Dawbarn *et al.*[2], who were responsible also for the microbiological assays reported here.

In all, these findings are in complete accord with the hypothesis that the syndrome of cobalt deficiency in the ruminant is the result of a metabolic defect arising from a profound deficiency of vitamin B_{12}. Ruminants apparently require much more vitamin B_{12} than other animals, but the reason for this is not yet clear. There is no evidence that cobalt performs any physiological function in the tissues of animals other than as a constituent of vitamin B_{12}. The details of these experiments will be published elsewhere.

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[1] Marston, H. R., and Lee, H. J., cf. Marston, H. R., *Physiol. Rev.*, 32, 66 (1952).
[2] Dawbarn, M. C., Hine, D. C., and Hughes, P. (see following communication).

Influence of Cobalt on the Production of Factors possessing Vitamin B_{12}-like Activity in the Fæces of Sheep

WE have estimated the vitamin B_{12} and similar factors in the urine and fæces of a number of sheep which were confined in metabolism cages and fed on cobalt-deficient rations, and which were employed by Marston and Smith[1] for the experiments referred to in the preceding communication.

During the collection periods referred to here, an aliquot of the urine passed each day was treated with cyanide, adjusted to *p*H 6·8 and stored at − 20° C. until composite samples were made up immediately before the assays were conducted. Aliquots of the fæces were taken each day and stored at − 20° C. in sealed containers until required.

The active material in the urine was measured by its capacity to promote the growth and lactic acid production of *Lactobacillus leichmannii* 313 under strictly controlled conditions. Extracts of the fæces were analysed by this method and also by the plate method of Harrison *et al.*[2], in which the test organism is a mutant of *Escherichia coli*. The results are shown in Tables 1 and 2. All animals had been on the deficient rations for 35 weeks before the treatments were begun. Period *A* was the 14 days immediately prior to treatment; period *B* was the 14 days beginning a fortnight after treatment was started. The first three animals were in an advanced stage of the cobalt-deficiency syndrome and their food intake in period *A* was less than 250 gm. per day; in period *B* animals No. 5009 (cobalt) and No. 7019 (vitamin B_{12}) consumed more than double this amount, whereas the intake of No. 7009 (untreated) remained practically unchanged. The second three animals had not become sufficiently depleted for their appetites to fail and consumed the whole of their rations, 1,050 gm. a day, in period *A*. The intakes of No. 5012 (cobalt) and No. 9072 (vitamin B_{12}) were unchanged in period *B*, but No. 9090 (untreated) began to lose its appetite[1].

Table 1. Effects of Administration of Cobalt and of Vitamin B_{12} on the 24-hr. Excretion of Vitamin B_{12}-like Substances in the Urine and Fæces of Sheep on Cobalt-deficient Rations

Sheep No.	"Vitamin B_{12}" in urine (μgm.) (*L. leichmannii*)		"Vitamin B_{12}" in fæces (μgm.) (*E. coli*)		(*L. leichmannii*)		Treatment in period *B*
	Period *A*	Period *B*	Period *A*	Period *B*	Period *A*	Period *B*	
7009	0·18	0·26	—	135	—	95	Nil
5009	0·15	2·9	110	2,500	50	320	1 mgm. cobalt a day (*per os*)
7019	0·83	12·7	100	310	60	115	50 μgm. vitamin B_{12} a day (parenteral)
9090	0·78	0·85	—	400	—	270	Nil
5012	0·23	3·1	490	3,700	245	720	1 mgm. cobalt a day (*per os*)
9072	1·03	6·1	590	520	250	280	50 μgm. vitamin B_{12} a day (parenteral)

Ford and Porter[3] have reported that several "B_{12}-like factors" occur in the fæces of calves, and that these differ in their capacity to promote the growth of the two micro-organisms *E. coli* and *L. leichmannii*. We have found that the estimate of vitamin B_{12} activity in sheep fæces obtained with *E. coli* as the test organism was always higher than that with *L. leichmannii*. The relation between the two measurements has been expressed as a ratio (Table 2).

TABLE 2. VITAMIN B_{12} EQUIVALENT PER GM. FÆCES AS VOIDED

During period *A*, the first three animals were in an advanced stage of the cobalt-deficiency syndrome and were refusing the greater part of their rations (ref. 1). The appetites of the other three had not begun to fail during period *A*, but during period *B* the untreated No. 9090 refused to consume the whole of the ration

Sheep No.	Period *A*			Period *B*			Treatment in period *B*
	Assay by *E. coli* (μgm.) (*C*)	Assay by *L. leichmannii* μgm. (*L*)	Ratio *C*/*L*	Assay by *E. coli* (μgm.) (*C*)	Assay by *L. leichmannii* (μgm.) (*L*)	Ratio *C*/*L*	
7009	—	—	—	0·66	0·46	1·4/1	Nil
5009	0·56	0·25	2·2/1	3·7	0·47	8/1	Cobalt (*per os*)
7019	0·77	0·46	1·7/1	0·75	0·28	2·7/1	Vitamin B_{12} (parenteral)
9090	—	—	—	0·45	0·30	1·5/1	Nil
5012	0·52	0·26	2·0/1	3·8	0·74	5/1	Cobalt (*per os*)
9072	0·50	0·21	2·4/1	0·46	0·25	1·8/1	Vitamin B_{12} (parenteral)

In the fæces of the cobalt-deficient sheep, the ratio was between 1·4 and 2·4 to 1. After the animals had been treated with cobalt (1 mgm. per day *per os*), each method of assay showed an increased concentration of 'vitamin B_{12}', and the ratio rose to 5 or higher. Injection of vitamin B_{12} (50 μgm. a day) into the animals had no appreciable effect on the vitamin B_{12}-like activity of a given weight of fæces.

It would seem that there are at least two "B_{12}-like factors" in sheep fæces and that their absolute and relative concentrations depend upon the level of available cobalt in the gut.

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[1] Marston, H. R., and Smith, R. M. (see accompanying communication).
[2] Harrison, E., Lees, K. A., and Wood F., *Analyst*, **76**, 696 (1951).
[3] Ford, J. E., and Porter, J. W. G., *Biochem. J.*, **51**, v (1952).