### The effect of treating ewes during pregnancy with cobalticoxide pellets on the vitamin $B_{12}$ concentration and the chemical composition of colostrum and milk and on lamb growth

BY M. W. O'HALLORAN\* AND K. D. SKERMAN

Nicholas Institute for Medical and Veterinary Research, Sherbrooke, Victoria, Australia

(Received 21 July 1960—Revised 13 September 1960)

The efficiency of a method of cobalt supplementation for ruminants using cobaltic oxide pellets of high specific gravity (sp.gr. 4) has been demonstrated by Dewey, Lee & Marston (1958). The presence of such a pellet in the rumen-reticulum of cattle substantially increases the liver store of vitamin  $B_{12}$  (Skerman, Sutherland, O'Halloran, Bourke & Munday, 1959).

Co supplementation of the ration of pregnant ewes has been shown to increase the vitamin  $B_{12}$  concentration in the milk and colostrum (Moinuddin, Pope, Phillips & Bohstedt, 1953; Harper, Richard & Collins, 1951). This paper reports an experiment on the effect of giving cobaltic-oxide pellets to pregnant ewes on the subsequent vitamin  $B_{12}$  content and the chemical composition of the milk, and on the growth of lambs born to these ewes.

### METHODS

Sheep. Two similar groups, each of twenty-five Southdown ewes, were selected on the basis of age, body-weight and origin. These ewes were grazed on pastures where lambs show signs of Co deficiency annually, but adult sheep are apparently normal (Skerman & Sutherland, 1957).

*Treatment.* One group was selected by the toss of a coin and treated with cobalticoxide pellets (5 g, 90 % cobaltic oxide, sp.gr. 4.0), now commonly known as Co bullets. Each treated ewe received one Co bullet. The ewes were treated on the day that rams were introduced.

Known parentage was required for stud purposes so the two rams used were each placed with one group of ewes each in separate paddocks. Rams were withdrawn after 8 weeks and thereafter all ewes were grazed together. During lambing, the farm caretaker kept the ewes under observation from 6 a.m. to 9 p.m. each day and noted the known or estimated time that each ewe lambed. Visits were made each day to identify and weigh lambs and to collect colostrum or milk samples as required.

Colostrum and milk samples. A sample of colostrum was taken from each ewe as soon as practicable after parturition, and the known or estimated time after parturition was

<sup>\*</sup> Present address: Department of Animal Husbandry, Roseworthy Agricultural College, Roseworthy, South Australia.

recorded. Further samples of colostrum were collected twice daily (at 9 a.m. and 4 p.m.), thus giving eight samples in all from each ewe during the first 4 days *post partum*. Thereafter milk samples were taken at 7, 14, 21 and 28 days after lambing. 'Let-down' of milk was stimulated by intramuscular injection of 5 i.u. posterior pituitary extract. The teat was wiped clean with 70 % (v/v) ethanol to minimize contamination of the sample. Milk stored in the teat was discarded and the sample then drawn from freshly let-down milk.

The object of the experiment was to determine the effect of treatment with Co bullets on the chemical constituents of ewe's colostrum and milk at known times after lambing. Therefore, for valid comparison of treated and control ewes, only samples from ewes that had lambed within 10 days of each other, and for which the time of parturition was known, were considered. Moreover, samples from treated ewes were confined to those from ewes that had retained the Co bullet. On this basis eight ewes in each group were suitable.

Portions of milk or colostrum were weighed as soon as possible after collection and stored at  $-15^{\circ}$  for determination of vitamin  $B_{12}$ , protein, total lipids and lactose. Owing to the viscosity of the samples of colostrum and early milk, measurement by weight was preferred to measurement by volume. As some time was to elapse before completion of all the assays, samples were selected for analysis at random, but were examined in complete sets, i.e. all samples from each ewe were examined at the same time. There was no significant decrease in the vitamin  $B_{12}$  concentration of milk samples stored at  $-15^{\circ}$  in the presence of KCN (2 mg/g) over a period of 6 months.

The livers of all lambs that died during the experiment were stored at  $-15^{\circ}$  for determination of vitamin B<sub>12</sub>. The time of death after lambing was noted and also whether or not the lambs had sucked.

Vitamin  $B_{12}$  concentration of colostrum and milk. Colostrum and milk samples were prepared for assay by the cyanide extraction (a papain digestion) method of Gregory (1954). The vitamin  $B_{12}$  activity of such milk extracts is due mainly to cyanocobalamin, the biologically active form of the vitamin. The filtrates were diluted to volumes suitable for analysis, and the pH was adjusted to 6.8 with N-NaOH. The test organism was *Lactobacillus leichmannii* 313 grown in Bacto  $B_{12}$  assay medium U.S.P. (Difco Laboratories). Cultures were maintained and inocula prepared as previously described (Skerman *et al.* 1959). Growth response was measured turbidimetrically in a Unicam spectrophotometer at 650 m $\mu$  with 1 cm glass cuvettes.

Vitamin  $B_{12}$  concentration in livers of lambs. The procedure described previously (Skerman *et al.* 1959) was used in the analysis of liver specimens from lambs.

Protein concentration of colostrum and milk. A weighed portion of milk was diluted with glass-distilled water to a volume appropriate for analysis, and nitrogen was determined by a micro-Kjeldahl procedure (Umbreit, Burris & Stauffer, 1957). The factor 6.38 was used to express nitrogen in terms of protein. All determinations were done in duplicate and most were completed within a few days of collection of the sample.

Lactose concentration of colostrum and milk. Colostrum and milk samples were

### Vol. 15 Effect of cobalt bullets on milk of ewes 1

deproteinized by precipitation with tungstic acid (Folin & Wu, 1919). The lactose concentration of the protein-free filtrate was determined by the anthrone method (Umbreit *et al.* 1957). All assays were done in duplicate.

Total lipid concentration of colostrum and milk. Ethanol and diethyl ether used for extraction of lipids from milk were purified by the method of Johnson, O'Halloran & Hewgill (1958). A portion of colostrum or milk (2 g) was added to 25 ml of a 1:3 mixture of diethyl ether and ethanol in a 30 ml wide-mouth McCartney bottle. The sample was thoroughly mixed and heated in a water-bath at  $60^{\circ}$  for 2 h. The extract was filtered and washed with ether-alcohol mixture and the total lipids were determined gravimetrically after the solvent had been distilled off in an atmosphere of oxygen-free nitrogen. All assays were done in duplicate.

Weight of lambs. Each lamb was weighed as soon as practicable after birth. Against each birth weight a note was made of the time in hours after the known or estimated time of birth when the weight was taken, and whether the lambs had dried and whether they had sucked. Later all birth weights were corrected to a standard value taken as 3 h after birth with dry coat and no sucking. These corrections were based on the data of Alexander (1956). Thereafter each lamb was weighed at 7, 14, 21 and 28 days after its birth. After the last lamb born had reached 4 weeks of age, all lambs were weighed on the same day at weekly or fortnightly intervals until the last lamb born was 12 weeks old. The weight of each lamb at 12 weeks of age was then computed from individual growth curves.

Retention of Co bullets. The treated ewes were examined by X-ray fluoroscopy 1 month after lambing, that is 6 months after the 'bullets' had been given. The Co bullet was still present in fourteen of the twenty-three surviving treated ewes. (An improved type of Co bullet (weight 10 g and sp.gr. 5.0), to give better retention in sheep, has recently been announced by the (Australian) Commonwealth Scientific and Industrial Research Organization (1959).)

#### RESULTS

Vitamin  $B_{12}$  concentration of colostrum and milk. The levels of vitamin  $B_{12}$  in the colostrum and milk of ewes treated with Co bullets was significantly (P < 0.001) greater than the levels found in untreated ewes (Table 1). This difference was maintained throughout the first 28 days of lactation. The concentration of vitamin  $B_{12}$  declined very rapidly during the first 4 days of lactation and thereafter there was a more gradual decline in both treated and control groups.

Protein concentration of colostrum and milk. Throughout the first 28 days of lactation the milk from ewes given Co maintained a significantly (P < 0.02) higher level of protein, as measured by total nitrogen content, than that of control ewes (Table 1). The protein content of the colostrum of both treated and untreated ewes was much higher than that of milk. This appears to be a feature of the colostrum of all species (McMeekin, 1954). The protein content of the ewe's milk became approximately constant at the 4th day of lactation, as with vitamin B<sub>12</sub>.

Total lipid concentration of colostrum and milk. The total lipid concentration was

M. W. O'HALLORAN AND K. D. SKERMAN

higher in colostrum than in milk (Table 1). The concentrations were higher for treated ewes, but the difference was not statistically significant.

Lactose concentration of colostrum and milk. There was an apparent reduction in the lactose content of the milk of the ewes given Co (Table 1), but the difference was not statistically significant.

## Table 1. Effect of treatment with cobalt bullets on the vitamin $B_{12}$ , protein, total lipid and lactose concentration of colostrum and milk of ewes

(Mean values with their standard errors for eight control and eight treated ewes)

Time after	Vitamin	$B_{12} (\mu g/kg)$	Protein (mg/g)		
(days)	Control	Treated*	Control	Treated†	
ò	35·2±3·76	102·1 ± 2·66	92·5 ± 5·99	$108.6 \pm 5.16$	
0.2	15.2±2.33	79 <b>·o</b> ±5·97	$57.3 \pm 5.75$	71·5±4·53	
1.0	10.2 ± 1.53	63·0±5·85	35.6±2.97	49.4±3.99	
1.2	11·6 ± 1·17	$50.5 \pm 6.91$	32.5 ± 2.20	42.0 ± 4.02	
2.0	8·1 ± 1·09	$38.3 \pm 5.67$	31.4±2.34	$46.8 \pm 3.84$	
2.2	6·4±0·55	28.7 ± 3.01	28.6±2.24	$40.5 \pm 2.67$	
3.0	5·6±0·65	23·3 ± 1·95	29·3 ± 1·95	39·4 ± 2·80	
3.2	5·6±0·63	25·4±3·21	28.7±1.92	39·4 ± 2·18	
7	2·2±0·42	23·1 ± 1·91	27.5±2.77	34·6 ± 2·39	
14	1.5 ∓ 0.01	21·1 ± 1·97	24·3 ± 1·87	34·8±1·78	
21	1.4 ± 0.02	17.0 ± 2.18	24·4 ± 2·77	31·4±3·54	
28	1.5 ∓ 0.01	11.6±0.54	24·0±2·17	29·9±2·99	
	Total lip	oid (mg/g)	Lactose (mg/g)		
	Control	Treated	Control	Treated	
0	51·4±5·01	48·4 ± 4·66	28·1 ± 2·99	19 <b>·7 ± 2·0</b> 6	
0.2	67·4 ± 3·44	70·4±6·93	31·1 ± 3·17	$28.5 \pm 2.08$	

	4 5	45 4 1 3 54	54.4 ± 2.30	$40.0 \pm 1.20$	45'5 ± 1'70	
	3.0	44°4±4°70	57·2±3·34	45°0±1°36	41·0±3·31	
	3.2	45 <b>·</b> 4±5 <b>·</b> 07	45·2±1·62	45·5±1·21	44·1 ± 2·46	
	7	43·5±4·46	49'I ± 2'77	51·6±1·77	51·6±2·78	
	14	45·5±2·39	55 <b>·o</b> ±3 <b>·</b> 79	50·8±0·90	48·9±1·32	
	21	43·1 ± 4·53	50·8±2·96	53·4±1·52	$53.3 \pm 1.14$	
	28	45°2±2°18	55·7±4·11	51·4±1·76	52·3 ± 1·57	
гч	1.0.	· · · · · · · ·		· · · · · · · · · · · · · · · · · · ·	· · ·	

 $73.1 \pm 6.65$ 

74·2±5·58

57·1 ± 3·37

36·1 ± 2·56

42.4 ± 2.75

42·2 ± 2·16

 $33.5 \pm 2.58$ 

37.1 ± 2.83

41.0 ± 1.98

\* The differences in favour of treatment were highly significant (P < 0.001) throughout the first 28 days of lactation.

 $\uparrow$  The differences in favour of treatment were significant (P < 0.02) throughout the first 28 days of lactation.

Vitamin  $B_{12}$  concentration in livers of lambs. Vitamin  $B_{12}$  concentrations in livers of four lambs that were born dead and of five lambs that died before sucking are shown in Table 2. The numbers were small, but there was a trend towards higher vitamin  $B_{12}$  concentrations in the liver of lambs born to ewes that retained the Co bullet throughout pregnancy.

Birth weights and growth of lambs. The weights of all lambs at birth, at 28 days and at 12 weeks of age are shown in Table 3. The mean birth weight of lambs from treated ewes was approximately 1.5 lb greater than that of lambs from control ewes. At

1.0

1.2

2.0

59.2 ± 3.33

59.0±5.14

51.7±4.12

28 days lambs from treated ewes were 2.25 lb and at 12 weeks 5.2 lb heavier than lambs from control ewes.

There was a striking difference in the appearance of the two groups of lambs when the final weighings were made, i.e. when the youngest lamb was 12 weeks and the oldest 16 weeks of age. On this occasion the lambs were separated for the first time into two groups, those from treated ewes and those from control ewes. The lambs from the treated ewes were obviously better as a group, being evenly grown with a healthy vigorous appearance, but there was a tail that appeared less thrifty. On the other hand, lambs from the control group were uneven in development and at least half had an unthrifty appearance. The few unthrifty lambs from treated ewes were found to be from treated ewes that had rejected the Co bullet.

# Table 2. Vitamin $B_{12}$ concentration ( $\mu g/g$ ) in livers of lambs that died before sucking

	NTf	Liver vitamin B <sub>12</sub>			
Class of ewe	lambs	Individual	Mean		
Control	3	0.47, 0.72*, 0.83*	0.67		
Bullet rejected	2	0.70, 0.87*	0.78		
Bullet retained	4	0.94, 0.94*, 1.10, 1.31	1.07		
	<b>*</b> E	Born dead.			

Table 3.	Mean birth	weights and	l weights (lb)	to 12 weeks	s of age of	lambs from
e	wes treated u	with cobalt b	ullets and fr	om untreated	d control e	wes

		Ram lambs			Ewe lambs					
Age of lamb Dams (week	Age of lambs (weeks)	Singles	Twins	Twins reared singly	All	Singles	Twins	Twins reared singly	All	All lambs
Control ewes	0 4 12	7·3(6) 19·9(3) 41·8(2)	6·4(9) 17·4(5) 32·1(5)	6·3(2) 17·6(2) 29·0(2)	6·7(17) 18·1(10) 33·4(9)	7·3(3) 22·8(2) 36·5(2)	6·5(5) 14·6(5) 26·5(5)	8·0(1) 22·6(1) 39·0(1)	6·9(9) 17·7(8) 30·6(8)	6·8(26) 17·9(18) 32·1(17)
Ewes that rejected bullets	0 4 12	8·4(5) 20·5(4) 38·1(4)			8·4(5) 20·5(4) 38·1(4)	8·6(2) 23·0(2) 41·5(2)		7·3(1) 14·5(1) 29·0(1)	8·2(3) 20·3(3) 37·3(3)	8·3(8) 20·4(7) 37·8(7)
Ewes that retained bullets	: 0 4 12	9·1(3) 22·7(3) 43·2(3)	9.8(1) 21.8(1) 35.1(1)		9·3(4) 22·5(4) 41·2(4)	8·5(3) 19·7(3) 38·5(3)	7·4(3) 16·4(3) 31·2(3)	6·5(2) 17·8(2) 35·8(2)	7·6(8) 18·0(8) 34·6(7)	8·2(12) 19·5(12) 37·0(11)

Figures in parentheses are the number of lambs included in mean.

These clinical observations are substantiated by values in Table 3, which gives the mean weights of lambs classified on the basis of sex and single or twin births. The ram lambs from ewes that retained their Co bullet made better weight gains than those from ewes that had rejected the bullet, and these in turn made better gains than the lambs from untreated ewes. The ewe lambs showed a similar trend. Those from treated ewes made better weight gains than those from control ewes, but there was little difference between lambs from treated ewes that retained the bullet and lambs from those that rejected the bullet.

103

The difference in weight gains of lambs was not examined statistically, owing to the small numbers in groups and the uneven distribution of sexes and single and twin births. However, visual inspection left little doubt that, on this farm where deficiency is known to occur, treatment of ewes with Co bullets during pregnancy resulted in better growth of lambs up to 12 weeks of age.

#### DISCUSSION

The concentration of vitamin  $B_{12}$  in the colostrum of treated and untreated ewes was much higher than in their subsequent milk samples. The concentration fell very rapidly during the first 4 days, particularly during the first 12 h *post partum*, and thereafter the decline was more gradual. Moinuddin *et al.* (1953) also found high concentrations of vitamin  $B_{12}$  in colostrum of ewes, falling markedly at 10 days *post partum* and decreasing further at 8 weeks.

Treatment of ewes with Co bullets during pregnancy resulted in significantly higher levels of vitamin  $B_{12}$  in their colostrum and milk in comparison with untreated controls. The levels were three to five times as high during the first 4 days of lactation. The rate of decline was less in treated ewes, so that at 28 days treated ewes had in their milk ten times as much vitamin  $B_{12}$  as untreated ewes. Moinuddin *et al.* (1953) similarly found that, at parturition, the vitamin  $B_{12}$  concentration in the colostrum of ewes given Co in mineralized salt was three to four times higher than in that of control ewes, and that higher levels were maintained in milk at 10 days and 8 weeks *post partum.* Shrimpton & Duckworth (1953) also found that the concentration of vitamin  $B_{12}$  in ewe's milk declined with advancing lactation, but the concentration was not raised by provision of Co in mineral licks or by drenches given from one to three times at intervals of 14 days.

It is difficult to compare our results with those of other workers because samples were assayed at different stages of lactation. Further, it is difficult to establish the normal concentration of vitamin  $B_{12}$  in ewe's colostrum or milk. Moinuddin *et al.* (1953) give 0.7 mg Co as the calculated mean daily intake of Co by their ewes given supplement, but note that ewes with the greatest Co intake had the highest levels of vitamin  $B_{12}$  in their milk. They concluded that the vitamin  $B_{12}$  content of blood and milk of ewes was dependent on the Co intake, presumably over and above the requirements for health.

The ewes in our experiment were clinically normal. Deficiency has never been evident in adult sheep on this farm, whereas lambs are affected annually. Thus the ewes may at times be suffering from subclinical deficiency, especially at parturition and early lactation, so that the output of vitamin  $B_{12}$  in milk of untreated ewes may be subnormal.

For precise comparison of vitamin  $B_{12}$  levels in milk, it would be desirable to know the daily Co intake, the liver vitamin  $B_{12}$  reserves and the stage of lactation of ewes in different experiments. Table 4 shows the vitamin  $B_{12}$  concentrations of colostrum and milk of ewes reported by various authors. The mean of the values for milk of ewes not receiving supplementary Co is approximately 1.8  $\mu g/l_{.}$ , which is similar to the 1.2  $\mu g/kg$  Vol. 15 Effect of cobalt bullets on milk of ewes

for milk of our control ewes. Further, the colostrum taken immediately after parturition from our control ewes had higher levels of vitamin  $B_{12}$  than those reported by Moinuddin *et al.* for colostrum of treated ewes, but much of the difference may be explained if there was as much as 12 h delay after parturition in the collection of the latter samples (Table 1).

These comparisons do suggest that our untreated ewes were relatively normal in vitamin  $B_{12}$  status, and that the marked increases in treated ewes were due to provision of Co in excess of requirements for health.

	1 2 20	U U			
		Time of	Mean vitamin $B_{12}$ concentration of milk $(\mu g/l.)$		
Authors	Form of Co supplement	sampling post partum	Control ewes	Treated ewes	
Shrimpton & Duckworth (1953)	Single pre- lambing drench	At 1 month At 1 month	1·3 2·3	1·3 2·5	
Harper et al. (1751)	Mineral salt	At 3–4 months	1.38	7·89 to 9·58	
Moinuddin et al. (1953)	Mineral salt	At parturition At 2 months	6 to 7 1 to 2	20 to 25 About 4	
Hart & Andrews (1959)	Co bullets	At 3 months	2.2	10.3	
This study	Co bullets	At parturition At 1 month	35·2* 1·2*	102* 11.6*	

Table 4. Vitamin  $B_{12}$  concentrations of milk of ewes treated with cobalt and of untreated control ewes reported by different investigators

\* Expressed as  $\mu g/kg$  (see p. 100).

Our mean vitamin  $B_{12}$  value of 11.6  $\mu$ g/kg for milk taken at 28 days *post partum* for Co-treated ewes agrees closely with that of 10.3  $\mu$ g/l. reported by Hart & Andrews (1959) for ewes treated with Co bullets and sampled at approximately 3 months *post partum*, and also with those of 7.89-9.5  $\mu$ g/l. at 3-4 months recorded by Harper *et al.* (1951) in ewes given cobalt in a mineral-salt supplement.

Shrimpton & Duckworth (1953) concluded, from work in Scotland, that provision of supplementary Co to ewes had no effect on the vitamin  $B_{12}$  concentration of their milk. However, in the experiments for which figures for milk of treated and control ewes are shown, the treated ewes received only one drench containing 21 mg Co as cobalt sulphate 4 weeks before lambing, so the lack of effect may be ascribed to insufficient continuity of Co supplementation. Moreover, on one farm they found a highly significant difference in the vitamin  $B_{12}$  concentration of milk of two groups of ewes, each receiving Co in a mineral mixture but depastured on land of strikingly different soil types. Although the authors discussed the possible effect of different availability of heather on the different soils, they did not comment on the cobalt content of soils and pastures or on the intake of mineral mixture by the two groups of ewes, which, if recorded, might have shown sufficient difference in Co intake to explain the difference in the vitamin  $B_{12}$  concentrations found.

Other investigators are in agreement that provision of supplementary Co to ewes

has a marked effect on the vitamin  $B_{12}$  concentration of their milk, which was about four times that in the controls.

The vitamin  $B_{12}$  requirement of a 50 lb lamb has been estimated at 100–300  $\mu$ g/day. Hart & Andrews (1959) have pointed out that a lamb cannot obtain this amount by drinking 1 l. of milk, even if the vitamin  $B_{12}$  concentration of the milk has been elevated by Co treatment of the ewe. This contention, based on the vitamin  $B_{12}$  concentration of milk taken at about 3 months *post partum*, is supported by our results. It focuses attention on the importance of the reserve of vitamin  $B_{12}$  in the liver of the lamb at birth, and on the importance of early establishment of vitamin  $B_{12}$  synthesis in the rumen of the lamb.

Nothing is known of the availability of vitamin  $B_{12}$  to newborn lambs or of their requirements of this vitamin. It is known that, in colostrum and milk, vitamin  $B_{12}$ is bound to protein (Gregory, 1954), and that during the first days of life protein, and in particular  $\gamma$ -globulin, is readily absorbed by the newborn animal (Hansen & Phillips, 1947). It is more than likely that at least during this stage the lamb absorbs vitamin  $B_{12}$  almost quantitatively. The requirements of newborn lambs are probably lower than the 100–300  $\mu$ g/day estimated for a 50 lb lamb. Hence a substantial proportion of the lambs' requirements of vitamin  $B_{12}$  may have been met by colostrum and milk (102  $\mu$ g/kg decreasing to 23  $\mu$ g/kg at 7 days) of treated ewes during the 1st week of our experiment.

The reason for increased protein concentration in the milk of treated ewes is difficult to explain. The effect could be attributed to better appetite and higher intake of nutrients with a subsequent reflection in the quality of the milk produced.

There was no significant effect of the treatment on lactose concentration of milk of either group of ewes.

There appeared to be a difference in favour of the treated group in total lipid content, but owing to the high degree of variability within the groups the effect was not significant.

The few analyses of the concentration of vitamin  $B_{12}$  in the liver of newborn lambs indicated that it was probably related to the Co status of the ewe. The newborn lambs of ewes treated with and retaining Co bullets had the highest liver stores of vitamin  $B_{12}$ . Lambs from ewes that had rejected bullets had slightly better liver stores of the vitamin than lambs from untreated ewes.

The relative birth weights and growth rates of lambs from treated and control ewes were obscured by disparity in sexes and twin births between groups. Overall, the lambs from treated ewes were heavier at birth and their subsequent gain in weight was better than that of lambs from untreated ewes. In similar experiments in New Zealand lambs born to ewes treated with Co bullets during pregnancy were somewhat heavier at birth and made better early growth than lambs from untreated control ewes (E. D. Andrews, personal communication, 1960).

Lambs from treated ewes had higher reserves of vitamin  $B_{12}$  in their livers at birth (Table 2), and received more vitamin  $B_{12}$  in colostrum and milk than lambs from control ewes (Table 1). Notwithstanding, we found that at the end of our experiment, when the youngest lamb was 12 weeks of age, there was evidence that the lambs from treated

1961

106

Vol. 15

ewes were suffering from a mild degree of subclinical Co deficiency. In the New Zealand work there was stronger evidence of this nature (E. D. Andrews, personal communication).

Thus, on Co-deficient pastures, the early growth rate of lambs can be increased by Co treatment of the ewe during pregnancy, but for optimum growth the lambs should also be treated at an early age, preferably at 8 weeks.

The increased growth of lambs from treated ewes might be attributable to several factors. In addition to increased quantities of vitamin  $B_{12}$  in the colostrum and milk of treated ewes, we found that the protein content was also significantly increased. No estimate was made of volume of milk produced, but it is probable that treated ewes also produced more milk, as milk production of cattle grazing Co-deficient pastures was increased by Co-bullet treatment (Skerman *et al.* 1959). Part of the difference could be attributable also to a sire effect, as different rams were mated with the treated and control groups of ewes. Such an effect could have operated in favour of, or against, the growth of lambs from treated ewes.

### SUMMARY

1. On a farm where adult sheep thrive but lambs suffer from cobalt deficiency annually, the effect of treating ewes with Co bullets during pregnancy on the vitamin  $B_{12}$  concentration and the chemical composition of their colostrum and milk and on the growth of their lambs was examined.

2. The mean vitamin  $B_{12}$  levels for colostrum of eight treated and eight control ewes were 102·1 and 35·2  $\mu$ g/kg, respectively. For milk samples taken at 28 days *post partum* the levels were 11·6 and 1·2  $\mu$ g/kg for treated and control ewes respectively. The difference in favour of treatment was highly significant (P < 0.001) and was maintained for the period of observations to 28 days *post partum*.

3. The decline in vitamin  $B_{12}$  concentration of colostrum and milk of ewes with increasing time *post partum* reported by other workers was confirmed. More detailed studies showed a precipitous fall in the first 12 h, a continuing rapid fall during the first 4 days and thereafter a more gradual decline.

4. There was a significant (P < 0.02) and sustained increase of about 10% in the protein concentration of colostrum and milk of the treated ewes.

5. The protein concentration of colostrum and milk declined with increasing time *post partum* at a rate about parallel to the decline in vitamin  $B_{12}$  concentration, reaching a relatively stable level after 4 days.

6. Treatment had no significant effect on the concentrations of either total lipids or lactose in colostrum or milk.

7. Analysis of livers from lambs that were born dead or died before sucking indicated that lambs from treated ewes had higher liver reserves of vitamin  $B_{12}$  at birth.

8. Treatment of ewes resulted in better growth and overall condition of lambs.

The authors wish to express their thanks for the technical assistance of R. J. Brockie, B. K. Osborn, R. M. Deacon and Dorothy V. White. They are indebted to Mr H. J. Gibbs, Ferney Creek, Victoria, for providing the sheep and facilities for this experiment.

### REFERENCES

Alexander, G. (1956). Aust. vet. J. 32, 321.

- Commonwealth Scientific and Industrial Research Organization (1959). 11th Annual Report for Year 1958-59, p. 48. Canberra: Commonwealth Government Printer.
- Dewey, D. W., Lee, H. J. & Marston, H. R. (1958). Nature, Lond., 131, 1367.
- Folin, O. & Wu, H. (1919). J. biol. Chem. 38, 81.
- Gregory, M. E. (1954). Brit. J. Nutr. 8, 340. Hansen, R. G. & Phillips, P. H. (1947). J. biol. Chem. 171, 223.
- Harper, H. E., Richard, R. M. & Collins, R. A. (1951). Arch. Biochem. Biophys. 31, 328.
- Hart, L. I. & Andrews, E. D. (1959). Nature, Lond., 184, 1242.
- Johnson, A. R., O'Halloran, M. W. & Hewgill, F. R. (1958). J. Amer. Oil Chem. Soc. 35, 496.
- McMeekin, T. L. (1954). In The Proteins, vol. 2, part A. [H. Neurath and K. Bailey, editors.] New York: Academic Press Inc.
- Moinuddin, M., Pope, A. L., Phillips, P. H. & Bohstedt, G. (1953). J. Anim. Sci. 12, 497.
- Shrimpton, D. H. & Duckworth, J. (1953). J. Sci. Fd Agric. 4, 301.
- Skerman, K. D. & Sutherland, A. K. (1957). Aust. vet. J. 33, 197.
- Skerman, K. D., Sutherland, A. K., O'Halloran, M. W., Bourke, J. M. & Munday, B. L. (1959). Amer. J. vet. Res. 20, 977.
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). Manometric Techniques, revised ed. Minneapolis, Minn.: Burgess Publishing Co.

1961

Printed in Great Britain

108