Preliminary Observations on the Relationship between Vitamin B_{12} Status and Ovine White Liver Disease

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Summary

Eight outbreaks of WLD were associated with primmary Co deficiency in the pasture. Both affected and unaffected sheep on these pastures were deficient in vitamin B_{12}. It appears that only Co deficient or B_{12} deficient sheep are susceptible to WLD, and that the condition can be prevented by appropriate topdressing. The question of a possible hepatotoxin being involved in the aetiology of WLD remains open. Serum B_{12} radio-assay appears to be more sensitive and reliable than either liver B_{12} assay or pasture analysis as an index of ovine Co/B_{12} status. It is suggested that pasture topdressing with Co SO_4 be considered if the mean serum B_{12} of representative sheep is less than 1000 pg/ml.

Introduction

Ovine White Liver Disease (WLD) is an hepatic lipodystrophy which has been recognised as a clinical entity in the northern and eastern North Island since at least 1967. (Martinovich and Cordes, pers comm.; Cordes and Gardner, 1971; Martinovich, 1974, 1977; and Davis, 1974). Since 1971 it has been recognised that affected sheep have low hepatic levels of vitamin B_{12} (Martinovich, 1977). WLD is associated with a wide range of clinical signs some of which are probably referable to Co deficiency. All or some of the following features may be present. ill thrift, anorexia, depression, serious ocular discharge, anaemia, photosensitivity, polioencephalomalacia and sometimes high mortality.

There are four possible explanations for the low B_{12} levels encountered in clinical WLD. These are:

(i) Primary cobalt (Co) deficiency
(ii) Impairment of intra-ruminal microbial B_{12} synthesis.
(iii) Defective intestinal absorption of B_{12}
(iv) Loss of hepatic B_{12} as a consequence of severe liver disease.

At the Whangarei Animal Health Laboratory we have used the Schillings test to study the intestinal B_{12} absorption of WLD and Control sheep. Although we have found considerable between sheep variation these differences are not referable to WLD.

As part of a wide ranging epidemiological study of WLD we have investigated the background of Co and B_{12} status of pastures and flocks in which the condition occurs. This paper reports preliminary observations which suggest that primary Co deficiency is an important factor in the pathogenesis of WLD. To a lesser extent they support the postulated loss of B_{12} from damaged liver. They do not exclude the possibility that the specific liver lesion is the result of a toxic insult.

Materials and Methods

All observations were made over the period November 1976 to December 1977

Pasture Co levels pertaining to 8 outbreaks of WLD are recorded with the month in which the sample was collected and in relation to the various outbreaks. One pasture sample from each of 13 paddocks on which WLD was occurring, or had occurred, was analysed for cobalt at the Ruakura Agricultural Research Centre. The only exception to this was pasture 3a from outbreak C from which two further samples were collected after 14 days. (3b and 3c). Each sample was composed of at least 20 sub-samples from random sites within the paddock.

In five of these outbreaks it was possible to compare the liver and serum B_{12} levels of affected and unaffected sheep. The liver B_{12} levels encountered were compared with those in control sheep from properties on which WLD is unknown. The serum levels were compared with those encountered on a property where a regular WLD problem appears to have been prevented by topdressing with cobaltised super phosphate (380g $CoSO_4$/ha).

The liver and serum samples from WLD properties did not necessarily derive from the same sheep. All affected sheep had clinical signs of WLD. All livers from affected sheep had histological lesions of WLD, and in every case serum samples from affected sheep showed biochemical changes consistent with WLD. The unaffected sheep from WLD properties were regarded as clinically normal. None of their livers showed the lesions of WLD, and the serum B_{12} levels all relate to sheep with no biochemical evidence of WLD. Twenty-three livers and 14 sera were examined from affected sheep and 24 livers and 17 sera from unaffected sheep. The 15 control livers derived from six properties, and the 16 control sera from one property as outlined above.

Microbiological vitamin B_{12} assay of all liver samples was done at the Wallaceville Animal Health Reference Laboratory. The serum B_{12} assays were done by means of a radio assay utilising competitive binding with human intrinsic factor at the Wallaceville Animal Research Centre

Results

The results of the pasture Co analysis are tabulated in Table 1. Liver B_{12} levels are tabulated in Table 2, and serum B_{12} levels in Table 3.

Discussion

None of the pastures contained more Co than the 0.10

parts per million (ppm) recommended by the National Research Council (1976). Outbreak C occurred within one week of the introduction of the sheep to pasture 3(a). This pasture was sampled twice more 14 days later and both samples were Co-deficient 3(b) 3(c). The sheep had been introduced from another Co deficient pasture.

The mean hepatic B_{12} level of the unaffected sheep on WLD properties is less than 30% of the mean for control sheep. Depending upon the standard applied the mean level for unaffected sheep is either deficient or on the borderline of deficiency. The mean hepatic B_{12} level for sheep with WLD is clearly deficient The mean serum B_{12} levels of both affected and non-affected sheep on WLD properties are clearly deficient and are only about 10% of the mean for control sheep.

These results indicate that the B_{12} levels encountered in WLD sheep are due to primary Co deficiency. They also suggest that WLD only develops in sheep of inadequate B_{12} status. This raises the question of whether WLD is solely the result of cobalt deficiency or whether the acute hepatic lipodystrophy is the result of exposure to an hepato-toxin. If the latter is the case it seems likely that cobalt or vitamin B_{12} are protective. There is epidemiological evidence suggesting that a toxin could be involved, and there are toxic conditions in which cobalt and/or vitamin B_{12} have a protective effect. High levels of dietary cobalt protect against phalaris staggers (Underwood 1977). There are also strong indications that, in Co deficient areas, Co supplementation limits the severity of facial eczema (Cornforth — pers. comm.).

On the other hand, "fatty livers" have frequently been described as a feature of Co deficiency in both grazing and housed sheep (Underwood 1977; Marston *et al* 1961; MacPherson *et al* 1976). Whether or not all Co deficient sheep suffer from a greater or lesser degree of WLD is open to speculation. This seems unlikely as the attack rate of WLD does not reflect the distribution and intensity of Co deficiency in New Zealand.

Whatever the true situation it appears from these results and from limited practical experience that Co topdressing of suspect pastures will prevent WLD. Pasture Co topdressing of suspect pastures will prevent WLD. Pasture Co analysis is less reliable than serum B_{12} analysis as a measure of the adequacy of Co/B_{12} nutrition of grazing sheep. Pasture Co can fluctuate dramatically over short periods, as in pasture 3 above. In monitoring the situation on a WLD property we have observed pasture Co to go from a "healthy" 0.46 ppm to a 0.04 ppm in less than 30 days. The sheep were deficient throughout.

Applying the "accepted" standards to the results of the tissue B_{12} analysis above it is evident that serum provides a better index of B_{12} status than does liver. Six out of 23 affected sheep, and 21 of 24 unaffected sheep, would not have been recognised as deficient on the basis of liver analysis. On the basis of serum analysis all of the unaffected sheep and all but one of the affected sheep would have been recognised as deficient. MacPherson *et al* (1976) have observed that serum levels of B_{12} take about six weeks to reflect the change to a Co deficient diet.

The slightly higher mean serum B_{12} levels of affected sheep may reflect leakage of hepatic B_{12}. Elevated levels of serum B_{12} have been associated with human hepatopathies (Jones and Mills 1956), and also occur in sheep with experimental *Phomopsis leptostromiformis* poisoning (Gardiner 1967). These observations underline the need to sample representative and preferably clinically normal animals if it is desired to establish the Co status of a flock.

The mean serum B_{12} of 2522 pg/ml in the sheep from a top-dressed WLD pasture compares with the 2040 pg/ml in a group of Flock House ewes (Andrews and Stephenson 1966). Levels up to 900 pg/ml have been associated with "incipient Co deficiency" (MacPherson *et al* 1976).

Levels around 1000 pg/ml have been observed in untreated sheep recovering from WLD. The protective effects of Co or B_{12} against hepato-toxins have already been mentioned. In addition it appears from our own and other observations (MacPherson *et al* 1976) that Co deficient sheep have decreased resistance to parasitism and infections. These observations and considerations as well as the basic requirements of preventative veterinary medicine are the reason for our recommending 1000 pg/ml as the cut-off point for "normal" seru B_{12} levels in sheep.

Experience with the treatment of WLD is limited. If possible the sheep should be moved to a new pasture. In one WLD therapy trial injection of vitamin B_{12} was shown to improve general condition and to relieve that component of the syndrome referable to "Cobalt Deficiency Disease" i.e. Anaemia, serous ocular discharge, chronic ill thrift etc. Neither parenteral B_{12} nor drenching with Co SO_4 appeared to hasten the improvement in the clinical biochemical parameters of WLD (Sutherland and Carbery — unpublished).

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Table 1. Cobalt Status of WLD Pastures

Outbreak	Pasture	Month	Co (p.p.m.)
A	1	Aug	0 02
B	2	Sept	0 08
C	3a	Sept	0 10
"	3b	Oct	0 04
"	3c	Oct	0 06
D	4	Nov	0 015
E	5	Nov	0 056
F	6	Dec	0 017
"	7	Dec	0 020
G	8	Dec	0 034
"	9	Dec	0 029
"	10	Dec	0 026
"	11	Dec	0 040
H	12	Dec	0 039
"	13	Dec	0 031

Table 2. Liver Vitamin B_{12} (ug/g)

Accepted level* 0 10
Recommended level** 0 20

Property	Sheep	N	Range	Mean
WLD	Affected	23	0 00-0 16	0 07
	Unaffected	24	0 06-0 25	0 18
Non WLD	Control	15	0 32-0 94	0 62

* Andrews 1971
** Level below which Whangarei AHL recommends Co supplementation

Table 3. Serum Vitamin B_{12} (pg/ml)

Accepted level* 450 pg/ml
Recommended level** 1000 pg/ml

Property	Sheep	N	Range	Mean
WLD	Affected	14	100-710	254
	Unaffected	17	53-420	193
Topdressed***	Control	16	620-4600	2522

* Andrews and Stephenson (1966)
** Level below which Whangarei AHL recommends Co supplementation
*** Two livers from this property and not included among the control livers had B_{12} levels of 0 55 and 0 70 ug/g

Reports on Group Discussions of Set Questions

Question 1: What changes in parasite control are needed with irrigation: consider all species of gastro-intestinal parasites?

While it was emphasised that each unit must be treated on an individual basis as each would have its own particular management problems, the general conclusion was that under regular irrigation conditions —

A. optimum conditions caused by the regular waterings exist for build up of parasite larvae;
B. clean pasture, except possibly following closure for hay and then only for a short period after, does not exist;
C. a drenching programme must therefore feature highly as a means of parasite control.

Any drenching programme must relate to the grazing management of a particular grazing unit. In this instance it was thought that a likely system would be a 4 paddock — 3 weekly rotation per grazing group, i.e., one week on a paddock, three weeks off. After weaning ewes would follow lambs through the rotation.

The epidemiology of gastro-intestinal parasitism on irrigated pasture should be determined This may be just monitored by faecal egg counts or a full scale investigation by worm counts and pasture larvae counts may be mounted.

Question 2: What recommendations on parasite control in lambs would you make to farmers in this area?

Drenching programme

A. Pre-lambing drench of ewes required, followed by movement of stock to fresh paddocks

B. Docking drench of lambs **essential**, probably also a ewe drench at this stage; again move to fresh paddock (ewe deaths due to parasites can be high during lactation).

C. Lambs from 6 weeks of age need to go on to a regular drenching regime and, if a species such as *Nematodirus* is a problem, this may need to be at fortnightly intervals because of the rapidity with which infectious larvae develop on the pasture. Early weaning could be considered to avoid drenching suckling lambs too frequently — (youngest lambs 8 weeks).

D. Ewes which are following the lambs in rotation, could be restricted to a tactical drenching programme.

E. Some drenching could be avoided during the grazing of the hay aftermath Ewe lambs would probably be the first to graze this. Strip grazing with an electric fence and prevention of back grazing could be used to make best use of this relatively clean pasture.

F. Pre-tup drenching of ewes should be carried out.

While such an intensive programme is expensive there seemed to be little scope for any effective means of preventing a rapid parasite build up by grazing control (hay conservation excepted) unless types of stock other than sheep were integrated into the farming system. Prophylactic drenching was therefore likely to yield best results under irrigation conditions with an all-sheep system

Question 3: What recommendations on parasitic control in ewes would you make in this considering the environmental changes produced by irrigation?

Specifically the pretupping drench, the pre-lambing drench, the post-lambing drench; crops could be used to decrease the pasture larvae build up. It is important to drench all ewes or all the lambs, not just the ill thrifty animals.

Long term recommendations: the area is an ecological entity which, while similar to other irrigated areas or naturally wetter areas, needs its own parasite populations and its fluctuations to be studied and identified. The work of Elizabeth Campbell would be a useful guide but no more than this

The depth of study could well include populations of parasite larvae, total worm counts for healthy and sick sheep at intervals throughout the year, haematological and biochemical examinations and pasture larvae counts.

Short-term recommendations: total worm counts, pasture rotation practices with strategic drenching and pasture larval counts Specific drenches should be given at specific intervals, probably four times a year. There will be a needofor considering whether ewes can follow young sheep safely on farms in this area.

Question 4: What problems in ewe thrift and production are likely to be encountered in developing irrigation areas with changes in husbandry and how should they be controlled?

Problems with ewe thrift and production will be caused by —

1. Increased stocking rate
2. Leaching effect of the irrigation water.
3. Changes in soil structure.
4. Changes in mirco-climate for parasites.
5. No shelter for the sheep.

Diseases to be considered include — facial eczema, foot conditions, footrot, fungal toxins, gastro-intestinal parasites, Johnes disease, liver fluke, nutrition problems pneumonia, salmonellosis, trace element deficiencies (cobalt, copper, iodine, magnesium and selenium)

Recommendations for the control of some of these conditions —

1. Stock conservatively during development
2. **Management** — the farmer should have a thorough understanding of husbandry and use a specialised farm consultant service including financial advice. Feed utilization should be watched closely.
3. **Nutrition** — evaluate pasture production and quality. Also monitor soil and animal mineral levels, and weigh ewes and hoggets at regular intervals to monitor growth. Watch for metabolic diseases such as hypocalcaemia.
4. **Parasite control** — use faecal egg counts and/or total worm counts from sheep sent to meat works to determine worm borders Silage and renewable crops can be used in integrated control programmes, also cattle

5. Use copper and cobalt in fertilizers.
6. **Johnes disease** — this disease is very difficult to control. Reliable tests for clinical cases and pre-clinical cases should be developed. Stock movement control should be considered.
7. **Liver fluke** — not likely to be a major problem. **L.columella** is not spreading greatly, possibly due to temperature difference. Fluke is easily diagnosed and controlled.
8. **Foot conditions** — this includes footrot, scald and foot abscess. Their importance will depend on the breeds of sheep and the types of soil. Irrigation will extend the season that footrot is a problem.
9. **Salmonellosis** — the increased stocking rate may increase the incidence of this disease in sheep. The stocking rate will need to be maintained and vaccines will be the most satisfactory method of control.

As no research was done when the irrigation was first introduced, Winchmore results were taken and applied although the area has a different soil type. The farmer needs to understand the problems associated with this approach.

The provision of extra topdressing for extra feed for extra stock which may produce extra metabolic disease needs to be understood. Parasite control methods differ considerably from those of dryland farming and Animal Production Society recommendations are not applicable in this situation.

Question 5: What changes in cattle health problems are likely to be encountered in a recently irrigated area and how should these be countered?

Very similar problems to those experienced in sheep are likely to be encountered. Bloat is likely to be a big problem on irrigated pasture. Hypomagnesaemia is also likely to be a problem.

Question 6: Ideally, what steps should have been taken to investigate this ill thrift problem in lambs and ewes?

A. Farm Problem

Autopsies and laboratory investigations have shown that these conditions are present on the property:—

1 Parasitism
2. Cobalt deficiency
3 White liver disease
4. Marginal copper deficiency
5. Further hepatopathies

We would ask for more information on —

1 Dry matter intake — are they getting enough to eat?
2. Trace elements — copper, selenium and cobalt status in ewes and lambs, preferably using blood or serum levels
3. Degree of parasitism.
4 Liver enzymes
5. Soil and pasture mineral levels

This extra information would enable us to give better advice on —

1. Pasture management
2. Topdressing
3. Parasite control
4. Trace element supplementation
5. Disease prevention

B. District Problem

The problem needs to be properly defined: how bad is it? How extensive is it? What areas and farms are affected? Farmer opinion should be obtained by interviews and/or questionnaires. This should be a continuing exercise. Drystock farmers should be included in areas which are changing to irrigation. Management factors should be studied in particular, including farms with no problems.

Surveillance of parasite and trace element levels of normal animals should be done on freezing works stock. All laboratory specimens should be processed and reported promptly, particularly where a problem is being investigated and the type of treatments used depend on what laboratory results are obtained.

Question 7: What recommendations have you for continuing investigations of this problem?

The problem is continual and multifactorial.

Nutrition — the quantity and quality of available pasture should be ascertained, and the stocking rate adjusted accordingly.

Parasites — "this is a parasites paradise". A detailed study of parasitism, its epidemiology and affects in this area should be made, preferably by a researcher. Methods to reduce pasture level contamination should be investigated.

Cobalt — normal levels of liver and serum vitamin B_{12} should be ascertained for the entire district. Optimal cobalt topdressing rates and frequency should be determined. Quicker analysis results are necessary for this work.

White liver disease — continue to monitor this condition.

Fungi — continue to monitor fungi using washed grass technique

General Discussion

This is an edited version of the general discussion, arranged in three sections for convenience.

Parasitism

A farmer's expenditure on drenches is usually low, far lower than on other items with an uncertain return, e.g. fencing, topdressing. In comparison to these, drenches are not expensive. Also in our environment we have to totally depend on drenches for parasite control. These facts can be used to encourage a farmer to use a drenching programme for parasite control.

Lamb drenching before weaning is becoming more common in both North and South Islands.

Regular faecal sampling of ewes and histopathology of the abomasum and intestines in slaughtered sheep can be used to assess parasite burden.

Faecal egg counts may give a false impression of a worm burden in a situation where rapid infestation allows a pathogenic burden to be reached before any worms mature and produce eggs. The prepatent period may be 10 to 80 days. Larval digests of abomasum and intestines may be necessary to detect these. Larval damage may affect mineral uptake, e.g. copper in cattle.

Trace Elements

(The meeting was fortunate in having Professor T. Walker, Soil Science Department, Lincoln College, sitting in on this discussion).

Copper deficiency was not involved in this problem. Copper response trials on this property and throughout South Canterbury had not shown any significant results Weight gain was the measured parameter in these trials, although there is no evidence that this is affected by copper deficiency in sheep, even in situations where other signs of copper deficiency are present.

It is not known what the optimal frequency of application of cobalt is. The recommendation in South Canterbury is at least once every three years, while Dr. Cornforth suggests that more frequent use may be necessary. There is no information on the upper limits of cobalt application, what effects might be expected, and what interactions with other trace elements might occur.

Serum vitamin B_{12} levels express the cobalt status of the pasture being grazed about 4 weeks prior to the sampling. This is a good diagnostic test of cobalt sufficiency.

There is overseas work on the effect of parasitism on the uptake of certain minerals. No work has been done on cobalt uptake. It could be expected that a parasitic abomasitis may depress intrinsic factor production so preventing vitamin B_{12} uptake.

The adequacy of herbage analysis on irrigated pasture was questioned. Experience from this problem confirmed that herbage analysis was not always a true indication of the mineral status of the animals grazing it.

Sheep grazing non-irrigated land normally ingest varying amounts of soil which act as a source of trace elements. In irrigated land the soil may be deficient in trace elements and also the herbage may have less "contaminating" soil so reducing the amount ingested by grazing animals.

The soil in the Morven-Glenavy-Waitaki plains area is old, poor and gravelly, Stewart and Lismore types Its age indicates that leaching has occurred, with resulting trace element deficiencies. Increased production from such soils using irrigation and fertilizers will accentuate such deficiencies unless they are rectified. Increased organic material will also increase production from these soils, again accentuating any mineral deficiencies present.

Border dyking disturbs the soil and may result, as it has in this scheme (Morven-Glenavy) with topsoil being buried. This topsoil contains the plant nutrients, and its loss results in a loss of plant nutrients. Unless completely balanced fertilizers are used pasture produced from these soils will show deficiencies, which will be accentuated by increased irrigation and topdressing

White Liver Disease and Other Hepatopathies

This condition is currently limited to sheep. About 1% of sheep with white liver disease (WLD) show polios encephalomalacia. Recently a vitamin B_{12} deficient steer showed polio encephalomalacia and one would speculate that under such conditions WLD may occur in cattle.

WLD does not occur on pasture with adequate cobalt levels. There is no indication of the cause of the hepatopathies seen in the ill thrifty ewes. They did not resemble WLD nor were the sheep vitamin B_{12} deficient.

The fungicide trial done on the farm after the WLD outbreak was not relevant to WLD. The fungicide used (Benlate) is mainly active against *Pithomyces chartarum*