

COBALT AND VITAMIN₁₂ IN RUMINANT NUTRITION: A REVIEW

S. E. SMITH AND J. K. LOOSLI

Dept. of Animal Husbandry, Cornell University, Ithaca, N. Y.

This review will not delve into details of either cobalt or vitamin B₁₂ metabolism, since these separate subjects have been extensively reviewed elsewhere. Cobalt in the nutrition of animals has been reviewed by Russell (61), Maynard and Smith (51), Beeson (10), Marston (48), and Underwood (72), as well as others. Vitamin B₁₂ in its many aspects has been reviewed by Zucker and Zucker (77), Smith (63), Ungley (73), Marston (48), Sebrell and Harris (62), Ford and Hutner (26), and Williams (76).

Cobalt. A devastating syndrome in ruminants, now recognized in many parts of the world, was demonstrated in 1934 to be the result of cobalt deficiency, first in pastures of certain areas, then in harvested forages, and later in selected mixed rations. The nonspecific symptoms, demonstrated by many different groups of workers, included inappetence, followed by a loss in production and an anemia. The anemia has been described by Smith *et al.* (65) to be normocytic and normochromic, and by Marston (48) to be macrocytic. A recheck of the type of anemia, by Smith (64), confirmed the earlier observation of a simple anemia, so it appears that there must be some fundamental difference between the cobalt deficiency observed by the New York workers and the Australian group. Postmortem findings in cobalt-deficient sheep have not been helpful in differential diagnosis, since the pathological alterations are typically those of general inanition, sometimes accompanied by a fatty degeneration of the liver and hemosiderosis of the spleen. Depraved appetite has sometimes been cited as a symptom of cobalt deficiency. In our observations in pen-raised sheep over a period of approximately eight years, involving over 400 animals, depraved appetite has never been a prominent sign of this deficiency. Apparently, this is in agreement with Marston's extensive observations, for he has not cited an abnormal appetite as being characteristic of cobalt deficiency under Australian conditions.

Since the anemia of cobalt deficiency characteristically follows the anorexia, and is the last symptom to return to normal, or approximately so, after cobalt therapy, it has been frequently stated that the anemia is probably not the primary sequela but is, rather, secondary to appetite failure. It will be recognized that this statement denies any direct action of cobalt on hemoglobin and/or erythrocyte formation. Since there is no conclusive evidence one way or the other on this point, it might more properly be left open, and thus serve as a challenge to others to delve into the problem in more detail than has been done in the past. Beyond this, the anemia has other interesting facets. Observations are in general agreement that the anemia of cobalt deficiency responds slowly and variably following either cobalt or vitamin B₁₂ administration, so much so that as a criterion of treatment-response we consider it secondary to appetite-response. More often

than not, hemoglobin never does quite reach normal levels following therapy, which suggests that some now-unrecognized factor is lacking.

Several lines of evidence early indicated that cobalt functioned in some manner associated with the ruminant stomach. There were early reports that non-ruminants, horses and rabbits, behaved normally on pastures that were cobalt-deficient for sheep and cattle. Laboratory animals, rats [Houk *et al.* (34)], and rabbits and guinea pigs [Thompson and Ellis (70)], failed to develop a cobalt deficiency, even though fed diets containing much less cobalt than cobalt-deficient pastures. Further evidence was furnished by varying the route of administration of cobalt salts. As early as 1944, McCance and Widdowson (47) mentioned a personal communication to the effect that cobalt given parenterally to cobalt-deficient sheep was not therapeutically effective. Marston and Lee (49) published data demonstrating that 1 mg. of cobalt fed per day to deficient lambs was therapeutic; whereas, the same amount given parenterally was not. This was confirmed by Smith *et al.* (65). On the other hand, Keener and Percival (37) and Ray *et al.* (57) reported slow responses in deficient lambs given cobalt salts parenterally but in quantities much larger than 1 mg. cobalt per day.

These varying observations seem best explained on a quantitative basis. With high-level cobalt injections, enough of the element reaches the rumen to stimulate a slow response. How much cobalt reaches the rumen is not clear. The fact remains, however, that a quantity (1 mg. per day) of cobalt which when given by mouth elicits a good response will not do so when given parenterally, and thus attention was focused on the intestinal tract as the probable site of cobalt needs. This and other evidence, some admittedly viewed from hindsight, has pinpointed the probable area of the intestinal tract that is involved, namely, the stomach. That the lower tract is not primarily involved, stems from the knowledge that the bile is a significant, though not the major path, of excretion. Furthermore, Phillipson and Mitchell (56) found that 0.1 mg. of cobalt per day which is effective when fed, is not effective when introduced directly into the duodenum. Quantities of cobalt much higher than 0.1 mg. daily, when injected into the duodenum or abomasum, did result in positive responses in cobalt-deficient lambs and were correlated with increased cobalt concentrations in the rumen ingesta. It seems reasonable that at least in this instance, cobalt reached the rumen by antiperistalsis.

Cobalt distribution throughout the body has been studied by use of radioactive cobalt administered in various ways [Comar and Davis (17), Comar *et al.*, (18), Rothery *et al.* (59), Keener *et al.* (38), and others]. The salient findings of these studies are, that cobalt salts when fed are eliminated chiefly (80%) through the feces, with only minor amounts (0.5%) emitted via the urine; that the liver is the organ of highest cobalt concentration, and that a significant excretion occurs through the bile. When injected intravenously, cobalt rapidly disappears from the blood and 65% is excreted in the urine and about 30% in the feces. When small amounts of cobalt⁶⁰ were injected, practically none was found in the rumen; whereas, much larger amounts permitted some accumulation in the rumen contents.

Cobalt-vitamin B₁₂ relationship. Since the best evidence up to 1948 indicated a rumen-cobalt interaction, it was but a short step to think of rumen microorganisms playing a role in the metabolism of this element. A study by Gall *et al.* (27) clearly demonstrated that in cobalt-deficient lambs the rumen bacteria were reduced from a control level of 54 billion per gm. of dry material, to 30 billion; the principal types of bacteria were less than in comparable cobalt-fed lambs, and mixed cultures of bacteria obtained from cobalt-deficient lambs failed to grow beyond dilutions of 10⁻⁹; whereas, cultures from positive control lambs continued to grow even at dilutions of 10⁻¹¹. From this preliminary study, it was evident that a shortage of cobalt significantly affected rumen bacteria — their total numbers, types present, and cultural qualities. It is unfortunate that this pilot study has not been expanded, for it would appear to be a fruitful field for research.

At this stage of progress, we set up the rather obvious working hypothesis that cobalt when fed is utilized by ruminal bacteria to produce some metabolically active compound, a shortage of which is the fundamental cause of the recognized symptoms of cobalt deficiency. A number of supplements and compounds were screened, but all were found to be inactive with the exception of commercial preparations of liver extract routinely used to treat pernicious anemia in man. Becker and Smith (8) reported that the daily subcutaneous injection of 15 U.S.P. units of liver extract was highly effective in curing cobalt deficiency. The same amount of liver extract was ineffective when given by mouth.

As this work was in progress, Rickes *et al.* (58) announced that vitamin B₁₂ was a cobalt-containing complex. It seemed evident that this vitamin must be at least one of the metabolically active products of cobalt metabolism. However, proof that vitamin B₁₂ was the primary functional form of cobalt in ruminants was slow in coming.

Merck and Company sent us a small supply (200 μg.) of crystalline B₁₂, which was immediately tested on cobalt-deficient lambs. The responses were disappointingly negative, which we now recognize were due to insufficient amounts. Since we had no information on the vitamin B₁₂ requirements of any species, we were forced to use as a guess the still fragmentary information on the amount required to elicit a favorable response in pernicious anemia patients. West (74) had reported responses in patients given as little as 3.6 μg., intramuscularly. Other lambs were fed much larger amounts of vitamin B₁₂ concentrate, but again the response was negative [Becker, Smith, and Loosli (9)].

Since it was impossible then to obtain more vitamin B₁₂, we decided to concentrate the active factor in liver extracts.

A commercial preparation of anti-pernicious anemia liver extract was separated into 24 fractions, using the counter-current distribution technique of Craig (20). Every third fraction was tested on cobalt-deficient lambs. It was found that fractions 12 to 18, inclusively, when injected resulted in positive responses that were maximal with Fraction 15. [Smith, Koch, and Turk (67)]. A sample of the original liver extract when autoclaved at pH 12, a treatment that destroyed vitamin B₁₂, was inactive when tested with deficient lambs. This

TABLE 1
Response of lambs to liver fractions and vitamin B₁₂

Treatment	Change in concentrate intake (<i>gm/day</i>)	Change in total body weight (<i>lb.</i>)	Change in hemoglobin (<i>gm/100 ml.</i>)	Total vita- min B ₁₂ in- jected/lamb (<i>μg.</i>)
Liver extract, B ₁₂ destroyed	- 48	-3	+0.1	0
Liver extract B ₁₂ destroyed + crystalline B ₁₂	+235	+6	+2.0	255
Crystalline B ₁₂	+202	+5	+1.2	300

information, combined with the demonstration that vitamin B₁₂ was also concentrated in maximum amounts in our Fractions 12-18, again strongly suggested that vitamin B₁₂ was the principal product of cobalt metabolism in the body. By this time, crystalline B₁₂ was available in large amounts, and it was restudied with cobalt-deficient lambs (results summarized in Table 1).

These data give ample evidence that cobalt-deficient lambs would respond to vitamin B₁₂ therapy if given enough, in this instance a total of 300 μg. over a two-week period. Furthermore, it was shown that the active factor in liver extract was vitamin B₁₂. The obvious conclusion was drawn that "vitamin B₁₂ is an important intermediary in cobalt metabolism in sheep." These observations were soon confirmed by Marston and Lee (50), Anderson and Andrews (2), and Hoekstra, Pope, and Phillips (32).

It is often asked if vitamin B₁₂ is the only important intermediary in cobalt metabolism. While we have no evidence now that other intermediaries are involved, future investigations may well demonstrate others. It is true that vitamin B₁₂ therapy of cobalt-deficient sheep appears to be complete, inasmuch as treated lambs respond quickly in appetite and weight gains and continue to do well so long as treatment is maintained. When vitamin B₁₂ administration is stopped, sheep relapse into typical cobalt deficiency. There can thus be little doubt that a cobalt deficiency in sheep is essentially a deficiency of vitamin B₁₂.

Previous observations [Becker (6) and Andrews and Anderson (3)] had suggested that vitamin B₁₂ was much more effective given parenterally, rather than by mouth. Further information was desired, since by this time vitamin B₁₂ feeding supplements were in general use for swine and poultry, and many wished to use this method of supplementing ruminants in cobalt-deficient areas. Kercher and Smith (41) found that 100 μg. of crystalline B₁₂ given by mouth was ineffective in curing cobalt-deficiency; whereas, 500 μg. per day was as effective as injecting about 15 μg. per day, suggesting that only some 3% of the orally administered B₁₂ was absorbed. Thus, parenterally administered vitamin B₁₂ is some 35 times more effective than that given orally. Certainly, in terms of feeding practice, cobalt supplementation is the more economical method.

Progressively more detailed studies of vitamin B₁₂ soon led to the realization that here was a group of related compounds, only some of which had biological activity. Brink *et al.* (13) reported that vitamin B₁₂ was a cyano-cobalt complex. This led Kaczka *et al.* (36) to designate all of the B₁₂ molecule, except the cyano

group, as cobalamin. This permitted the naming of specific compounds of the B₁₂ family as cyano-cobalamin, hydroxo-cobalamin, etc. Some of these modifications occur naturally, whereas others can be prepared from cyano-cobalamin in simple chemical transformations in which the cyano group is replaced by a variety of anions. All members of this group seem to be readily converted to the classical vitamin B₁₂ itself (cyano-cobalamin) by cyanide treatment.

One of these substitution compounds, vitamin B_{12a}, now termed hydroxo-cobalamin, was tested with cobalt-deficient lambs by Koch and Smith (44). It was found to be as therapeutically effective as cyano-cobalamin.

More recently, a further series of the vitamin B₁₂ group has been isolated from natural materials. Unlike the cobalamins, these compounds are not convertible to cyano-cobalamin by cyanide treatment and, furthermore, they have little or no biological activity. An excellent discussion of these cobalt-complexes has been given by Ford and Hutner (26).

Kercher (40) tested one of these naturally occurring compounds, pseudo-B₁₂ of Dion, Calkins, and Piffner (21). When injected in amounts as high as 500 μ g. into cobalt-deficient lambs, the response was negative. Similar negative results were reported for calves treated with pseudo-B₁₂ by Hopper and Johnson (33). The presence of pseudo-vitamin B₁₂ and other B₁₂-like compounds introduces many difficulties, inasmuch as some of these compounds are microbiologically, though not biologically, active. Thus, any assay of vitamin B₁₂ activity must be carefully described, since the total B₁₂ values found will depend on the assay organism and other conditions of the test. According to Ford and Hutner (26), only about 10% of the total B₁₂ of rumen contents and feces of calves is made up of the cobalamins, the remainder consisting of B₁₂-like compounds. Such knowledge should make one very cautious in interpreting the nature of so-called "B₁₂-like fractions of tissues isolated chemically," unless the composition of such fractions is further detailed by selected microbiological, chromatographic, and spectrophotometric methods. Progress is being made in the search for microorganisms such as *Ochromonas*, that respond largely, or possibly only, to biologically active vitamin B₁₂.

Vitamin B₁₂ content of tissues and ingesta. It was early shown that rumen contents and feces of ruminants are relatively rich sources of vitamin B₁₂. Hale *et al.* (28) showed a positive correlation between the cobalt intake and the B₁₂ concentration of rumen contents of sheep. By the chick assay, the rumen contents of a cobalt-supplemented sheep were found to contain a minimum of 60 μ g. of vitamin B₁₂ per 100 g. dry material; whereas, the rumen ingesta of a cobalt-deficient sheep contained much less. Hoekstra *et al.* (31), using *Lactobacillus leichmannii* as the assay organism, found 9 μ g. of vitamin B₁₂ per 100 g. dry rumen contents in cobalt-deficient sheep, compared to 130 μ g. in cobalt-fed sheep. Moinuddin and Bentley (53), using the same assay organism, found 100 μ g. of vitamin B₁₂ per 100 g. dry steer feces, and further reported that this microbiologically active B₁₂ was fully active for the rat. On the other hand, only 70% of the B₁₂ activity of rumen liquor was utilized by the rat.

Kercher and Smith (43), also using the *L. leichmannii* assay, found an average of 104 μg . of vitamin B₁₂ per 100 g. dry rumen contents of cobalt-fed animals, compared to 15 μg . in cobalt-deficient lambs. Rumen contents of cobalt-fed ruminants is thus one of the richest natural sources of this vitamin and, as might be expected, the vitamin B₁₂ concentration is a function of the amount of cobalt ingested. As might be anticipated, cattle feces are also high in vitamin B₁₂. Teeri *et al* (69) observed total excretions to average about 2,200 μg . per cow per day and about 540 μg . per heifer per day. Some 99% of the excreted B₁₂ was in the feces. Since cows in this study consumed 47.6 μg . per day, excretion exceeded intake some 50-fold.

The cobalt and vitamin B₁₂ content of milk has been the subject of several investigations. Early, Archibald (5) showed that the cobalt content of cow's milk could be increased by feeding relatively massive doses (500 mg. per day) of cobalt salts. Hartman and Dryden (29) failed to find an increase in vitamin B₁₂ in the milk of cows fed supplemental cobalt. Collins *et al.* (15) reported a range of from 2.6 to 3.8 μg . of vitamin B₁₂ per liter in market milk from 23 to 57 herds. Furthermore (13), there was no breed difference among Holstein, Jersey, and Guernsey cows; pasteurized milk was as rich in the vitamin as raw milk; colostrum was higher than milk. The milk of rats contains much more vitamin B₁₂ than any species studied so far. In India, Sreenivasmurthy *et al.* (67) found values of from 2.7 to 9.0 for cow's milk, 2.8 to 4.0 for buffalo milk, and 0.05 to 0.16 μg . of vitamin B₁₂ per liter for human milk.

Rusoff and Haq (60) found a range of from 1.69 to 4.12 μg . of vitamin B₁₂ per liter of cow's milk, which did not change when the ration was supplemented with either chlortetracycline or vitamin B₁₂. Collins *et al.* (15) reported that the vitamin B₁₂ content of goat's milk was increased the first week after parturition, when the ration was supplemented with a cobalt-containing salt mixture.

Couch *et al.* (19) reported the following values on the vitamin B₁₂ concentration of the whole blood of various species: calf, 0.9, cow 0.5, sheep 0.7, rabbit

TABLE 2
Cobalt and vitamin B₁₂ contents of milk

Species	Cobalt ($\mu\text{g}/\text{l}$)			Vitamin B ₁₂ ($\mu\text{g}/\text{l}$)		
	Mean	Range	Reference	Mean	Range	Reference
Whole milk, market	0.6	0.2-1.1	47	3.2	2 -25	14, 16
Holstein cows	—	—	—	8.0	4 -16	4, 60
Jersey cows	—	—	—	7.6	—	4
Sheep	—	—	—	1.4	0.07- 0.15	16
Goats	—	—	—	0.1	—	16
Buffalo	—	—	—	—	2.8 - 4.0	67
Human	—	—	—	0.4	0.1 - 1.5	16
Sow	—	—	—	1.0	0.03- 2.7	16
Rat	—	—	—	—	11 -95 ^a	16
Dog	—	—	—	—	0.7 -13.0	16
Horse	—	—	—	0.02	—	16
Colostrum						
Cow	5	—	47	28	21 -49	4, 16
Goat	—	—	—	4.2	1.3 - 8.5	16

^a The lower value was for rats fed an all-plant diet, and the higher value for rats fed the plant diet plus 10 μg . vitamin B₁₂ per 100 g. of diet.

10, horse 2.1, and man 0.8 μg . per ml. Since then, many other reports have been made which will not be reviewed here, except as the blood level of ruminants varied with the cobalt intake. Hoekstra *et al.* (31) found that the vitamin B₁₂ activity of the blood of sheep, in early cobalt deficiency, averaged 0.47 μg . per ml., compared to 2.3 and 4.3 in the blood of cobalt-supplemented sheep. These observations were extended by Kercher and Smith (42), who found that Western feeder lambs at purchase averaged 0.92 μg . of vitamin B₁₂ per ml. of whole blood, and that this level progressively decreased to a low of 0.03 μg . when the lambs were fed a cobalt-deficient ration. There was a highly significant decrease in the blood vitamin B₁₂ levels, considerably before the classical symptoms of anorexia, loss in body weight, and decreased hemoglobin level were observed.

In further studies, Kercher and Smith (43) reported blood vitamin B₁₂ levels of: 0.11 μg . per ml. for cobalt-deficient lambs, 0.09 for cobalt-deficient lambs treated by cobalt injections, and 0.62 for lambs fed 1 mg. of cobalt per day. It appears that for the first time we have a criterion, namely, blood vitamin B₁₂ level, that will be of specific help in positively and quickly diagnosing a cobalt deficiency, something that can not be stated for such nonspecific symptoms as anorexia, loss in production, and an anemia.

Kercher and Smith (43) studied the vitamin B₁₂ concentration (*L. leichmannii* assay) and total content of various segments of the gastrointestinal tract and various tissues, in lambs that were either cobalt-deficient or were treated with cobalt salts orally or parenterally. Cobalt given orally resulted in significant increases in the vitamin B₁₂ concentration of the blood, kidney, pancreas, adrenal glands, the contents of the rumen-reticulum, omasum-abomasum, duodenum-jejunum, and ileum. No increases, except in the contents of the large intestine and caecum, were observed in those lambs given cobalt salts intravenously or subcutaneously. This observation is consistent with the knowledge that cobalt is excreted via the bile into the intestinal tract, and thus is available to microorganisms for the synthesis of B₁₂ in the lower tract. No evidence was obtained of tissue synthesis of vitamin B₁₂ from injected cobalt, contrary to the report of Monroe *et al.* (54).

Some miscellaneous observations of Kercher and Smith (42) and Kercher (40) were, that cobalt-deficient lambs did not respond to injections of folic acid; the vitamin B₁₂ was not spared by the feeding of chlortetracycline, and that the simultaneous administration of an "intrinsic factor" concentrate, along with suboptimal amounts of vitamin B₁₂, did not give a positive response. From very limited numbers of observations, Tribe *et al.* (71) reported a delayed response in cobalt-deficient sheep treated with penicillin.

Requirements of ruminants. The minimum cobalt requirement of sheep was closely approximated in the late thirties, chiefly by New Zealand workers. These studies are well summarized by Russell (61). It was found that pastures containing 0.07 p.p.m. cobalt on a dry weight basis were on the borderline of adequacy. More recently, Marston (48) re-evaluated the cobalt requirement of sheep

and came to the conclusion that a total daily ingestion of 0.07 to 0.08 mg. cobalt is close to the quantity that will completely fulfill the needs for normal health. This amount is furnished by a ration containing 0.08 to 0.10 p.p.m. cobalt on a dry weight basis. Unfortunately, the requirement of cattle has not been determined so precisely. The earlier New Zealand studies suggested that cattle may have a lower requirement than sheep. It was estimated that pastures containing 0.04 p.p.m. on a dry weight basis were about borderline for cattle. In the absence of better data, it would appear that cobalt intakes adequate for sheep should also be adequate for cattle, but, obviously, final conclusions must await further studies.

The first attempts to treat cobalt-deficient lambs with vitamin B₁₂ yielded negative results. It is now realized, as previously mentioned, that the amounts of the vitamin used were suboptimal. These observations led to the frequent statement that ruminants must have a high vitamin B₁₂ requirement, a statement with which the authors disagree. The following table summarizes the vitamin B₁₂ requirement of a number of different species. Since it is desirable to include for comparison the requirement of man, at least in terms of the pernicious anemia patient, we have selected from the literature the vitamin requirement when it is given parenterally rather than orally.

TABLE 3
Vitamin B₁₂ requirement of different animals (parenteral administration)

Species	Body weight	Reported requirements	Calculated daily requirement per lb. of body wt. (μg)	Reference
Rat	Av. 150 g.	0.125 μg . per day (fed thyroid)	0.37	Emerson (25)
Chick	Av. 120 g.	0.3 μg . weekly	0.16	Stokstad <i>et al.</i> (68)
Pig		0.6 μg /kilo body weight	0.27	Nesheim <i>et al.</i> (55)
Pig		0.26 μg /kilo body weight	0.12	Anderson and Hogan (1)
Sheep	Av. 60 lb.	150 μg . per 2 weeks	0.18	Smith <i>et al.</i> (66)
Human	150 lb.	1 μg . per day ^a	0.007	Bethell <i>et al.</i> (12)

^a To relieve pernicious anemia.

Although there is variation in the reported requirements, and these data will in some cases be modified by further studies, it appears clear that the vitamin B₁₂ requirement of the sheep is approximately of the same magnitude as that of other animals. The outstanding exception is man, at least insofar as remission of pernicious anemia is concerned.

By feeding a synthetic milk low in vitamin B₁₂, Draper *et al.* (22) developed a vitamin B₁₂ deficiency in dairy calves characterized by cessation of growth, poor appetite and, in some cases, incoordination. Using similar techniques, Lasiter *et al.* (45) confirmed and extended these observations. In addition to growth retardation and poor appetite, they reported a white-spotted kidney condition.

Under these experimental conditions, calves required between 20 and 40 μg . vitamin B₁₂ per kilogram of dry matter consumed. These figures are a little higher than those estimated to be required by the chick (15 μg . per kilo diet), reported by Stokstad *et al.* (68), and by pigs (20 μg . per kilo diet), reported by Nesheim *et al.* (55).

Cobalt toxicity. Excessive intakes of cobalt are toxic to many species, causing a polycythemia. In ruminants, the toxic level has fortunately proven to be very high and greatly in excess of requirements, so that overenthusiastic dosing of cattle and sheep is unlikely to cause trouble.

Ely, Dunn, and Huffman (24) found that cobalt fed in excess of 40 mg. daily per 100 lb. of body weight was harmful to dairy calves. The same authors later reported (23) that 30 mg. or more of cobalt per 100 lb. of body weight, given by intravenous injection, was also toxic. The symptoms observed were lacrimation, salivation, dyspnea, incoordination, and excessive defecation and urination. Furthermore, the injection of methionine previous to cobalt injections markedly reduced the severity of the reaction. Keener *et al.* (39) found that dairy calves were able to consume up to about 50 mg. of cobalt daily per 100 lb. body weight before untoward symptoms appeared; that is, loss of appetite, decreased water consumption, lack of muscular coordination, and some increase in erythrocyte concentration, i.e., a mild polycythemia.

Josland (35) drenched a few sheep with 5 mg. cobalt per kilogram of body weight and observed erratic responses ranging from a polycythemia to a mild anemia. Becker and Smith (7) found that, by drenching sheep with graded amounts of cobalt chloride, levels up to 160 mg. of cobalt per 100 lb. of body weight daily were tolerated with no abnormal signs, over a period of eight weeks. Levels above 160 mg. were associated with depressed appetite, loss in weight and, at very high levels, an anemia. No evidence of polycythemia was obtained.

Miscellaneous. On occasion, therapeutic value has been claimed for cobalt treatment of a number of diseases—sterility, milk fever, ketosis, brucellosis, and others. As early as 1948, Henderson (30) reported the successful treatment of 12 cases of ketosis with cobalt sulfate. The latest report of which the authors are aware is that of White (75). So far, these claims are based on faulty or inadequate experimental techniques, especially the lack of adequate controls. If cobalt plays some role in the prevention or cure of ketosis, it remains to be shown. Similar statements can be made for its possible role in the treatment of sterility, milk fever, and brucellosis. Berman *et al.* (11) failed to find that supplementing the ration with trace-mineral elements, including cobalt, altered the course of brucellosis in dairy cattle.

The question of availability of cobalt from various sources has been inadequately studied. One would presume a ready availability of cobalt for ruminants, even from highly insoluble compounds, such as the raw mineral limonite, which was successfully used in the earliest studies. Keener *et al.* (38), using radioactive cobalt, showed appreciable absorption of cobalt carbonate by sheep. Mittler (52), using radioactive cobalt oxide, found that cobalt was well absorbed and distrib-

uted throughout the body of lambs. However, it should be recognized that these studies were more qualitative than quantitative, and much further work remains to be done.

Cobalt supplementation, as a means of practical control is, in this country, largely by direct addition to the ration. Amounts that have been recommended vary widely. For addition to the concentrate portion of the ration, the amounts most commonly added have been 2 to 4 g. of soluble salts as the sulfate, chloride, or nitrate per ton of concentrate feed. Where the carbonate is used, half this amount furnishes approximately the same amount of cobalt. The fine powdery nature of the carbonate is preferred by many feed mixers. When cobalt salts are added to a general mineral mixture, recommendations commonly have suggested 0.5 to 1.0 oz. of cobalt salts per 100 lb. of total mixture. This may be fed free-choice or added to the concentrate mixture at a level of 2%. Where such recommendations have been followed, the problem of cobalt deficiency in ruminants appears to have been eliminated.

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