Some metabolic aspects of vitamin B_{12} deficiency in sheep

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1. Studies were made with pair-fed vitamin B_{12}-deficient and vitamin B_{12}-treated or cobalt-treated ewes fed a Co-deficient diet in cages. Measurements were made of body-weight changes and some observations were made of energy and nitrogen metabolism. The effects of oral Co on energy and nitrogen metabolism were examined in sheep fed the Co-deficient diet, but not yet deficient of vitamin B_{12} in the tissues.

2. The rate of loss of body-weight of vitamin B_{12}-deficient sheep was significantly faster ($P < 0.01$) than that of pair-fed sheep given 50 μg vitamin B_{12}/d by injection.

3. In a limited number of observations of pair-fed sheep no significant differences were found in retention of combustible energy from the diet, but excretion of faecal nitrogen was higher in deficient animals than in animals receiving vitamin B_{12} or Co.

4. There was no significant effect of supplementary Co on energy metabolism, nitrogen metabolism, production of methane or digestibility of fodder in sheep that were not deficient of vitamin B_{12}.

Cobalt deficiency in sheep is a wasting disease in which the animal dies in a state of inanition, and, apart from anaemia and fatty liver, without any obvious pathological changes (Marston, 1952; Underwood, 1962). There is a sharp fall in food intake accompanying the fall in body-weight (Marston & Smith, 1952; Marston, 1970) and the symptoms found are largely those of starvation. Treatment with oral Co or injected vitamin B_{12} is effective either in preventing the appearance of symptoms or in restoring deficient animals to health.

The present experiments with pair-fed animals were undertaken to determine whether the loss in body-weight was due solely to loss of appetite, or whether, in addition, the deficient animal failed to metabolize its fodder efficiently. It was found that the deficient animal lost weight substantially faster than the pair-fed control, and further definition of the metabolic inefficiency implied by this finding was sought in a few studies of energy and nitrogen excretion and of energy expenditure in pair-fed animals.

Although not highly significant, the results indicated that both faecal nitrogen excretion and fasting energy expenditure were greater in deficient animals than in pair-fed controls. Within small limits of error there was no effect of supplementary Co *per se* on the digestibility of the fodder or the metabolism of the animal and the conclusion is reached that the observed symptoms of Co deficiency were entirely due to a lack of vitamin B_{12} in the tissues.

EXPERIMENTAL

Animals and diet. These were as described by Smith & Marston (1970), the full ration consisting of 1000 g wheaten hay-chaff and 50 g of gluten/d. The hay-chaff fed contained 0.02–0.04 μg Co/g (dry) and the gluten less than 0.02 μg Co/g (dry).

After a training period in cages, and in some instances an initial depletion of vitamin B_{12} reserves on the experimental diet in open-air pens, animals were maintained in collection cages (Marston, 1935) for periods of up to 4 years. If both oral Co (1 mg Co/d given as a drench at the time of feeding) and vitamin B_{12} (50 μg cyanocobalamin/d injected intramuscularly) were withheld, normal health appeared to be maintained for 20–35 weeks and the full daily ration (1050 g moist weight, or about 940 g dry weight) was consumed. Thereafter a progressively increasing proportion of the ration was left uneaten, the animals lost weight and, if not treated, died in an emaciated condition in a further 20–35 weeks.

Pair-fed animals. The time required to produce deficiency symptoms (loss of appetite) varied widely and so pair-fed animals were produced and maintained individually from a pool of fifteen to twenty animals fed the Co-deficient diet. Animals to be pair-fed were selected on the basis of similar age, similar food intakes (generally in the range 500–800 g/d but in some instances the full food intake of 1050 g/d), and similar body-weights, both animals having generally consumed the deficient diet for a similar time. One animal was then treated continuously with Co or vitamin B_{12} and its food intake restricted to that consumed by its deficient counterpart on the previous day. Food residues were collected and weighed daily and the appropriate mixture of hay-chaff and gluten was calculated on a moist-weight basis. In periods when nitrogen and combustible energy excretion were measured, food residues were dried at 110° for 24 h before weighing and food intakes calculated on a dry-weight basis. In these periods treated animals were pair-fed 2 d after their deficient counterparts. No food residues were left by treated animals during these periods and only very rarely in the entire series of experiments.

Body-weight changes in pair-fed animals. During the 5-year period reported in Table 1, thirty-five pairs of animals were produced in which the treated animals received vitamin B_{12}. Observations to be reported were restricted to periods of falling food intake in the range 800 g/d–200 g/d. Observations were rejected if body-weight differences at the beginning of the period of observation exceeded 3 kg. All observations on eight of the pairs were rejected for this reason. After pair-feeding was established, a stabilization period of 4 weeks was allowed before observations began and, if the animals were shorn, a further 4 weeks stabilization was allowed before observations were resumed. Rates of body-weight loss were calculated from weekly body-weight values as linear regressions, and in the statistical analysis these were weighted inversely as their variances. The analysis was performed on regressions from twenty-seven pairs, and a distinction was made between first observations off pasture and repeat observations on the same pair of animals after temporary recovery of the deficient member on treatment with vitamin B_{12}.

Energy and nitrogen metabolism. During the 14 d periods in which combustible energy and nitrogen retention were measured, samples of fodder components, food residues and excreta were collected and analysed as described by Marston (1948). Measurements of respiratory exchange were made in the open-circuit calorimeters described by Marston (1948). Heat production is reported either as kcal/24 h or as kcal/$W^{0.73}$ per 24 h.

Chemical estimations. Co in fodder was estimated by the method of Marston & Dewey (1940). Blood haemoglobin was measured by the alkaline haematin method standardized by the van Slyke determination of oxygen-carrying capacity and by iron content (Marston & Lee, 1948). Nitrogen was estimated by a Kjeldahl procedure, and combustible energy with an Emerson bomb calorimeter.

RESULTS

Body-weight changes in pair-fed animals. Two examples of pair-fed vitamin B_{12}-deficient and vitamin B_{12}-treated animals maintained for long periods on the deficient diet are shown in Fig. 1. The deficient member of the pair was periodically treated for 2–4 weeks with 50 μg vitamin B_{12}/d before again being allowed to become deficient. Blood haemoglobin was measured from the time pair-feeding began and shows the typically more pronounced degree of anaemia found in deficient animals than in pair-fed animals receiving vitamin B_{12}. Although the response in food intake and body-weight of the deficient animal to vitamin B_{12} was immediate, the haemoglobin response was frequently delayed.

In Fig. 1 the rate of body-weight loss is perceptibly faster in deficient than in treated animals. Values for rates of body-weight loss were obtained in forty-nine observations on twenty-seven pairs of animals over a 5-year period. The mean period of observation was 9·6 weeks. Table 1 summarizes the results for fifteen of these pairs, and shows the individual rates for first observations off pasture. In the final column are shown the ratios between the weighted mean rates for deficient and treated animals respectively for repeat observations like those shown in Fig. 1.

Statistical analysis of all the regressions showed that variation between pairs (in first observations) did not differ significantly from variation within pairs (in repeat observations) and that the regressions for deficient and treated animals each comprised a homogeneous population. The values for all forty-nine observations were therefore pooled to give 74 degrees of freedom. The mean rate of body-weight loss for all observations on deficient animals (0·5464 kg/week $\pm$ 0·0224 (SEM)) was significantly greater ($P < 0·01$) than that for all observations on treated animals (0·4365 kg/week $\pm$ 0·0251 (SEM)). The corresponding degree of significance for the mean rates shown in Table 1 for first observations was $P < 0·05$, and for the repeat observations indicated in the final column was also $P < 0·05$.

The rate of loss of body-weight varied greatly between deficient animals and reflected the rate of loss of appetite, but there was no correlation between these rates and the corresponding ratios. From the mean rates for all observations given above, the deficient animals lost weight 25% faster than the treated animals.

Nitrogen and energy retention in pair-fed vitamin B_{12}-deficient and Co-treated or vitamin B_{12}-treated ewes. Three pairs of animals were used when food intakes had fallen to less than 400 g/d. In two pairs the treated animal received vitamin B_{12} and, in the third pair, Co given by mouth. Two sets of measurements were made with the latter pair.

Results are shown in Tables 2 and 3. Partly owing to a degree of selective rejection of flake-gluten by the deficient animals, food intakes were not perfectly matched and nitrogen intakes of treated animals were about 2 % higher than those for their deficient counterparts while combustible energy intakes were about 4 % lower.

For statistical analysis, values for excretion were expressed as fractions of actual intakes, and on this basis there were no significant differences between deficient and treated animals in any of the quantities measured except faecal nitrogen. Expressed as a fraction of ingested nitrogen, this was significantly higher ($P < 0{\cdot}05$) in deficient than in treated animals and the effect was consistent for both vitamin B_{12}-treated and Co-treated controls.

Energy expenditure of vitamin B_{12}-deficient and pair-fed vitamin B_{12}-treated animals. Measurements of energy expenditure by respiratory exchange in sheep are not highly accurate unless the animals are thoroughly stabilized on a particular plane of nutrition, but with pair-fed deficient and treated sheep the plane of nutrition was constantly falling. In addition, while the deficient animal was listless and ate its food slowly, the

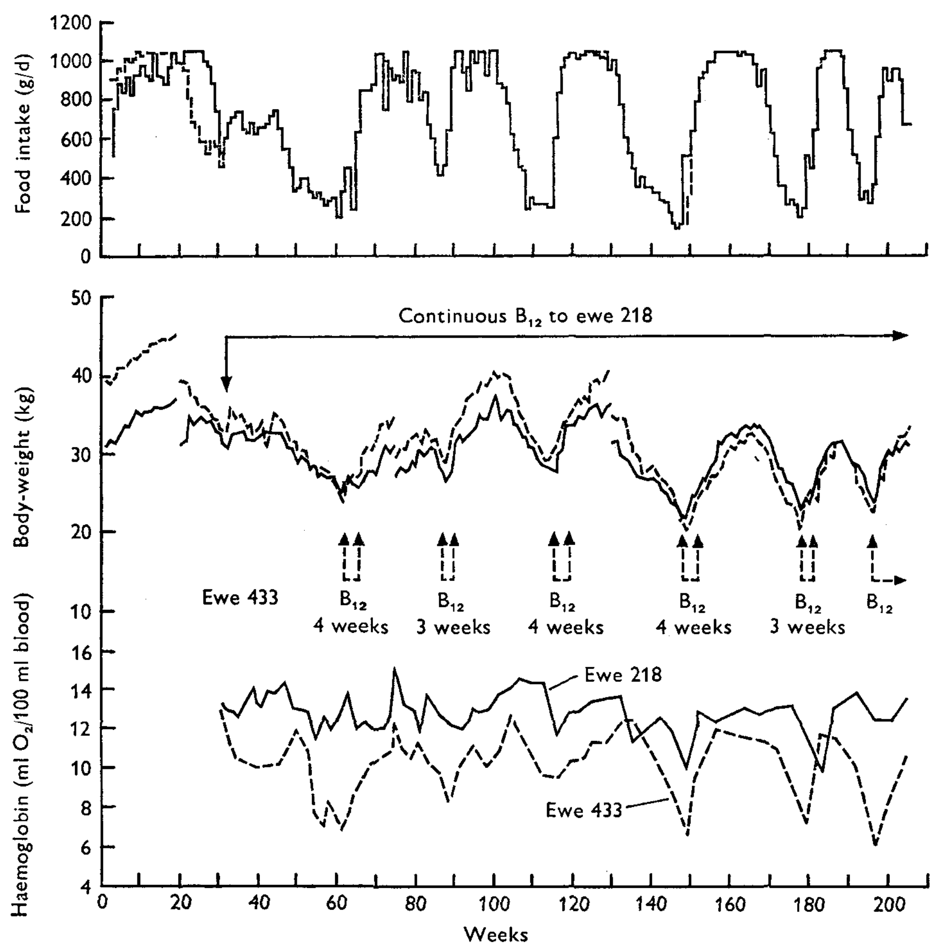

Fig. 1 A. For legend see opposite page.

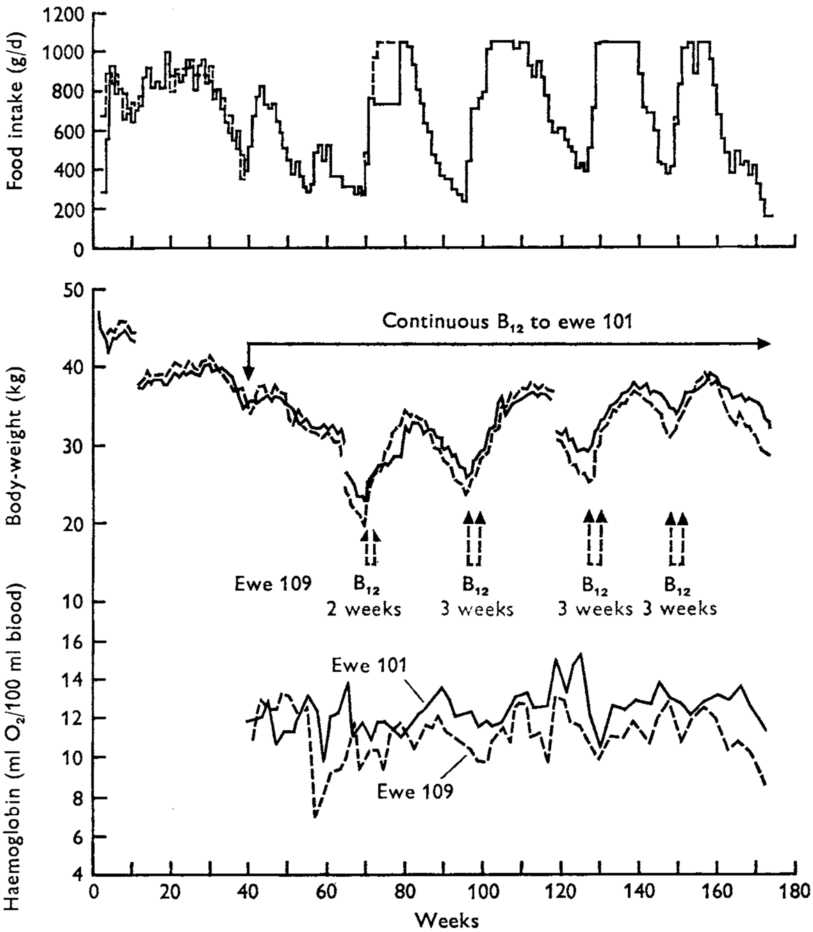

Fig. 1 B

Fig. 1. Food intake, body-weight and haemoglobin of pair-fed vitamin B_{12}-deficient and vitamin B_{12}-treated ewes. The deficient animals are represented by broken lines, the pair-fed animals injected with 50 μg vitamin B_{12}/d by solid lines. Breaks in the body-weight curves were caused by shearing. During the periods indicated by arrows, deficient animals were treated for 1–4 weeks with 50 μg vitamin B_{12}/d; treatment of the other animal was continuous from the time of pairing as shown by the upper arrow. (A), ewes 433 (deficient) and 218 (treated), 18 months of age at week 0; (B), ewes 109 (deficient) and 101 (treated), 30 months of age at week 0.

pair-fed treated animal consumed its daily ration within a few hours. These factors combine to make measurements of energy expenditure of doubtful value but, nevertheless, a few such measurements were made and are reported below. Urinary nitrogen was not measured and energy expenditure was calculated from total oxygen consumption using simple respiratory quotients.

Table 4 shows the energy expenditure of the pair-fed animals no. 433 and no. 218 (Fig. 1 A). The measurements were made after 60 weeks on the Co-deficient diet and 27 weeks after pair-feeding began. The animals were 32 months of age; no. 433 weighed 27·2 kg and no. 218 weighed 26·0 kg. Although energy expenditure was

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Table 1. *Body-weight losses of vitamin B_{12}-deficient ewes and of pair-fed ewes injected with 50 μg vitamin B_{12}/d*

(Rates of body-weight loss were calculated as linear regressions from weekly body-weight values for the periods shown. Regressions were weighted inversely as their variances and the weighted means were shown with their standard errors. Further observations on the same animals were shown as ratios of the weighted mean rates for the numbers of observations in parentheses)

			Food intake			Body-weight					
						Vitamin B_{12}-deficient ewes		Treated ewes			
Animal no. deficient; treated	Age* (years)	Time paired before observation period (weeks)	Start of observation period (g/d)	End of observation period (g/d)	Duration of observation period (weeks)	Initial (kg)	Rate of loss (A) (kg/week)	Initial (kg)	Rate of loss (B) (kg/week)	Ratio, A:B for first observations	Ratio, A:B of mean rates of further observations on same animals
074; 240	2	4	420	210	6	32·2	0·689	34·6	0·739	0·93	1·14 (1)
076; 278	3	4	440	310	13	34·6	0·405	33·1	0·281	1·44	—
	3·5	25	650	360	10	26·0	0·424	27·8	0·238	0·78†	
259; 080	3	4	730	330	13	37·0	0·472	39·4	0·484	0·97	—
	3·5	22	490	230	16	28·3	0·396	29·1	0·242	1·63†	
114; 282	3·5	4	630	240	11	35·4	0·846	38·3	0·633	1·34	—
013; 284	3·5	9	550	260	7	34·9	0·677	33·8	0·542	1·25	—
109; 101	3·5	10	500	290	6	36·4	0·725	35·6	0·486	1·49	2·05 (3)
263; 325	2·5	20	540	340	17	32·2	0·133	30·5	0·085	1·57	1·06 (2)
362; 385	2·5	22	750	460	9	37·1	0·487	38·2	0·347	1·41	—
438; 363	2·5	20	690	280	13	30·4	0·264	30·9	0·193	1·37	—
205; 341	2·5	15	510	360	5	29·2	0·597	30·0	0·600	1·00	1·23 (3)
433; 218	2·5	16	550	300	13	32·9	0·539	31·1	0·408	1·32	1·17 (5)
144; 410	3	5	420	350	10	28·0	0·205	28·1	0·111	1·84	1·07 (2)
158; 074	3·5	4	470	400	3	35·0	1·270	34·1	0·950	1·34	—
	3·5	12	550	320	13	30·0	0·547	30·9	0·413	1·33†	
438; 436	4	4	480	220	13	36·7	0·639	38·3	0·506	1·26	—
122; 084	1·5	22	400	220	12	19·5	0·335	18·6	0·220	1·52	—
Means	—	12·4	543	304	10·6	32·0	0·458 ± 0·034	32·4	0·343 ± 0·039	—	—

* To the nearest 0·5 year.

† Resumed first observation 4 weeks after shearing.

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Table 2. *Combustible energy and nitrogen retention by pair-fed vitamin B_{12}-deficient and vitamin B_{12}-treated ewes*

(Ewes 5009 and 7019 were 6 years old, weighing 36 kg and 39 kg respectively after 40 weeks on the Co-deficient diet; they had been pair-fed and 7019 was treated with 50 μg vitamin B_{12}/d for 15 weeks. Ewes 9090 and 7009 were 5 years old, weighing 30 kg and 28 kg respectively after 48 weeks on the deficient diet; they had been pair-fed and 7009 was treated with 50 μg vitamin B_{12}/d for 9 weeks)

	Ewe 5009, deficient			Ewe 7019, treated			Ewe 9090, deficient			Ewe 7009, treated		
	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)
Food offered	8293	11·25	2563	3851	5·23	1189	10456	13·69	3246	4969	6·45	1542
Food residues	4301	6·13	1334	None	None	None	5352	7·41	1653	None	None	None
Net intake	3992	5·12	1229	3851	5·23	1189	5104	6·28	1593	4969	6·45	1542
Faeces	1652	1·62	524	1539	1·54	487	2353	2·49	742	2110	1·94	663
Urine (ml)	7534	3·60	60	17800	4·03	52	5810	5·40	84	10800	4·56	43
Output in visible excreta	—	5·22	584	—	5·57	539	—	7·89	826	—	6·50	706
Balance	—	−0·10	+645	—	−0·34	+650	—	−1·61	+767	—	−0·05	+836

similar in the two animals when fed it appeared to fall more rapidly in the treated animal on fasting.

Two further measurements, each of 24 h duration, were made on a second pair of animals on the 1st day of fast and are given in Table 5. The animals were 3 years of age and had been fed on the Co-deficient diet for 53 weeks when the first measurements were made. Methane was not measured. Energy expenditure on the 1st day of fast was again higher in the deficient animals.

Table 3. *Combustible energy and nitrogen retention by pair-fed vitamin B_{12}-deficient and Co-treated ewes*

(Ewes 9134 and 7049 were given the Co-deficient diet at 3 years of age and pair-fed, and 7049 was treated with 1 mg Co/d after 36 weeks. Two 14 d collection periods were held at 41 weeks and 63 weeks. At 41 weeks the body-weight of 9134 was 31 kg and of 7049 33 kg, and at 63 weeks body-weights were 23 kg and 27 kg respectively. Both animals died at 66 weeks)

	Period 1					
	Ewe 9134, deficient			Ewe 7049, treated		
	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)
Food offered	9846	13·50	3057	3621	5·44	1130
Food residues	6012	8·10	1857	None	None	None
Net intake	3834	5·40	1200	3621	5·44	1130
Faeces	1527	2·17	483	1474	1·42	460
Urine (ml)	7225	4·62	64	4546	3·90	60
Output in visible excreta	—	6·79	547	—	5·32	520
Balance	—	−1·39	+653	—	+0·13	+611
	Period 2					
	Ewe 9134, deficient			Ewe 7049, treated		
	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)	g (dry) in 14 d	N (g/d)	Combustible energy (kcal/d)
Food offered	7052	9·51	2168	2358	4·63	744
Food residues	4528	4·94	1386	None	None	None
Net intake	2524	4·57	782	2358	4·63	744
Faeces	979	1·39	308	952	1·04	293
Urine (ml)	9650	4·41	48	9030	3·98	34
Output in visible excreta	—	5·80	356	—	5·02	327
Balance	—	−1·23	+426	—	−0·39	+417

The three sets of results constitute only two independent observations and the difference between deficient and treated animals although consistent, is not statistically significant ($0·05 < P < 0·1$). The findings are, therefore, no more than indicative of a higher fasting energy expenditure in deficient animals on the 1st day of fast.

Digestibility of fodder and energy utilization with and without supplementary Co in animals not deficient of vitamin B_{12}. Two animals were used, one receiving 1 mg Co/d by mouth and both consuming a maintenance ration of the Co-deficient diet. After one set of measurements was completed Co treatment was reversed, the animals were again stabilized and the measurements repeated. The maximum periods on the deficient diet without Co (8 weeks for no. 077 and 6 weeks for no. 113) were not long enough seriously to deplete the tissues of vitamin B_{12} and appetite did not fail. The daily ration (800 g wheaten hay-chaff and 50 g gluten) maintained the animals at constant weight throughout the experiment, no. 113 at 42 ± 1 kg and no. 077 at 38 ± 1 kg.

Table 4. *Respiratory exchange and energy expenditure of a vitamin B_{12}-deficient ewe and a pair-fed ewe treated with 50 µg vitamin B_{12}/d*

	Ewe 433, deficient				Ewe 218, treated			
Treatment	Oxygen deficit (l/d)	Carbon dioxide increase (l/d)	Methane evolved (l/d)	Energy dissipated as heat (kcal/$W^{0.73}$d)	Oxygen deficit (l/d)	Carbon dioxide increase (l/d)	Methane evolved (l/d)	Energy dissipated as heat (kcal/$W^{0.73}$d)
Fed (350 g fodder)	200	179	9·4	86·9	187	169	10·6	84·1
Fasted: day 1	170	127	4·2	72·7	138	109	3·1	61·2
day 2	162	114	0·7	72·3	151	108	2·3	66·3
day 3	157	98	1·1	69·1	142	94	1·2	62·3

Table 5. *Energy expenditure on the 1st day of fast of a vitamin B_{12}-deficient ewe and a pair-fed ewe treated with 50 µg vitamin B_{12}/d*

	Ewe 072, deficient		Ewe 141, treated	
Time after pairing (weeks)	Body-weight (kg)	Energy dissipation (kcal/$W^{0.73}$d)	Body-weight (kg)	Energy dissipation (kcal/$W^{0.73}$d)
12	20·3	66·9	21·0	59·4
14	20·0	67·6	20·4	58·6

After 4 weeks stabilization of both animals, with no. 113 receiving Co, the energy and nitrogen available from the ration were determined from collection of excreta over 14 d. During the subsequent 14 d, eight 24 h measurements of respiratory exchange were made on each animal. Treatment was then reversed and after 14 d stabilization the above procedure was repeated except that ten instead of eight measurements of respiratory exchange were made. During the 14 d periods in which excreta were collected, daily determinations of faecal dry weight were made and these, together with the replicate measurements of respiratory exchange, were used in statistical analysis of the results. That 14 d without Co is more than sufficient to deplete the rumen of Co and vitamin B_{12} was shown by Smith & Marston (1970), and the rapidity of the response in food intake and body-weight of deficient animals to Co given orally (Marston & Smith, 1952; Marston, 1970) indicates that the reverse is also true.

The results for energy and nitrogen retention from the diet are shown in Table 6, and for energy expenditure in Table 7. The statistical analysis showed that there was no significant effect of orally administered Co on any of the quantities measured.

Table 6. *Effect of 1 mg cobalt/d given by mouth on nitrogen and energy retention by sheep eating the Co-deficient ration but not yet deficient of vitamin B_{12}*

	Ewe 077					
	Period A, untreated			Period B, treated		
	g (dry)/d	N (g/d)	Combustible energy (kcal/d)	g (dry)/d	N (g/d)	Combustible energy (kcal/d)
Ration: chaff	690	8·53	2920	677	8·62	2930
gluten	45·5	6·08	255	45·5	6·08	255
Food residues	0	0	0	0	0	0
Net intake:	735·5	14·61	3175	722·5	14·70	3185
faeces	319 ± 7·0	4·34 ± 0·10	1404 ± 31·0	323 ± 7·0	4·62 ± 0·10	1415 ± 30·8
urine	—	8·18	130	—	8·10	126
Output in:						
visible excreta	—	12·52 ± 0·10	1534 ± 31·0	—	12·72 ± 0·10	1541 ± 30·8
methane	—	—	176 ± 3·4	—	—	199 ± 2·2
Total output	—	12·52 ± 0·10	1710 ± 31·1	—	12·72 ± 0·10	1740 ± 30·9
Retained	—	2·09 ± 0·10	1465 ± 31·1	—	1·98 ± 0·10	1445 ± 30·9

	Ewe 113					
	Period A, treated			Period B, untreated		
Ration: chaff	690	8·53	2920	677	8·62	2930
gluten	45·5	6·08	255	45·5	6·08	255
Food residues	0	0	0	0	0	0
Net intake:	735·5	14·61	3175	722·5	14·70	3185
faeces	311 ± 7·0	4·04 ± 0·09	1359 ± 30·8	299 ± 7·0	4·07 ± 0·10	1319 ± 31·1
urine	—	8·55	128	—	8·68	132
Output in:						
visible excreta	—	12·59 ± 0·09	1487 ± 30·8	—	12·75 ± 0·10	1451 ± 31·1
methane	—	—	206 ± 15·1	—	—	226 ± 5·8
Total output	—	12·59 ± 0·09	1693 ± 34·3	—	12·75 ± 0·10	1677 ± 31·6
Retained	—	2·02 ± 0·09	1482 ± 34·3	—	1·95 ± 0·10	1508 ± 31·6

DISCUSSION

It was clear from previous work (Marston, 1970) that either Co given orally or injected vitamin B_{12} was capable of preventing the loss of appetite and body-weight suffered by sheep given a Co-deficient diet. The capacity of deficient animals to respond immediately to short periods of treatment with vitamin B_{12} is shown in Fig. 1 to be sustained for periods approaching 4 years, and since only small amounts of injected vitamin B_{12} enter the rumen (Smith & Marston, 1970) it may be concluded that the deficiency symptoms are predominantly due to lack of vitamin B_{12} in the tissues. That they may be ascribed entirely to this cause is suggested by the values in Tables 6 and 7 where the presence of additional Co (and hence of cobamides) in the rumen had, within small limits of error, no effect either on the digestibility of the fodder or on the energy or nitrogen metabolism of the animal.

These findings do not support the suggestion (Ford, Kon & Porter, 1952; Porter, 1953) that relatively high concentrations of cobamides may be necessary for normal

Table 7. *Effect of cobalt given by mouth on respiratory exchange of sheep eating the Co-deficient ration but not yet deficient of vitamin B_{12}*

(The values refer to the same experiment as those in Table 6. Eight measurements of respiratory exchange were made in period A, ten in period B. The results are mean values with their standard errors. The energy balance is derived from the retained (available) energy value in Table 6 minus the energy dissipated as heat. Both animals maintained constant weight throughout the experiment, 077 at 38 ± 1 kg and 113 at 42 ± 1 kg)

	Respiratory exchange			Protein metabolism		Energy metabolism		
Animal no. and treatment	Oxygen deficit (l/d)	Carbon dioxide increase (l/d)	Methane evolved (l/d)	N in urine (g/d)	Non-protein respiratory quotient	Dissipated as heat (kcal/d)	Available from diet (kcal/d)	Balance (kcal/d)
077; period A, no cobalt	307 ± 2·2	287 ± 2·8	18·7 ± 0·4	8·18	0·90 ± 0·01	1497 ± 12	1465 ± 31	−32 ± 33
period B, 1 mg Co/d	303 ± 1·0	292 ± 2·7	21·1 ± 0·2	8·10	0·92 ± 0·01	1487 ± 5	1445 ± 31	−42 ± 31
113; period A, 1 mg Co/d	291 ± 2·4	288 ± 4·1	21·8 ± 1·6	8·55	0·95 ± 0·01	1433 ± 12	1482 ± 34	+49 ± 36
period B, no cobalt	290 ± 2·2	289 ± 3·2	24·0 ± 0·6	8·68	0·95 ± 0·01	1429 ± 11	1508 ± 32	+79 ± 34

ruminal fermentation. It is not improbable that some microbial functions in the rumen do necessitate vitamin B_{12} or other cobamides, but the minimum requirements for effective fermentation can evidently be met at a level of dietary Co too low to support the requirements of the animal's tissues for vitamin B_{12}. Synthesis of methane in the rumen, for example, probably depends, at least in part, on the participation of cobamides (Stadtman & Blaylock, 1966; Blaylock, 1968), but in the present work the absence of supplementary Co had no effect on methane production (Table 7), and in the vitamin B_{12}-deficient animal methane production was reduced only in proportion to food intake (Table 4 compared with Table 7). The reduction in bacterial counts in the Co-depleted rumen (Gall, Smith, Becker, Stark & Loosli, 1949), if it occurred in the present experiments, did not appear to affect the availability of energy or nitrogen from the diet.

The relative rates of body-weight loss of pair-fed deficient and vitamin B_{12}-treated animals (Table 1) clearly establish the more rapid loss of weight by the former. The wide variation in individual ratios between pairs was also found in repeat measurements of single pairs and probably reflects the imprecision of body-weights in ruminants. That the difference between deficient and treated animals represents a loss of tissue substance and not of water is indicated by the similarity of muscle composition in deficient animals and Co-treated animals on restricted food intake (Holmes, 1965). From Fig. 1 it is clear that these losses are made good when the deficient animal is treated with vitamin B_{12}. The extent of the metabolic inefficiency in deficient animals implied by these findings appears to be characteristic of the deficiency state and to be maintained through successive cycles of deficiency.

With a variable and falling daily food intake, conditions for measurement of energy and nitrogen metabolism in pair-fed animals were not ideal, and only tentative conclusions can be reached from the limited number of observations made. The finding of a higher faecal nitrogen excretion together with a higher fasting energy expenditure in deficient than in pair-fed treated animals, however, is consistent with the higher rate of body-weight loss in the former.

The major cause of body-weight loss in the vitamin B_{12}-deficient sheep was loss of appetite, but a substantial metabolic inefficiency existed as well. The extent of the metabolic inefficiency showed no correlation with the rate of loss of appetite (as reflected in body-weight losses) and there is therefore no evidence to suggest that these two manifestations of vitamin B_{12} deficiency were causally related.

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