CCXXXIV. THE INFLUENCE OF COBALT ON PINE DISEASE IN SHEEP

By HAROLD HARTMANN CORNER
AND ALEXANDER MARTIN SMITH

From the Edinburgh and East of Scotland College of Agriculture

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PINE disease in sheep is prevalent in certain parts of the Cheviot Hills on both the Scottish and English sides of the border, and a preliminary paper by Greig *et al.* [1938] described the nature of the disease and its occurrence. Evidence was submitted to show that it is an anaemia, chlorotic in type, and that it may be controlled by the administration of Fe compounds.

Nearly 200 pining sheep on various farms in the area have been treated with iron ammonium citrate or crude iron oxide during the past 3 years, a course of treatment lasting about 3 weeks being sufficient to bring about recovery in 95 % of the cases. These results confirmed the conclusion reached earlier that these Fe compounds were of direct curative value in combating the anaemia and restoring the sheep to health.

Fe compounds are also effective in preventing the disease. Hill sheep in pining areas readily acquire a taste for iron oxide put into boxes with salt, and on many farms where this practice has been adopted the disease has been almost entirely abolished. Ewes which have received the crude iron oxide have been kept experimentally for 2 years on severe pining land without a change of pasture and have remained quite free from the disease.

As previously reported, however, it was observed that although Fe therapy proved effective it was not always followed by an increase in the blood haemoglobin and, further, the composition of soils and herbage from the area lent support to the view that Fe itself was not responsible for controlling the disease. In his study of enzootic marasmus in Australia, Underwood [1934] showed that the affected sheep had apparently no difficulty in absorbing ample quantities of Fe from food, for there was a higher concentration than normal of Fe in the liver, kidneys and spleen, but they lacked something necessary for the utilization of the Fe. It was shown that this disease, which resembles pine, could be cured by an extract, free from Fe, of the limonite commonly used by farmers and it was suggested that the disease was due, therefore, not to a deficiency of Fe in the food but to some trace element or elements associated with the Fe.

In the present investigation, attention was directed to Cu of which a trace was present in the iron ammonium citrate, but treatment of pining animals in the field with a dilute solution of CuSO_4 was ineffective. This result was not unexpected, because the analyses of soils and herbage from the area did not reveal any correlation between disease and Cu content.

Soils and herbage. At the beginning of 1935 samples of soil and herbage were taken from eight localities in the area in question and fresh samples were obtained from the same places in July. Altogether 73 samples of soil, to a depth of about 4 in., and 90 samples of herbage, including grass, heather and hay, were analysed, and a summary of the results is given in Table I.

In work of this nature it has been usual for investigators to determine the constituents in a strong HCl extract of the soil, but it was felt that a closer approximation to the material available to plant and animal would be obtained by preparing a less acid extract; the analyses were, therefore, carried out on the filtrates obtained by extracting 10 g. soil with 250 ml. ammonium oxalate-oxalic acid solution containing 200 mg. equivalents of HC_2O_4^- per l. and of pH 3.2-3.3. It was also considered desirable to express the results in terms of volume rather than of weight of soil, on account of the variation in soil type encountered, so the apparent densities of the soils were determined and the results calculated to a volume basis. Samples of the herbage were ignited at a dull red heat and the ash was extracted with dilute HCl.

Fe was determined by means of thiolacetic acid and Cu with sodium diethyl-dithiocarbamate, both colorimetrically, using a Lange photocell comparator. There is an indication that both soils and herbage from the non-pining land at D are better supplied with Fe and Cu than those from the pinning land at D but they are no better supplied than those from the pinning area A. Determinations of Mn, by the periodate method were no more successful in throwing light on the problem and it appeared to be unlikely that pine, if it were a deficiency disease, was controlled by any of the three elements Fe, Cu or Mn. Attention was then directed to cobalt.

Cobalt as a factor. Marston [1935] and Lines [1935] had reported favourable results from the administration of minute quantities of Co to sheep suffering from "coast disease" in Southern Australia. This was followed by a paper by Underwood & Filmer [1935] in which Co was reported to give positive results in cases of enzootic marasmus. Then it was discovered that "bush sickness" in New Zealand could be successfully controlled, both in South Island and in North Island, by the use of Co; this work has been summarized by Hopkirk [1938]. In view of these results, an analysis of the crude oxide of iron used in the experiments in the Cheviot region was made, and this showed that Co was present to the extent of over 50 parts per million. It seemed probable, therefore, that the good results obtained from Fe compounds in the control of pine disease might similarly be due to the presence of Co.

Soils. Several publications on the relationship between sheep disease and Co have appeared in the last 2 years, and in a recent paper Kidson [1938], summarizing the available data on the Co content of soils, shows that figures varying between 0.0 and over 300 p.p.m. have been obtained. These are for concentrated HCl extracts and are therefore not strictly comparable with the results in Table I, but it would appear that the Border soils are generally very low in Co. The method of analysis [Kidson *et al.* 1936] is based upon the depth of orange colour formed by the reaction of Co with nitroso-*R*-salt and is subject to considerable error when the amount of Co in solution is of the order 0.001 mg., but it will be observed that the soils from Farm C and Lower land D (non-pining) contain more Co per unit volume than the others.

Herbage. The analyses of pastures in New Zealand [Askew & Dickson, 1937; Askew & Maunsell, 1937] have shown that the dry matter of unhealthy pastures usually contains less than 0.07 p.p.m. Co, whereas, for good quality pastures, the Co content is generally greater than 0.07 p.p.m. Paterson [1937], on the other hand, has recently suggested that 0.2 p.p.m. is insufficient and that 0.45 p.p.m. is sufficient in the pastures of Devon. The values given in Table I for the herbage lie between 0.20 p.p.m. and quantities less than 0.05 p.p.m. and those samples from C and D are rather better than the others. It would be quite impossible at present to draw a line showing the limiting value and there

Table I. *Average figures for minor elements in soils and herbage**

Place	Soil							
	Spring				Summer			
	Fe	Cu	Mn	Co	Fe	Cu	Mn	Co
A. Pining farm, hill	92	29	80	1.2	193	48	147	—
Pining farm, field <i>a</i>	218	36	21	1.8	201	49	23	1.7
Pining farm, field <i>b</i>	222	48	47	2.0	145	32	34	2.0
B. Pining farm, peat	48	18	0.3	1.5	41	11	0.5	0.8
C. Non-pining farm, peat	141	36	2	2.8	68	16	0.8	0.8
Non-pining farm, mineral soil	248	34	64	5.4	519	59	108	3.7
D. Upper land (pining)	62	20	4	1.7	70	23	7	1.6
Lower land (non-pining)	229	36	46	3.0	298	43	20	3.7

Place	Herbage							
	Spring				Summer			
	Fe	Cu	Mn	Co	Fe	Cu	Mn	Co
A. Pining farm, hill	18	49	116	0.15	7	42	69	0.10
Pining farm, field <i>a</i>	—	—	—	—	8	44	91	
Pining farm, field <i>b</i>	—	—	—	—	—	—	—	
B. Pining farm, peat	12	24	54	Tr.	13	15	90	Tr.
C. Non-pining farm, peat	15	23	45	Tr.	13	16	56	Tr.
Non-pining farm, mineral soil	21	39	72	0.17	14	41	63	0.11
D. Upper land (pining)	20	54	33	0.17	6	35	51	0.07
Lower land (non-pining)	33	50	42	0.20	8	38	69	0.12

* Soils: Cu and Co p.p.m. by volume; Fe and Mn p./100,000 by volume.
 Herbage: Cu and Co p.p.m. by weight; Fe and Mn p./100,000 by weight.
 Tr. signifies less than 0.05 p.p.m.

is no definite relationship between soils and herbage. It is interesting to observe that if, as indicated by the New Zealand experiments, from 0.1 to 1.0 mg. Co per week is necessary to maintain health, a foodstuff containing only 0.1 p.p.m. cobalt (i.e. 1 mg. Co in 22 lb.) might be regarded as being adequately supplied with this minor element. Attention is directed to the fact, however, that there is a tendency, as shown by Askew & Maunsell [1937], for the Co content to fall during the growing season, so that individual results may be liable to mis-interpretation. It was obviously necessary to test whether Co was the limiting factor in the health of the sheep by administering a supplement of Co to affected animals.

Preliminary field tests with cobalt. In May 1937 four pinning Cheviot ewes were confined on a piece of pinning hill land which had been found by previous tests to accentuate the trouble in a very marked degree. The ewes were given doses of 3 mg. Co per head in the form of CoCl_2 (Analar) in distilled water. This dosage was given daily for 1 week, and thereafter every second day, a total of 12 doses being administered to each sheep. Within 10 days an improvement in the appearance and vigour of the sheep was noted, and after 17 days recovery was regarded as being complete. All four ewes nursed lambs throughout the test and these also improved along with their mothers. The ewes increased, on the average, by 7.87 lb. live weight per head in 3 weeks from the date of commencement of the test. They were thereafter returned to their hill grazings and continued to make good progress throughout the summer.

In June 1937 a further three pinning ewes were treated on similar lines and these also made a rapid recovery. They increased in live weight, on the average, by

8.56 lb. per head in 3 weeks. In 6 weeks the increase amounted to 15.4 lb. per head.

As 3 mg. Co per day had proved effective in the above instances it was decided to try smaller doses. A test was made on a pining farm in a different part of the Cheviot area during August, 1937. Five pining hogs and one pining ewe were treated with 1 mg. Co per head per day. Within 3 weeks they had lost all symptoms of the disease and were considered to be effectively cured.

As these preliminary tests had proved successful it was arranged to carry out a properly controlled experiment.

Controlled experiments with cobalt. Twenty Cheviot weaned lambs, or hogs, which were healthy and free from pining, were divided into two groups on 16 August 1937, and enclosed on a section of pining hill land at an elevation of 800 ft. Both groups ran together and received no feeding except grass. An experiment on the same land during the previous season had shown an incidence of pining to the extent of 73 % within 2 months. The conditions were, therefore, favourable to the manifestation of the disease. The treatment given was as follows:

Group 1. 10 hogs; 1 mg. Co per head per day for 14 days.

Group 2. 10 hogs; no treatment.

Co was given in the form of CoCl_2 (Analar) dissolved in distilled water. The animals were dosed individually, the doses being spread over a period of 3 weeks, this being equivalent to the length of time normally occupied by a change off pining land.

The treated hogs made good progress and remained entirely healthy. The untreated hogs, on the other hand, soon began to develop symptoms of the disease. While all were healthy on 16 August, 8 out of 10 were pining by 11 September, and by the end of September only one hog remained in a healthy state. The average live weights of the hogs were as follows:

	16 August lb.	5 October lb.	Increase lb.
Treated group	38.35	46.25	7.9
Untreated group	36.4	39	2.6

The treated group gained 7.9 lb. live weight per head over the period, while the untreated group only gained 2.6 lb. There was an increase in the colour of the conjunctiva of the treated group, as ascertained by comparisons with a Tallqvist haemoglobin scale, while in the case of the untreated group, there was a decrease in the colour which was found on the average to amount to 9 %. It was clearly demonstrated by this test that the administration of 1 mg. Co per day for 14 days had entirely prevented the onset of pining.

The procedure next followed was to dose the untreated group, all of which, except one, were now affected with the disease. It was decided to attempt to cure the disease by doses of 0.5 mg. Co per head per day. Accordingly, 14 doses were given at this rate, spread over 3 weeks, commencing on 5 October. By 21 October quite a marked improvement was noted in the majority of the group, and within 1 month they had all recovered except two which had been particularly badly affected. These were given five additional doses of 3 mg. Co per day, after which recovery was rapid.

All the hogs continued to run on the same section of pining land till 9 December and received no hand feeding of any description. Owing to heavy snow and frost thereafter, however, it was necessary to feed hay, the produce of

pining land. This treatment continued till 15 February, after which concentrates were fed and the experiment was terminated.

The original pining group increased in live weight by 3.2 lb. per head between 5 October and 9 December and, considering the severe conditions at this altitude of 800 ft., this afforded good proof of their recovered vitality.

While this experiment was in progress an outbreak of pining occurred among ewes and hogs on the same hill farm, 30 animals being affected. Treatment was administered by giving for 3 weeks 1 mg. Co per day mixed with salt, together with a little concentrated food. As some of the sheep would not eat this mixture, they were given doses in liquid form. Recovery took place in all cases.

DISCUSSION OF RESULTS

The influence of Co in animal nutrition has been a subject of investigation by several workers. Waltner & Waltner [1929] found that the addition of Co to the diet of rats produced a marked increase in red blood cells. A similar result was noted in dogs by Mascherpa [1930], and other workers have confirmed these results. Beard & Myers [1929] found that Co was a useful supplement in curing anaemia in rats produced by feeding them with cow's milk, but Cunningham [1931] doubted whether a true anaemia was developed in rats fed on this diet.

Dixon [1937] showed that a weekly drench containing 0.8 mg. Co gave a good response in the control of bush sickness, and the effect was augmented by inclusion of 0.18 mg. Ni later in the treatment, when presumably the "primary deficiency of cobalt was satisfied". Filmer & Underwood [1937] found that 0.1 mg. Co per day was effective in controlling enzootic marasmus in Western Australia, whilst Marston *et al.* [1938] have shown how Cu, although useless alone, has a marked effect when administered along with Co in the treatment of coast disease of Southern Australia.

In the present investigation it has been shown by analysis of herbage that the amount of Co available to sheep on pining land is very small. The administration of 14 mg. of Co per sheep, however, entirely prevented the disease for 6 months on severe pining land. The consumption of Co over the whole of this period was less than 0.1 mg. per day.

Regarding therapeutic results from Co, a cure was repeatedly effected in the experiments described above by giving 14 doses of 1 mg. each, spread over a period of 3 weeks and the cure remained effective for many months. Attempts to deal with the disease with 0.5 mg. doses were only successful where pining was in its early stages.

It may be concluded from these experiments, therefore, that for preventive and curative purposes 1 mg. doses of Co are necessary to ensure reliable results in the treatment of pine disease in sheep, and that a minimum of 14 doses is desirable. The cost of the material is only about one penny for 30 sheep. These results have been obtained by administering the Co in solution to the animals individually, but the most practicable method of treating hill sheep is to allow the stock access to a mineral mixture containing an adequate amount of CoCl_2 . The whole of the stock on a pining farm were treated in this manner, during the winter and spring of 1937-38, with complete success. The application of CoCl_2 mixed with fertiliser or sand to pining land merits consideration, however, in some of the more accessible places.

SUMMARY

1. Pine disease in sheep in the Cheviot region of Scotland is a nutritional anaemia which can be cured and prevented by the administration of cobalt in the form of cobalt chloride.

2. The disease is not due to a deficiency of Fe or Cu or Mn.

3. The beneficial results previously obtained from the feeding of Fe compounds may be attributed to the presence of Co in the Fe compounds used.

4. The administration of 1 mg. Co per day for 14 days is sufficient to prevent the disease on severe pining land for a period of 6 months. A similar quantity is effective as a cure.

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