in all fractions (%)
Whole rumen contents (387 g, 15·5 % dry matter) (sample A)	Strained through two thicknesses of cheese-cloth under gravity	Wet vegetable matter with associated organisms (223 g, 25·4 % DM)	—	—	—	6·1	96	95
		Fluid with suspended organisms (164 g, 2·1 % DM)	—	—		0·23	4	
Whole rumen contents (sample B)	Strained through stainless-steel gauze and fluid centrifuged 30 min at 21000*g* and 0°	Original whole rumen contents	3·07	100	—	5·47	100	—
		Centrifugal supernatant fluid	—	—		0·21	4	
Whole rumen contents (sample B)	Strained through stainless-steel gauze, then through gossamer. Retained solids repeatedly shaken in artificial saliva (pH 7, containing KCN) and strained to recover organisms. Fluid and washes combined	Washed fibrous matter	0·29	11	87	0·45	11	75
		Combined strained fluid fractions	2·37	89		3·67	89	
Suspended micro-organisms in rumen fluid diluted with artificial saliva (pH 7, containing KCN) (combined strained fluid fractions of sample B)	Centrifuged 30 min at 21000*g* and 0°.	Supernatant fluid	0·11	5	92	0·34	9	98
		Sediment	2·07	95		3·26	91	
	Saturated with toluene, incubated 90 min at 39° and pH 6·6, then chilled and centrifuged 30 min at 21000*g* and 0°.	Supernatant fluid	0·53	24	92	1·61	43	102
		Sediment	1·64	76		2·14	57	
	Saturated with toluene, adjusted to pH 2 with hydrochloric acid, incubated 90 min at 39°, then chilled and centrifuged 30 min at 21000*g* and 0°	Supernatant fluid	2·34	94	105*	3·45	93	101
		Sediment	0·15	6		0·26	7	

DM, dry matter. * Recovery of added cyanocobalamin 101 %.

of the activity in whole rumen contents could be regarded as freely detached from fibrous solids.

Distribution of vitamin B_{12} activity between micro-organisms and fluid in rumen contents (obtained through a fistula) of a sheep eating 1050 g/d of the Co-deficient diet supplemented with 1 mg Co/d is shown by the results of the analysis of sample B. The sample was taken 4 h after feeding and drenching with Co. With the *L. leichmanii* assay only 4% of the vitamin B_{12} activity remained in the supernatant fraction after straining and high speed centrifugation. From the *Ochromonas* assay almost 90% of the

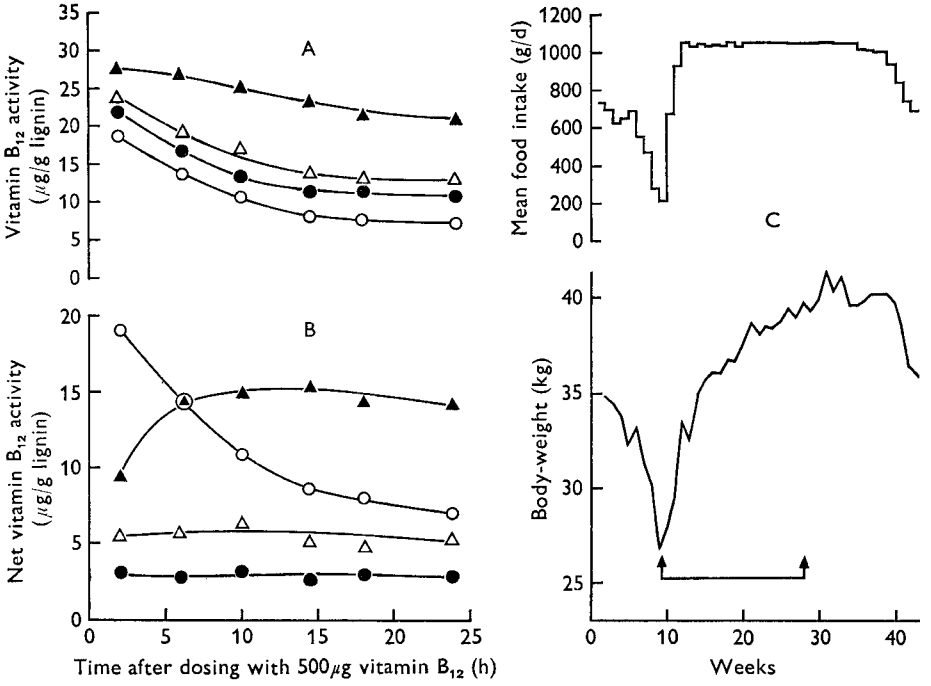

Fig. 2. Degradation of cyanocobalamin introduced into the rumen of sheep. C shows the response in food intake and body-weight of a severely vitamin B_{12}-deficient animal to a daily drench of 500 μg cyanocobalamin started at the first arrow and discontinued at the second. A and B show the results of microbiological estimations of vitamin B_{12} activity in rumen contents after the first dose. Activity was estimated by *Ochromonas malhamensis* (○), *Lactobacillus leichmanii* (●), *Escherichia coli* (tube method) (△), and *E. coli* (plate method) (▲). The total activities are shown in A, and in B the total activity for *Ochromonas*, together with the net activities for the other organisms after subtracting the *Ochromonas* activity.

vitamin B_{12} could be released from fibrous solids by repeated mechanical shaking (four times each for 4 min) in McDougall's (1948) artificial saliva (containing 0·005% KCN and adjusted to pH 7 with H_3PO_4). Most (95%) of this activity was sedimented on centrifuging and was presumably associated with bacteria. The washed fibrous solids were seen by microscopic examination to contain some residual adherent bacteria.

Incubation for 90 min at 39° (pH 6·6 in the presence of toluene) led to release of

about one-quarter of the vitamin B_{12} from the bacteria in the fluid phase, but when incubated at pH 2 (HCl) almost all (94 %) of the vitamin B_{12} was released.

Degradation of cyanocobalamin introduced into the rumen. An animal (no. 9047) was fed on the Co-deficient diet until it was severely deficient of vitamin B_{12} and was then treated orally with 500 μg cyanocobalamin/d. The response is shown in Fig. 2C. Treatment was stopped after 4 months, and 7 weeks later the vitamin B_{12} reserves created by this treatment had been depleted to the point where appetite again failed. (Recovery was immediate on subsequent treatment with 50 μg cyanocobalamin/d by injection.)

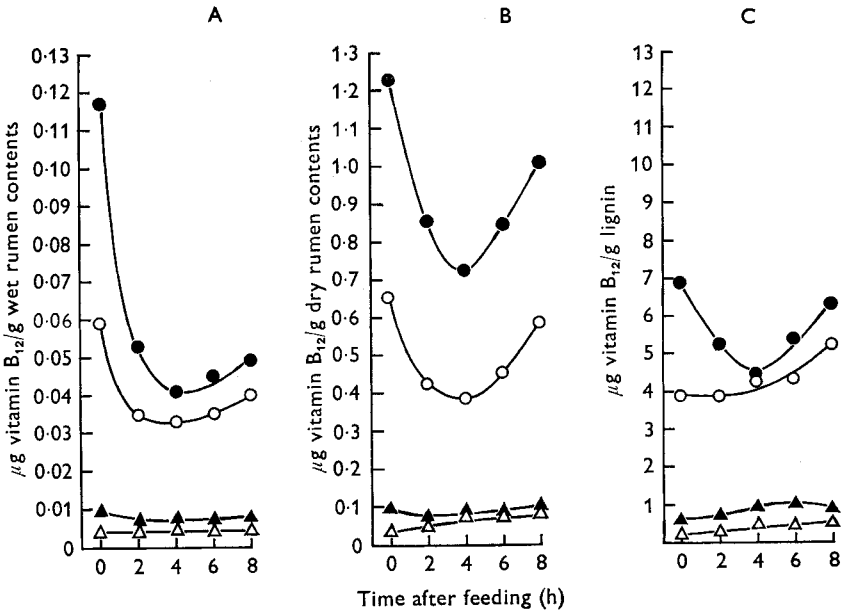

Fig. 3. *Ochromonas* vitamin B_{12} activity in whole rumen contents of sheep after feeding, expressed as μg cyanocobalamin/g wet matter (A), dry matter (B) or lignin (C), both with and without supplementary cobalt. ○, animal no. 110, +Co; ●, no. 193, +Co; △, no. 110, −Co; ▲, no. 193, −Co.

Although the daily drench of 500 μg cyanocobalamin permitted absorption of sufficient vitamin B_{12} for the animal's needs, little remained for storage. Using the values of Marston (1970) for prophylactic doses of injected cyanocobalamin on this diet, one may calculate that absorption probably lay between 6 μg and 12 μg/d, or between 1 % and 3 % of the dose.

Degradation of cyanocobalamin in the rumen was detected in samples of rumen contents taken through a fistula at intervals for 24 h after the first dose. This dose was introduced directly into the rumen and the animal was starved for 24 h. The ratios of vitamin B_{12} activity to lignin for the four assay procedures are shown in Fig. 2A, and in Fig. 2B the same results for vitamin B_{12} measured with *Ochromonas* are compared with the activity of factors other than cobalamins active for the other organisms. These were calculated by subtracting the *Ochromonas* vitamin B_{12} to lignin ratios from the corresponding ratios obtained by the other methods.

The rapid decline in the ratio of *Ochromonas* vitamin B_{12} to lignin over the first 10 h was accompanied by an increase in net *E. coli* (plate) vitamin B_{12} to lignin ratio, but there was little change in either net *E. coli* (tube) or net *L. leichmanii* vitamin B_{12} to lignin ratio.

Production of vitamin B_{12} in the rumen. No completely satisfactory method has been devised for measuring the rate of passage of an unabsorbed substance produced in the rumen of a fed animal. In the present work approximate rates of production of vitamin B_{12} have been calculated from *Ochromonas* vitamin B_{12} to lignin ratios in the rumen 4 h after feeding, by multiplying by the daily intake of lignin. This method is subject to criticism, and the results, particularly in animals receiving Co, must be regarded as minimum values.

Fig. 3 and Table 2 show the vitamin B_{12} activity in rumen contents at different times during the first 8 h after feeding, both with and without supplementary Co. Both animals were stabilized for at least 14 d with or without Co before the measurements were made. Use of the lignin ratio reduced but did not eliminate the effects of rate of consumption of food, and in animals receiving Co the vitamin B_{12} to lignin ratio tended to reach a minimum value 4–6 h after feeding.

In the experiment shown, the hay-chaff contained 86% dry matter and 0·03 μg Co/g (dry) and lignin was 11·1% of the dry matter. From the lignin intake and the vitamin B_{12} to lignin ratio 4 h after feeding, the minimum rates of production of vitamin B_{12} for animals 110 and 193 respectively were 406 and 423 μg/d when Co was given and 46 and 92 μg/d when Co was withheld. The results for the Co-depleted rumen are also regarded as minimum values because part of the vitamin B_{12} in rumen contents is not associated with lignin-bearing solids (Table 1) and Hogan & Weston (1967) and Weston & Hogan (1967) have shown that the rate of turn-over of water through the rumen is faster than that of fibrous solids. The latter error applies to all measurements reported but is probably small. Table 1 shows that vitamin B_{12} is fairly closely associated with lignin-bearing solids in the rumen and suggests that lignin is a reasonably appropriate marker in terms of distribution.

Fig. 4 shows the effects of withdrawal of Co on vitamin B_{12} production in the rumen of two sheep (no. 9029 and no. 9047) previously stabilized for 16 weeks and 2 weeks respectively on 1 mg Co/d. Periodic samples were taken for 112 d, during which time the mean food intake of no. 9029 was 920 g/d and of no. 9047 was 800 g/d. Calculated from lignin intakes, the minimum rate of vitamin B_{12} production fell from 600–750 μg/d to about 50 μg/d within 5 d of withdrawal of Co. The latter value was maintained for the remainder of the experiment and reflects synthesis from the Co in the fodder (0·01 μg Co/g (dry)). In animal 9047 the vitamin B_{12} to lignin ratio did not fall until the 2nd day after the final drench.

In a further experiment with no. 9029, Co was withdrawn after daily drenching for 4 months. The full ration of 1050 g/d was consumed and vitamin B_{12} production fell from a minimum of 480 μg/d to 50 μg/d at 6 d and 65 μg/d at 14 d after withdrawal of Co. The diet contained 0·04 μg Co/g (dry).

Production of other factors with vitamin B_{12} activity in rumen contents. The increases in *Ochromonas* vitamin B_{12} to lignin ratios in the rumens of both Co-deficient and

Table 2. *Vitamin B_{12} activities in rumen contents of sheep during 8 h after feeding*

Animal no.	Treatment	Time after feeding (h)	Food intake over preceding 2 h (g)	Lignin content of dry matter in rumen (%)	Vitamin B_{12} activity			
					Ochromonas (μg/g) (dry)	*E. coli* (plate) (μg/g) (dry)	*E. coli* (tube) (μg/g) (dry)	*L. leichmanii* (μg/g) (dry)
193	1 mg cobalt/d	0	0	17·9	1·23	13·9	2·5	—
		2	686	16·3	0·86	9·8	1·8	—
		4	364	16·6	0·73	9·0	1·6	—
		6	0	15·8	0·85	10·0	1·9	—
		8	0	16·0	1·01	12·6	2·1	—
193	Rumen depleted of Co	0	0	19·7	0·102	0·61	0·33	0·27
		2	756	17·3	0·078	0·79	0·30	0·23
		4	294	16·9	0·097	1·03	0·37	0·31
		6	0	15·6	0·090	1·16	0·41	0·34
		8	0	15·8	0·108	1·25	0·45	0·35
110	1 mg Co/d	0	0	19·5	0·66	13·4	2·9	2·1
		2	548	16·2	0·43	7·5	1·9	1·4
		4	349	15·8	0·39	6·8	1·6	1·2
		6	133	16·0	0·46	7·6	1·8	1·5
		8	16	17·5	0·59	9·6	2·3	1·9
110	Rumen depleted of Co	0	0	19 0	0·039	0·30	0·12	—
		2	499	17·2	0·051	0·51	0·20	—
		4	345	15·6	0·074	0·96	0·37	—
		6	169	15·9	0·071	0·89	0·33	—
		8	13	16·1	0·082	1·22	0·43	—

Co-supplemented sheep, shown in Fig. 3, clearly demonstrate synthesis of vitamin B_{12}. In both instances simultaneous synthesis of other cobamides may be inferred from Table 2 where the ratios to *Ochromonas* vitamin B_{12} of the activity for the other test organisms may be calculated and shown to be maintained. In Table 3 are summarized the results of all measurements made of vitamin B_{12} activity in rumen contents in the present series of studies. In addition to the summarized results of Table 2, the values in

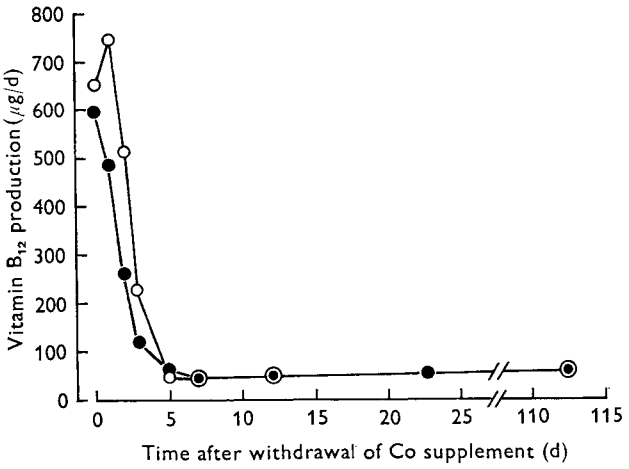

Fig. 4. Minimum rates of production of vitamin B_{12} in the rumen of sheep after withdrawal of a cobalt supplement, estimated from *Ochromonas* vitamin B_{12} to lignin ratios in rumen contents 4 h after feeding. Both animals received 1 mg Co/d by mouth before withdrawal on day 0. ●, animal 9029; ○, animal 9047.

Table 3 include the published results of Hine & Dawbarn (1954) and Dawbarn, Hine & Smith (1957). As in Fig. 2, the results for *E. coli* (plate), *E. coli* (tube) and *L. leichmanii* have been expressed as net activities by subtracting the *Ochromonas* values in order to express the activities of factors other than the cobalamins. In Table 3 the Co-treated animals had received 1 mg Co/d for at least 14 d and the Co-depleted animals had been deprived of supplementary Co for at least 7 d. Both groups subsisted on the Co-deficient diet (0·01–0·04 μg Co/g (dry)). Pasture-fed animals consumed a mixed grass–legume diet containing about 0·15 μg Co/g (dry).

Characteristic differences occurred not only between animals with and without Co (1 mg/d) on the Co-deficient diet, but also between Co-treated animals and grazing animals on sound pastures. Some of these features were noted by Hine & Dawbarn (1954).

Rumen contents of Co-deficient animals were characterized by low activities of vitamin B_{12} measured by all methods. The Co-treated animals showed a high net *E. coli* (plate) activity, and although this was not significantly higher than that in grazing animals, the ratio net *E. coli* (plate) to *Ochromonas* was significantly higher than that in either Co-deficient or pasture-fed animals. The high activity for *Ochromonas* in rumen contents of pasture-fed animals, although not significantly greater than that in Co-treated animals, contributed to the significantly lower ratios net *E. coli* (plate) to

Table 3. *Vitamin B_{12} and vitamin B_{12}-like substances in the rumen contents of sheep*

(Values from Table 2 and from the published results of Hine & Dawbarn (1954) and Dawbarn *et al.* (1957); mean values with their standard errors for the number of observations shown in parentheses)

Vitamin B_{12} activity	Cobalt-treated (Co+) (μg/g) dry	Cobalt-depleted (Co−) (μg/g) dry	Pasture-fed (P) (μg/g) dry	Significance of differences between Co+ and Co−	P and Co−	Co+ and P
Net *E. coli** (plate)	11·06 ± 1·53 (8)	0·577 ± 0·077 (6)	7·28 ± 1·28 (5)	$P < 0·01$	$P < 0·01$	NS
Net *E. coli** (tube)	1·62 ± 0·22 (8)	0·20 ± 0·032 (6)	1·04 ± 0·15 (5)	$P < 0·01$	$P < 0·01$	NS
Net *L. leichmanii**	0·890 ± 0·123 (7)	0·117 ± 0·032 (5)	0·78 ± 0·18 (5)	$P < 0·01$	$P < 0·05$	NS
Ochromonas	0·814 ± 0·088 (8)	0·075 ± 0·005 (6)	2·32 ± 0·67 (5)	$P < 0·01$	$P < 0·05$	NS

	Ratios of activities†			Significance of differences		
	Co+	Co−	P	Co+ and Co−	P and Co−	Co+ and P
Net *E. coli* (plate) / *Ochromonas*	13·84 ± 1·33 (8)	7·65 ± 0·86 (6)	3·96 ± 0·75 (5)	$P < 0·01$	$P < 0·05$	$P < 0·01$
Net *E. coli* (tube) / *Ochromonas*	2·19 ± 0·45 (8)	2·65 ± 0·44 (6)	0·60 ± 0·13 (5)	NS	$P < 0·01$	$P < 0·05$
Net *L. leichmanii* / *Ochromonas*	1·27 ± 0·27 (7)	1·52 ± 0·39 (5)	0·36 ± 0·09 (5)	NS	$P < 0·05$	$P < 0·05$

* Obtained by subtracting *Ochromonas* activity from total activity.
† Means of individual ratios.

Ochromonas, net *E. coli* (tube) to *Ochromonas* and net *L. leichmanii* to *Ochromonas* in the former, and this feature was characteristic of the grazing animals.

Ruminal production and faecal and urinary excretion of vitamin B_{12} by pair-fed Co-deficient and Co-treated ewes at low food intake. Two fistulated ewes (no. 9151 and no. 9029) were fed on the Co-deficient diet until food intake fell, when no. 9029 was treated with Co and pair-fed with no. 9151 (Smith & Marston, 1970). After 8 weeks, when food intake was 330 g/d, samples of rumen contents were taken and faeces and urine collected daily from the following day for 14 d. Further samples of rumen contents were taken on the 15th day.

Table 4. *Estimated minimum rates of ruminal production of vitamin B_{12} in pair-fed vitamin B_{12}-deficient and cobalt-treated ewes*

Animal no.	Treatment	Food intake (g/d) (dry)	Lignin intake (g/d)	Vitamin B_{12} in rumen contents (μg/g lignin)	Estimated minimum rate of production of vitamin B_{12} in rumen (μg/d)
9151*	Standard Co-deficient diet	220	23·1	0·37	9
9029	1 mg Co/d and pair-fed with 9151	216	22·6	5·6	127

* Severely vitamin B_{12}-deficient.

The mean value of the vitamin B_{12} to lignin ratio in the rumen contents was used to estimate ruminal vitamin B_{12} production over the 14 d collection period, and faecal and urinary excretion of vitamin B_{12} was determined from pooled samples of the excreta (Dawbarn & Hine, 1955). The wheaten hay-chaff used contained 11·0% lignin and 0·04 μg Co/g, both on a dry-weight basis.

Results are shown in Tables 4 and 5. In the deficient animal, daily faecal excretion of vitamin B_{12} was greater than estimated ruminal production, but both quantities were small. In the Co-treated animal, faecal excretion of vitamin B_{12} was somewhat less than the minimum estimated ruminal production. In both instances urinary excretion of vitamin B_{12} was small.

Production of vitamin B_{12} in relation to soluble Co concentration in the rumen. A 5-year-old ewe (no. 110) was stabilized for several months at full food intake with supplementary Co before Co was withdrawn. For several days before the final Co supplement, samples of rumen contents were taken 4 h after feeding for estimation of Co, lignin and vitamin B_{12}. The final supplement (1 mg Co) contained 1·0 mCi ^{60}Co and was introduced direct into the rumen at the time of feeding. Samples of rumen contents were taken 4 h later and then daily for 8 d 4 h after feeding. Immediately after taking each of these samples a sample of rumen fluid was withdrawn with a syringe and centrifuged for 30 min at 0° and 13000 *g*. The supernatant fluid was essentially free from micro-organisms and was used to estimate ^{60}Co and dry matter.

The samples of whole rumen contents were analysed for total Co, ^{60}Co, dry matter, lignin and *Ochromonas* vitamin B_{12}.

The measured water content of the two kinds of sample together with the lignin concentrations in whole rumen contents allowed the ^{60}Co values to be expressed as ^{60}Co to lignin ratios for each. On this basis the rate of decline of ^{60}Co with time was

Table 5. *Vitamin B_{12} excretion in relation to food intake of pair-fed vitamin B_{12}-deficient and cobalt- or vitamin B_{12}-treated ewes*

Expt no.	Animal no.	Treatment	Period of pair-feeding or recovery (weeks)	Food intake* (g (dry)/d)	Excretion of vitamin B_{12}†	
					Faeces (μg/d)	Urine (μg/d)
1	9134	Vitamin B_{12}-deficient	—	180	34	—
	7049	1 mg Co/d and pair-fed with 9134	27	168	86	—
2	9151	Vitamin B_{12}-deficient	—	220	18	0·07
	9029	1 mg Co/d and pair-fed with 9151	8	216	92	0·76
3	5009	Vitamin B_{12}-deficient	—	285	21	0·09
	7019	50 μg cyanocobalamin/d by injection and pair-fed with 5009	15	275	57	15·2
4	9090	Vitamin B_{12}-deficient	—	365	44	0·11
	7009	50 μg cyanocobalamin/d by injection and pair-fed with 9090	9	355	66	17·7
5	0077	Co-depleted in rumen‡	—	727	127	0·26
	0113	1 mg Co/d and pair-fed with 0077	4	727	260	3·6
6	0113	Co-depleted in rumen‡	—	723	120	0·35
	0077	1 mg Co/day and pair-fed with 0113	2	723	300	2·7
7	5009	Vitamin B_{12}-deficient	—	196	5	0·17
	5009	500 μg cyanocobalamin/d by mouth	6–32 d	509	120	0·24

* From the values of Smith & Marston (1970).
† From the values of Dawbarn & Hine (1955).
‡ Not deficient of vitamin B_{12} in their tissues.

found to be accurately exponential in both (except for the first supernatant sample taken 4 h after ^{60}Co was given). The half-time for decline of ^{60}Co in the supernatant fraction (20·9 h) was somewhat longer than that in whole rumen contents (17·5 h) and in consequence, although only 1·2% of the ^{60}Co was present in the supernatant fraction 28 h after the dose, 8 d later the amount had risen to 3%. This may have been due to a greater proportion of the supernatant ^{60}Co being present as cobamides as the Co concentration fell. These may not have been so tightly bound by the micro-

organisms as was ionic Co. In the first sample (4 h), 3% of the ^{60}Co in whole rumen contents was in the supernatant fraction. Under somewhat similar conditions Tosic & Mitchell (1948) found about 20% of administered Co in the supernatant fraction 10 h after feeding.

The concentration of Co in the supernatant fraction was calculated from the ^{60}Co content by assuming the specific activity (μCi $^{60}Co/\mu$g Co) to be the same as that measured in corresponding samples of whole rumen contents. These values, expressed as μg Co/g water, are shown in Fig. 5, together with the values for vitamin B_{12} and total Co in whole rumen contents expressed as ratios to lignin.

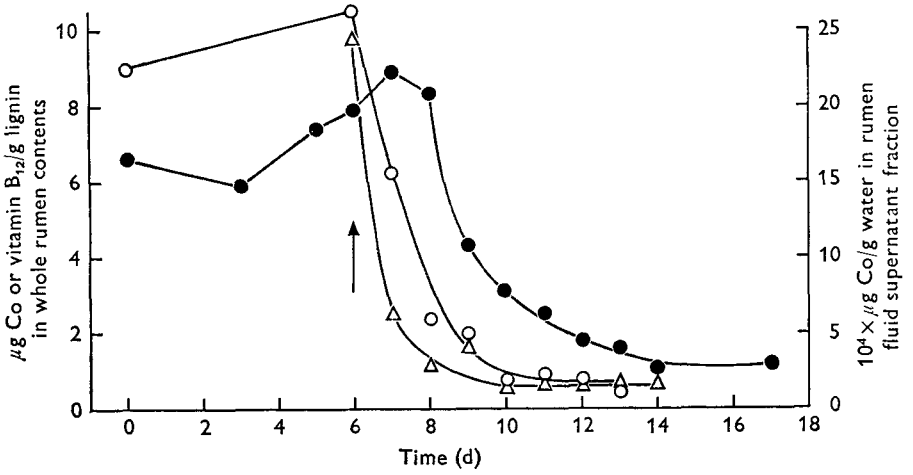

Fig. 5. Concentrations of cobalt and vitamin B_{12} in rumen contents of sheep before and after withdrawal of a Co drench (1 mg/d) on day 6. ○, total Co; △, Co in solution; ●, *Ochromonas* vitamin B_{12} expressed as cyanocobalamin.

Although both total Co and supernatant Co fell rapidly after the final dose, vitamin B_{12} concentrations did not fall until 2 d later when supernatant Co was less than 0·5 ng/ml and total Co less than 20 ng/g of wet rumen contents. The final concentration of Co in the depleted rumen at 8 d was steady at less than 0·2 ng/ml in the supernatant fraction and about 5 ng/g wet rumen contents (the value for the supernatant fraction was previously reported incorrectly as 20 pg/ml; Marston, Allen & Smith, 1961). The Co content of the chaff used was 0·53 μg Co/g lignin and this value was approached in the depleted rumen.

Calculated from daily lignin intakes, the estimated minimum rate of production of vitamin B_{12} in this experiment fell from 730 μg/d when Co was given to 110 μg/d 8 d after it was withdrawn.

Efficiency of conversion of Co into vitamin B_{12} in the rumen. From the Co to lignin and vitamin B_{12} to lignin ratios in whole rumen contents (Fig. 5) the fraction of the total Co present as vitamin B_{12} (cobalamins) may be calculated. These values are shown in Fig. 6 as a function of the Co to lignin ratio. Although the errors in this estimation became considerable at low Co concentrations, the efficiency of conversion of Co into vitamin B_{12} was obviously higher when Co was depleted.

It was clear from early work (Hale *et al.* 1950; Hine & Dawbarn, 1954) that ruminal synthesis of vitamin B_{12} was more efficient from small quantities of dietary Co than from supplementary Co, and the present results suggest an approximately linear decrease in efficiency from about 15% in the rumen of animals depleted to about 3% when 1 mg Co/d was given.

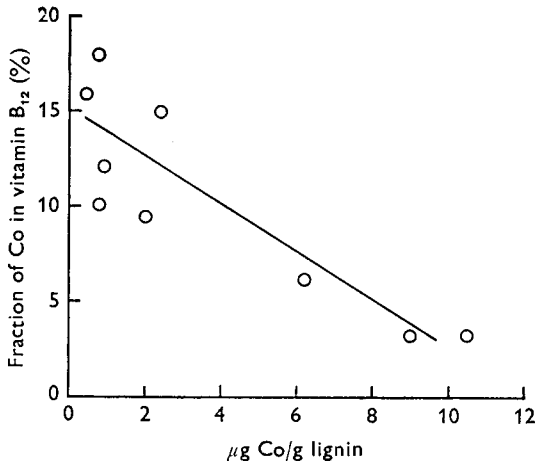

Fig. 6. Relationship between the cobalt concentration in rumen contents of sheep and the percentage present as cobalamins, derived from the values shown in Fig. 5.

Excretion of vitamin B_{12} in faeces and urine. Dawbarn & Hine (1955) have reported values for mean daily faecal and urinary excretion of vitamin B_{12} by pair-fed Co-deficient and Co-treated or vitamin B_{12}-treated ewes over 14 d periods. Nitrogen and combustible energy retention for the same periods were reported (except for the fistulated animals 9151 and 9029) by Smith & Marston (1970). The combined results for food intake and vitamin B_{12} excretion are summarized in Table 5. In the vitamin B_{12}-deficient animals the severity of the deficiency is indicated by food intake which had fallen from about 940 g (dry)/d. In Expts 5 and 6, both animals were restricted to the food intakes shown, and in periods without Co these animals were not seriously depleted of vitamin B_{12} in the tissues (Smith & Marston, 1970).

Also reported by Dawbarn & Hine (1955) and shown in Table 5 are faecal and urinary excretion of vitamin B_{12} by a severely deficient animal (no. 5009) before and after treatment with 500 μg cyanocobalamin/d by mouth. The animal responded to this treatment with increased food intake and body-weight gains, but when the treatment was withdrawn after 9 months it immediately relapsed and had evidently retained barely sufficient vitamin B_{12} for its daily needs. Steady levels of excretion were attained by the 5th day, and the means of the values 6, 13 and 32 d after treatment was begun are given in Table 5. If the requirement of injected cyanocobalamin is about 6 μg/d (Marston, 1970), only about 1% of the dose can have been absorbed even though a little more than 20% appeared in the faeces.

As noted by Dawbarn & Hine (1955), urinary excretion in animals fed on this diet

with or without supplementary Co was seldom more than 1% of faecal excretion, but when cyanocobalamin was injected (50 μg/d) 30–36% was excreted in the urine and most of the remainder in the faeces.

Table 6. *Distribution of ^{58}Co in tissues and alimentary tract contents after subcutaneous injection of* 500 μg [^{58}Co]*cyanocobalamin* (13 μCi) *over* 20 *d into a vitamin* B_{12}*-deficient sheep*

	Tissues			Contents of alimentary tract			
Organ, tissue or section of alimentary tract	Wet weight (g)	Specific activity (nCi/g) (wet)	Percentage of total administered dose	Wet weight (g)	Dry matter (%)	Specific activity (nCi/g) (dry)	Percentage of total administered dose
Liver	401	5·35	16·5	—	—	—	—
Kidneys	81	8·42	5·3	—	—	—	—
Spleen	55	1·45	0·6	—	—	—	—
Lungs	385	1·03	3·1	—	—	—	—
Brain	95	0·40	0·3	—	—	—	—
Heart	158	0·35	0·4	—	—	—	—
Skeletal muscle	—	0·13	—	—	—	—	—
Bone marrow	—	< 0·05	—	—	—	—	—
Blood	—	0·08	—	—	—	—	—
Gall bladder	2	0·72	—	—	—	—	—
Gall bladder contents	2·4	0·59	—	—	—	—	—
Oesophagus	37	0·27	< 0·1	—	—	—	—
Rumen	472	2·90	10·5	3150	16·4	0·12	0·5
Reticulum	119	2·05	1·9	330	12·6	< 0·05	< 0·1
Omasum	133	3·06	3·2	161	17·2	0·33	< 0·1
Abomasum	178	0·81	1·2	286	10·4	0·54	0·1
Duodenum	9	1·18	< 0·1	12	6·2	3·06	< 0·1
Ileum 1	40	2·24		69	10·5	1·81	
2	43	1·21		98	10·3	2·19	
3	20	1·42		73	10·9	1·47	
4	25	1·49	3·8	75	7·6	2·19	0·8
5	75	1·71		140	8·0	2·22	
6	33	1·10		93	7·5	1·42	
7	41	1·53		109	6·6	0·44	
8	44	1·51		92	7·0	0·41	
Caecum	144	0·82	0·9	580	11·4	0·64	0·4
Colon 1	58	0·71		75	14·5	0·55	
2	53	0·63	1·3	58	18·2	0·68	0·3
3	58	0·86		50	23·5	0·86	
4	80	0·58		44	21·7	0·68	
Rectum	89	0·40	0·3	34	27·4	0·51	< 0·1

Excretion and tissue distribution of [^{58}Co]*cyanocobalamin injected into a vitamin* B_{12}*-deficient sheep.* A vitamin B_{12}-deficient ewe (no. 830, body-weight 24 kg, food intake 440 g/d) was injected subcutaneously with 25 μg (0·65 μCi) [^{58}Co]cyanocobalamin/d for 20 d and was killed on the 21st day. Food intake increased to 880 g/d and body-weight to 27 kg during treatment.

Daily measurements of faecal and urinary ^{58}Co showed that during the 21 d 19·9% of the labelled vitamin B_{12} was excreted in the faeces and 13·3% in the urine. In a previous similar experiment lasting for 7 d (animal no. 377), 7·2% of the injected

label appeared in the faeces and 8·4% in the urine. Net retention of injected vitamin B_{12} by the tissues of deficient animals was therefore high, but the distribution of excreted vitamin B_{12} between faeces and urine was similar to that of repleted animals (Table 5).

Table 7. *Distribution of ^{60}Co in tissues and alimentary tract contents after intravenous injection of 304 μg 5′deoxyadenosyl[^{60}Co]cobalamin (21·9 μCi) over 10 d into a vitamin B_{12}-deficient sheep*

Organ, tissue or section of alimentary tract	Tissues: Specific activity (nCi/g) (wet)	Tissues: Dry matter (%)	Contents of alimentary tract: Dry matter (%)	Contents of alimentary tract: Specific activity (nCi/g) (dry)
Liver	19·6	24·0	—	—
Kidneys	17·0	17·6	—	—
Spleen	5·0	23·6	—	—
Pancreas	3·6	21·0	—	—
Submaxillary gland	3·9	21·0	—	—
Adrenals	3·4	19·1	—	—
Ovary	4·2	16·0	—	—
Thyroid	5·2	21·8	—	—
Lung	2·0	19·9	—	—
Heart	1·0	11·6	—	—
Skeletal muscle	0·2	29·0	—	—
Bile	0·03	—	—	—
Rumen	4·3	17·5	12·6	0·3
Reticulum	4·1	14·9	—	—
Abomasum	2·2	21·7	9·9	1·2
Duodenum	3·7	18·1	6·5	9·3
Ileum 1	3·3	14·9	8·8	15·7
2	3·5	16·6	11·6	12·6
3	3·8	16·5	6·1	10·4
4	2·9	13·1	8·1	5·1
5	0·8	8·8	8·6	3·3
6	0·3	9·0	8·7	3·5
7	3·5	15·3	8·4	2·5
8	2·8	13·0	8·5	1·3
Caecum	2·3	18·9	14·1	1·7
Colon 1	3·0	15·2	17·7	1·0
2	2·7	21·0	22·2	1·0
3	2·1	20·3	22·6	0·8
Rectum	1·8	32·2	23·8	1·1

The distribution of labelled vitamin B_{12} in tissues and alimentary tract contents of animal 830 was determined at slaughter. Immediately after death the small and large intestines were ligated in sections and the contents of each section gently extruded and collected. The contents of the remainder of the alimentary tract were separated and collected without ligation. After extrusion of contents, the sections of gut were rinsed out with isotonic saline and retained for analysis. Table 6 shows the results for alimentary tract contents on a dry-weight basis and for tissues on a wet-weight basis.

Excretion and tissue distribution of 5′-deoxyadenosyl[^{60}Co]cobalamin in a vitamin B_{12}-deficient sheep. A vitamin B_{12}-deficient ewe (no. 519, body-weight 27 kg, food intake 260 g/d) was injected intravenously with 30·4 μg (2·19 μCi) 5′-deoxyadenosyl[^{60}Co]cobalamin/d for 10 d and killed on the 11th day. Food intake increased to 640 g on the

10th day of treatment and body-weight to 30 kg. Samples of tissues and alimentary tract contents were collected at slaughter as before and in addition a sample of liver was chilled and homogenized in 0·25 M-sucrose for centrifugal separation of intracellular components (Smith, Osborne-White & Russell, 1965).

Table 8. *Subcellular distribution of ^{60}Co in liver after intravenous injection of 5′-deoxyadenosyl[^{60}Co]cobalamin injected into a sheep*

(A sample of the liver referred to in Table 7)

Starting material	Derived fraction	^{60}Co recovered from 14·7 g liver (nCi)	Nitrogen recovered from 14·7 g liver (mg)	Activity (nCi/mg N)
Whole liver	Total	270	393	0·69
Homogenate in 0·25 M-sucrose	Nuclear-free homogenate (600 *g* supernatant)	171	233	0·73
	Nuclear fraction (600 *g* sediment)	95·2	176	0·54
Nuclear-free homogenate	Washed mitochondria	89·2	58·4	1·53
	Mitochondrial wash fluid	2·4	19·7	0·12
	Microsomes	4·4	40·9	0·11
	Soluble fraction	68·5	125·8	0·55
Washed mitochondria	Centrifugal supernatant from mechanically disrupted mitochondria*	59·3	21·9	2·71

* Mitochondria disrupted at top speed in a Servall Omnimixer for 5 min before centrifuging for 1 h at 54000 *g*.

Table 7 shows the distribution of labelled cobalamins in tissues and tract contents, and Table 8 shows the distribution in the liver homogenate. At the end of the 10 d period 5·7% of the injected labelled material had been excreted in the faeces and 2·5% in the urine. The liver retained 25·6% of the injected coenzyme, and a large proportion of this (52% of the activity of the nuclear-free homogenate) was firmly retained within the mitochondria. There was no evidence of accumulation by microsomes. The nuclear fraction had a lower activity relative to nitrogen than did the whole homogenate and although this fraction was heavily contaminated with unbroken cells and cell debris, it is concluded that ^{60}Co did not accumulate in the nuclei.

DISCUSSION

With animals receiving 1 mg Co/d on full or near-full feed, the six estimates of minimum production rates of vitamin B_{12} ranged from 410 to 730 μg/d, the lower values corresponding in general to high rates of feeding during the 4 h before sampling. These values represent a range of minimum efficiencies of conversion of Co into vitamin B_{12} from 1·7% to 3·1% based on total daily Co intakes. From Fig. 6 the fraction of ruminal Co present as vitamin B_{12} under these conditions was about 3%. The agreement between this value and the highest of the estimated minimum efficiencies provides the basis for an assessment of ruminal vitamin B_{12} production at about

700 μg/d. In the single ewe studied at low food intake (Table 4), the same Co supplement produced a similar vitamin B_{12} to lignin ratio in rumen contents, suggesting that vitamin B_{12} production was limited by food intake rather than by Co at this level. The rough correlation between food intake and daily faecal excretion of vitamin B_{12} in animals receiving Co (Table 5) supports this contention.

In the Co-depleted rumen the minimum estimates of vitamin B_{12} production ranged from 46 to 110 μg/d on full feed, the variation depending in part on the Co content of the diet. Based on dietary Co intakes, the corresponding efficiencies ranged from 5% to 18% (mean 12%), and from Fig. 6 the fraction of Co present as vitamin B_{12} in rumen contents was about 15%. From these values the efficiency of conversion of dietary Co into vitamin B_{12} with the feed used is assessed at 13 ± 5%, and this did not appear to be altered at low food intake.

The efficiency of production of vitamin B_{12} from Co was undoubtedly affected by the nature of the diet. Measured by microbiological assay, rumen contents from pasture-fed animals contained a significantly greater proportion of their total vitamin B_{12} activity as cobalamins than did animals on the experimental diet either with or without Co. This is consistent with the electrophoretic results of Dawbarn *et al.* (1957), but the high *E. coli* (plate) activity in animals on the experimental diet supplemented with Co is unexplained. Of the cobamides and cobinamides known to be present in rumen contents (Porter, 1953, 1957) only Factor B (cobinamide) leads to this kind of response (Smith, 1965); Dawbarn *et al.* (1957) found little Factor B in rumen contents of Co-treated animals.

The estimated efficiency of absorption of cyanocobalamin given orally (1–3%) is consistent with previous estimates (Kercher & Smith, 1955; Marston, 1970) but cyanocobalamin in the rumen was fairly rapidly destroyed and little more than 20% of the daily dose appeared in the faeces. From the *E. coli* (plate) response, Factor B appeared to be a major product of degradation of cyanocobalamin in the rumen, and the virtual absence of Factor B from rumen contents of pasture-fed or Co-treated animals (Dawbarn *et al.* 1957) suggests that bacterially bound vitamin B_{12} in the rumen may not be so readily degraded. This is particularly apparent in pasture-fed animals where high *Ochromonas* vitamin B_{12} levels were accompanied by relatively low net activities for the other test organisms.

Destruction of vitamin B_{12} produced from Co in the rumen cannot be reliably assessed from faecal excretion because of possible synthesis in the lower gut (Kercher & Smith, 1956), but daily faecal vitamin B_{12} in Co-treated animals was about half the estimated ruminal production. In the absence of supplementary Co, faecal excretion of vitamin B_{12} may have been affected in addition by a net loss of vitamin B_{12} from the tissues. In severely deficient animals at low food intake, mean daily faecal vitamin B_{12} represented 12% of dietary Co but in animals not depleted of tissue vitamin B_{12} this fraction was 21% and probably exceeded ruminal production. Destruction of ruminal vitamin B_{12} undoubtedly occurred during passage through the alimentary tract, but it may not have been as extensive as that of cyanocobalamin given by drench.

Absorptive efficiencies of vitamin B_{12} may be calculated for both Co-treated and Co-deficient animals. By extrapolation from liver storage of vitamin B_{12} in sheep on

graded doses of injected cyanocobalamin, Marston (1970) estimated that about 34 μg vitamin B_{12}/d were absorbed in animals receiving 1 mg (or 10 mg) Co/d. (This is a maximum value that neglects excess urinary excretion of injected cyanocobalamin.) From the present estimate of ruminal production (700 μg/d), this represents an absorptive efficiency of about 5%. For animals on a diet containing 0·03 μg Co/g (dry), the minimum prophylactic dose of injected cyanocobalamin was about 6 μg/d, and of oral Co was about 40 μg/d. On the assumption that 13% of dietary Co was converted into vitamin B_{12}, the efficiency of absorption was again about 5%.

Using these estimates, an assessment may now be made of the total requirements of sheep for vitamin B_{12} either from the minimum requirement for Co (70 μg/d, Marston 1970) or from the minimum requirement for injected cyanocobalamin on a diet containing 0·03 μg Co/g (dry). The total requirement is assessed at about 11 μg/d.

The site of absorption of vitamin B_{12} appears to be the small intestine, as in other animals. Substantial amounts of labelled vitamin B_{12} were secreted into the duodenum and then reabsorbed throughout the length of the ileum. Transitorily high levels of vitamin B_{12} activity for *Ochromonas* or *L. leichmanii* have been reported in the small intestine of sheep (Hine & Dawbarn, 1954; Kercher & Smith, 1956).

After injection of labelled cobalamins the specific activities (μCi/g (dry)) of tract contents immediately above and below the small intestine were about the same, indicating that most of the vitamin B_{12} secreted into the duodenum was reabsorbed. This is in marked contrast to the poor absorption of vitamin B_{12} coming from the rumen and presumably released from bacteria in the acid conditions of the abomasum (Table 1). The poor absorption of vitamin B_{12} coming from the rumen is unexplained but, in view of the high efficiency of reabsorption, it would not appear to be due to competition for intrinsic factor by other cobamides.

The distribution of labelled cobalamins in alimentary tract contents provides no evidence for absorption of vitamin B_{12} below the small intestine and shows that little injected vitamin B_{12} entered the rumen. The activities in bile in the two experiments were widely divergent and with injected 5′-deoxyadenosyl[^{60}Co]cobalamin the activity was much lower than that in the duodenum and upper ileum. No conclusions can be reached as to the means of secretion of vitamin B_{12} into the duodenum.

The distribution in tissues of the two labelled cobalamins was similar and in general resembled that in other animals, with liver and kidneys the chief sites of retention (Reizenstein, 1959; Gräsbeck, Ignatius, Järnefelt, Lindén, Mali & Nyberg, 1961; Rosenblum, Reizenstein, Cronkite & Meriwether, 1963). The relatively high amounts of labelled cyanocobalamin in kidneys may reflect the higher urinary excretion of this substance. Injected labelled 5′-deoxyadenosylcobalamin was strongly retained by the liver, and the distribution in liver cells was similar to that in rat liver (Newman, O'Brien, Spray & Witts, 1962). A feature of interest is the relatively high activity found in the walls of the rumen. The epithelium of this tissue, like liver mitochondria, actively metabolizes propionate (Pennington & Sutherland, 1956; Smith *et al.* 1965), a reaction sequence that involves 5′-deoxyadenosylcobalamin (Marston & Smith, 1961).

It is probable that faeces, rather than urine, is normally the major vehicle of excretion of vitamin B_{12} released from the tissues (Dawbarn & Hine, 1955), the balance between

absorption and excretion being established in the small intestine. Urinary excretion of injected labelled 5′-deoxyadenosylcobalamin was substantially less than that of cyanocobalamin and liver retention was higher. It is possible that the relatively high urinary excretion of cyanocobalamin reflects a slower uptake by tissues of this form of vitamin B_{12}.

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