INDUCTION OF THIAMINE DEFICIENCY IN SHEEP,
WITH LESIONS SIMILAR TO THOSE OF
CEREBROCORTICAL NECROSIS

By

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INTRODUCTION

Acute thiamine deficiency has been produced in lambs and calves by feeding a
thiamine-free diet (Draper and Johnson, 1951). Once the rumen flora develops,
bacterial synthesis of the B-vitamins is able to meet the metabolic requirements of
these animals (Kon and Porter, 1954); mature ruminants do not need an exogenous
source of these essential nutrients (Virtanen, 1963).

Homogastric animals develop avitaminosis B, when fed a complete diet mixed with
low-temperature dried green bracken frond powder (Weswig, Freed and Haag, 1946).
The reason for this became clear when Evans, Jones and Evans (1950) found that
pteridophytes, e.g. the bracken fern (*Pteridium aquilinum,* and horse-tail (*Equisetum
arvense*)), contain an enzyme which destroys thiamine (thiaminase I, EC 2.5 1.2,
thiamine-base, 2-methyl-4-aminopyrimidine-5-methenyl transferase). Ruminating
animals do not develop clinical thiamine deficiency when fed bracken frond diets
analogous to those which induce the condition in simple-stomached animals. Cattle
succumb to the bone-marrow poison also present in this plant, to the effects of which
this species is particularly sensitive (Evans, Evans and Hughes, 1954; Evans, Evans,
Thomas, Watkin and Chamberlain, 1958). Sheep can tolerate high concentrations of
this toxin and homogastric animals (e.g. rat, pig and horse) even higher.

Whilst investigating the quantitative distribution of thiaminase I in the bracken
plant including seasonal variations in enzymic activity, it was discovered that the level
of this enzyme in the rhizome is several times higher than in the frond (on average
10 to 30 times, depending on the age of the frond).

This paper reports the induction of acute thiamine deficiency in mature
sheep by the inclusion of a potent source of plant thiaminase I (bracken rhizome
powder) in a balanced diet for this species; the terminal histopathological
lesions are indistinguishable from those described in cerebrocortical necrosis
(CCN).

MATERIALS AND METHODS

*Animals and housing.* Welsh Mountain wethers (1 to 2 year old) were used. During
the initial series of experiments, the animals were individually housed in wooden crates,
which allowed 24 h. urine and faeces collections to be made separately. Later experi-
ments, to determine the minimum concentration of rhizome powder required to
induce thiamine deficiency, were conducted with the animals housed individually in
pens sprinkled with sawdust.

*Diets and feeding regime.* Bracken rhizomes, exposed after ploughing, were collected
in February, hosed down to removed extraneous soil, dried in a current of warm air (35 to 40°C) and milled, (2.5mm. mesh). Thiaminase was inactivated in a portion of the rhizome powder by spreading it on trays and autoclaving at 15 lb. in² for 30 min. These powders were incorporated in various proportions (per cent. by weight) in the diets. In the initial experiment, bran and milled concentrate pellets were used; subsequently, the bran was replaced by dried grass powder and a small amount of molasses dissolved in the requisite quantity of water added for pelleting at room temperature. Where stated, hay was also offered; water ad lib. was available to all animals.

Composition of the diets. In the 1st expt. the dietary components were as follows. Bran: crude protein, 14-6 per cent.; ether soluble extractives, 0.5 per cent.; thiamine, 3.7 µg./g. Rhizome powder: crude protein, 6-6 per cent.; ether soluble extractives, 5.4 per cent.; crude fibre, 20.7 per cent.; ash, 9.4 per cent. Concentrate pellets: crude protein, 18.0 per cent.; fibre, 6.0 per cent.; oil, 2.5 per cent. (Hill Ewe Nuts, Lever's Feeds Ltd.). Hay: crude protein, 8.1 per cent.; ether soluble extractives, 11.0 per cent.; thiamine, 1.3 µg./g.

Two wethers were placed on the experimental diets (not pelleted), composed of (a) bran 50 per cent., milled concentrate 25 per cent., rhizome powder 25 per cent. (b) bran 33 per cent., milled concentrate 33 per cent., rhizome powder 33 per cent. Two “control” wethers received diets mixed in the same proportions, except that the rhizome powder was replaced by milled hay. After consuming their daily ration, all animals were offered hay.

In the 2nd expt., 3 wethers were placed on pelleted diets mixed* in the following proportions (per cent.), with a view to observing the outcome of the replacement of bran by milled dried-grass of 18 per cent. crude protein content.

<table>
<thead>
<tr>
<th></th>
<th>Bran</th>
<th>Dried grass</th>
<th>Cake</th>
<th>Rhizome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep A</td>
<td>50</td>
<td>—</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Sheep B</td>
<td>33</td>
<td>—</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Sheep C</td>
<td>—</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

The 3rd expt. was designed to test the effect of autoclaving the rhizome powder, thus inactivating thiaminase, on sheep. Two wethers were placed on each of the following pelleted diets:

**Diet A**

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>Proximate analysis</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Dried grass</td>
<td>33</td>
<td>Crude protein</td>
<td>17.8</td>
</tr>
<tr>
<td>Powdered cake</td>
<td>33</td>
<td>Crude fibre</td>
<td>20.1</td>
</tr>
<tr>
<td>Rhizome powder</td>
<td>33</td>
<td>Ether extract</td>
<td>1.7</td>
</tr>
<tr>
<td>Molasses</td>
<td>2</td>
<td>Ash</td>
<td>7.8</td>
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</table>

**Diet B**

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>Proximate analysis</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried grass</td>
<td>33</td>
<td>Crude protein</td>
<td>18.0</td>
</tr>
<tr>
<td>Powdered cake</td>
<td>33</td>
<td>Crude fibre</td>
<td>17.9</td>
</tr>
<tr>
<td>Autoclaved rhizome powder</td>
<td>33</td>
<td>Ether extract</td>
<td>1.4</td>
</tr>
<tr>
<td>Molasses</td>
<td>2</td>
<td>Ash</td>
<td>9.8</td>
</tr>
</tbody>
</table>

The diets in the 4th expt. were analogous to those in the 3rd, except that the rhizome and autoclaved rhizome components were reduced to 15 per cent. and 5 per cent. respectively, with a consequent increase in the dried grass portion. All the

* The components of the diets were homogenized in an automatic food mixer, with the requisite amount of molasses solution added to give the right consistency for pelleting by a hydraulic press.
diets were pelleted and fed to wethers housed in individual pens; one of each active rhizome group was allowed access to hay.

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Rhizome diet (15%)</th>
<th>Autoclaved rhizome diet (15%)</th>
<th>Rhizome diet (5%)</th>
<th>Autoclaved rhizome diet (5%)</th>
<th>Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>2</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Thiaminase I assay. The levels of this enzyme in plant tissues, and various contents of the alimentary tract and faeces, were measured by the radio-chemical method of Edwin and Jackman (1970, 1974), expressing results in the form: thiaminase activity = μg thiamine decomposed/min./g. sample.

Thiamine estimations. These were made by the thiochrome method after suitable pre-treatment of the samples (Freed, 1966).

Pyruvate estimations. The method of Friedmann and Haugen (1943) and the more specific LDH/NADH enzymic method of Czok and Lamprecht (1970) using Boehringer reagents (The Boehringer Corporation London Ltd.) were employed.

Pyruvate-kinase. The method of Beisenherz, Boltze, Bucher, Czok, Garbacte, Avendt and Pfeider (1953) using Boehringer kit reagents was employed.

Blood sampling and haematology. Blood was taken from the jugular vein using Vacutainer tubes (heparinized, 10 ml.) supplied by Becton-Dickinson U.K. Ltd. (York House, Wembley, Middlesex). Cellular elements were enumerated by standard procedures (Holman, 1953; Archer, 1965).

RESULTS

Distribution and Seasonal Variation of Thiaminase I Activity in the Bracken Plant (Pteridium aquilinum)

Figure 1 illustrates the variation in the level of thiaminase I in different parts of the plant and how this alters during the critical phases of the growing season. During the winter months, the rhizomes possess a high level of activity; this falls steadily to about one-third the value from January to April and then rises again from May onwards—reaching its peak in the autumn. The very young frond-buds in April also show a high level of activity; this drops sharply during May as the aerial parts of the plant unfold. Bracken rhizomes gathered in the autumn and winter months therefore contain 20 to 30 times as much thiaminase I per unit weight as green fronds harvested in June.

Experiment 1* (1963). Thiamine deficiency was produced in the 2 sheep receiving the rhizome diets in 45 and 46 days respectively. Initially, the rhizome containing rations (A and B) were consumed avidly (0.91 kg. and

* This experiment had been preceded by a feeding trial using analogous rhizome diets with the object of attempting to produce the haemorrhagic syndrome of "cattle bracken poisoning" in the sheep; previous experience had indicated that dried frond diets had to be fed to sheep for lengthy periods (150 to 250 days) before significant haematological changes occurred. The induction of clinical thiamine deficiency in 6 weeks was a surprise, and caught us unprepared technically to follow the biochemical changes involved in this "gratuitously" induced avitaminosis B$_1$ (Humphreys, 1963).
0.68 kg./day respectively), but by the 36th day, anorexia set in, the faeces became structureless and loose, and within a few days both sheep lost condition and developed ataxia. On days 45 and 46, the animals were found leaning against the side of the crate unable to move; within a few hours the sheep were prostrate and started to exhibit a marked opisthotonus. The biochemical lesions characteristic of avitaminosis B, in simple-stomached animals had also developed (including a depression in blood thiamine, from a normal value of 7 to 8 μg. to 3μg. and a raised pyruvate from 2 mg. to 6 mg./100 ml. whole blood). Thiamine (100 mg.) was administered i.v. to both animals on the evening of day 47. By the following morning the sheep were standing up and moving about in the pens to which they had been transferred, although they were somewhat unsteady showing incoordination of the hind limbs. The blood pyruvate was coming down towards normal values. The dose of thiamine was repeated intramuscularly (20 mg.) for 4 consecutive days; the appetite returned and both animals made a dramatic recovery.

Parameters measured during the induction of acute thiamine deficiency in one of the wethers on the rhizome diet are shown in Fig. 2; those for a control wether fed a ration which differed only by the substitution of milled hay for the rhizome component of the diet, remained within normal limits. It can be seen that the experimental wether developed the biochemical abnormalities characteristic of thiamine deficiency; furthermore, these responded to thiamine therapy. Within the span of time required to induce acute thiamine deficiency by feeding this rhizome-containing ration (6 to 7 weeks), the sheep showed none of the haematological changes characteristic of cattle bracken poisoning—even in the most sensitive elements, total leucocytes and platelets. At this time (1963), we were unaware of any connection between thiamine deficiency and the then recently described naturally occurring disease of ruminants called cerebro-
cortical necrosis (CCN). As both animals showing clinical thiamine deficiency responded to thiamine therapy, no post-mortem information or brain histopathology was obtained.

![Graph showing changes in haematological and biochemical parameters of sheep during the induction of acute thiamine deficiency by the bracken rhizome diet.](image)

**Fig. 2.** Haematological and biochemical parameters of sheep during the induction of acute thiamine deficiency by the bracken rhizome diet. (a) (-O-) Leucocytes $\times 10^3$ per cmm.; (-O-) platelets $\times 10^5$ per cmm. (b) (-O-) blood thiamine (ug per 100 ml.); (-O-) blood pyruvic acid (mg. per 100 ml.); (-O-) urinary thiamine (mg. per 24 h. sample $\times 10$); (-O-) thiamine therapy (100 mg. initially, then 20 mg. per day).

**Experiment 2 (1971).** The objectives of this experiment were: (a) to confirm the results of the previous experiment, (b) to feed the rhizome powder at 2 dose levels (25 per cent. and 33 per cent. of the diet), (c) to observe the effect of replacing the bran by green dried grass, (d) primarily, to obtain the post-mortem picture and brain histopathology of thiamine deficiency in sheep induced in this particular way. The 3 sheep were housed in crates and given 1 kg. of the pelleted rations daily; food consumption, haematology and blood pyruvate were recorded throughout. There were no haematological changes.

* We are grateful to Mr D. C. Ostler, V.I.O. and Mr T. Jones, Asst. V.I.O., Veterinary Investigation Department, Bryn Adda, Bangor for conducting the post-mortem examinations. The brains of the 2 sheep allowed to succumb were sent to the Veterinary Laboratory, Weybridge, for comparison of the histopathology with that of CCN.
food intake was satisfactory until anorexia set in and blood pyruvate rose to abnormal values. The individual case history of each animal was as follows.

Sheep A received the 25 per cent. rhizome-bran diet. Thirty-six days after commencement, the animal developed ataxia and the blood pyruvate rose to about 5 times the normal value. This sheep showed no scouring or anorexia preceding the symptoms, although irregular doughy stools were observed at times. It was able to respond to light and moving objects, i.e. absence of amaurosis, until it reached a severe state of hyperaesthesia to be followed by opisthotonus, irregular breathing and clonic spasms. The sheep was killed by i.v. injection of pentobarbitone sodium on the 37th day from the commencement of the experiment and a post-mortem examination carried out immediately. The sheep had consumed a total of 5.3 kg. rhizome, averaging 151 g./day.

Sheep B which received the 33 per cent. rhizome-bran diet took about 40 days to show the acute clinical symptoms of thiamine deficiency, the blood pyruvate reaching 6.3 mg./100 ml. blood. At this point 20 mg. thiamine was given i.v. daily for 4 days by which time the animal had recovered its appetite, appearing normal and alert.

Sheep C which received the 33 per cent. rhizome-dried grass, had consumed its ration regularly for 22 days, but then exhibited anorexia and scouring. Six days later amaurosis, trismus and hyperaesthesia developed. If the animal was placed on its feet, it remained in a stationary position until tonic extensor spasms resulted in rearing on its hind legs and falling backwards, breathing irregularly and pronounced opisthotonus. It was killed on the 28th day and a post-mortem examination carried out without delay. The animal had consumed a total of 6 kg. rhizome, averaging about 216 g./day.

At post-mortem examination the brains of sheep A and C showed areas of yellowish discolouration in the cerebral cortex and some swollen gyri visible to the naked eye. Superficially, these appeared similar to those seen in field cases of CCN. The histological changes of both brains were confirmed as CCN by the Central Veterinary Laboratory, Weybridge without previous knowledge that they were from experimental animals.

Experiment 3 (1972). The effect of inactivating thiaminase I in the rhizome on its ability to induce thiamine deficiency and the lesions of CCN was studied. The enzyme was destroyed by autoclaving the rhizome at 15 lb./in² for 30 min. Two wethers were placed on the 33 per cent. rhizome green dried grass-cake diet and another 2 on autoclaved rhizome diet. Sheep 1 and 2, on active thiaminase-rhizome diet, developed clinical thiamine deficiency in 38 and 36 days respectively; sheep 3 and 4, on inactivated thiaminase-rhizome diet, remained normal even after feeding for 70 days.

The active rhizome powder decomposed 0.08 µg. thiamine/min./mg. and after autoclaving, 0.006 µg. thiamine/min./mg. The efficiency of the inactivation process depends on how good the contact is between the live steam and rhizome powder. To promote inactivation the dry rhizome powder was spread on tiers of trays in the autoclave and thus avoided undue steam condensation.

Sheep 1 was destroyed for pathological investigation. Table 1 gives the thiamine content of various tissues, and the thiaminase levels of samples taken from different parts of the alimentary tract. The whole brain was placed in
neutralized formol-saline and taken to the Central Veterinary Laboratory, Weybridge where a diagnosis of CCN, based on the histopathological lesions, was made.

Sheep 2. When this animal exhibited clinical symptoms and the blood pyruvate was raised to more than 5-fold the normal value, thiamine (20 mg.)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thiamine (mg/100 g. fresh tissue)</th>
<th>Thiaminase (µg thiamine decomposed/min./ml.* filtered fluid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart muscle</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Spinal column</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Normal values</td>
<td>0.5–0.1</td>
<td></td>
</tr>
<tr>
<td>Rumen</td>
<td>0.0037</td>
<td></td>
</tr>
<tr>
<td>Abomasum</td>
<td>0.0392</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>0.3875</td>
<td></td>
</tr>
</tbody>
</table>

* A known weight of intestinal contents was diluted with buffer and the assay carried out as described in Methods; the figures appear to be related to the dry matter content, but as no markers were used they merely show thiaminase activity throughout the alimentary tract.

FIG. 3. Daily food consumption of sheep fed rhizome and autoclaved rhizome diets. (Expt. 3). (■) Sheep 1 on active rhizome diet developed acute thiamine deficiency and was killed on day 40 for biochemical and pathological investigation; (□) sheep 2 on active rhizome diet developed acute thiamine deficiency but recovered with thiamine therapy; (●), (○) sheep 3 and 4 on autoclaved rhizome diet (which inactivates thiaminase I) showed no anorexia and remained healthy.
was given i.v.; within 24 h. the blood pyruvate returned to within the normal range. The sheep made a dramatic clinical recovery after thiamine therapy had been given for a further 3 days. The total amount of rhizome consumed in 36 days was 10.7 kg., i.e. an average of 300 g./day.

Sheep 3 and 4. Feeding of these 2 animals on the autoclaved-rhizome diet lasted for over 70 days; they continued to gain weight, had a good appetite and appeared normal in every way. They showed none of the biochemical lesions associated with thiamine deficiency. By this time, each had consumed about 22.5 kg. of inactivated rhizome, i.e. an average of 320 g./day. Figures 3, 4 and 5 give the daily food consumption, blood pyruvate levels and faecal thiaminase I activity respectively, of the 4 sheep throughout the experiment. From these, it is clear that sheep 1 and 2, on active rhizome diet, developed anorexia which became pronounced on day 35; concomitant with this, the blood pyruvic acid was raised and clinical symptoms of acute thiamine deficiency appeared. The faeces showed appreciable thiaminase I activity. Sheep 3 and 4 on autoclaved rhizome diet maintained a good appetite and showed none of these abnormalities. Inactivation of thiaminase I in the rhizome by autoclaving rendered this diet innocuous to sheep.

Experiment 4 (1973/74). This experiment has been in progress for 322 days. Wether 1 on 15 per cent. rhizome diet developed symptoms of thiamine
deficiency on day 95, with anorexia, ataxia and circling aimlessly in its pen. Blood pyruvate and pyruvate-kinase rose sharply; the syndrome became progressively more acute. Thiamine therapy was delayed in order to gauge its effectiveness in reviving really advanced cases of the disease. After a further 2 days, a massive dose of the vitamin, 100 mg. i.v., was given to the recumbent animal. Within a few hours there was some improvement; blood pyruvate and pyruvate-kinase fell. During the next 7 days on thiamine therapy the appetite never recovered and in spite of drenching with fluids including some fresh rumen liquor, it died on day 119. The brain at post-mortem showed visible lesions typical of CCN which was histologically confirmed. This outcome is reminiscent of our experience with some field cases of CCN brought down from the hills to our laboratory at Bangor; delay after showing clinical symptoms decreases the chances of thiamine therapy being successful, in spite of the fact that blood pyruvate can be restored to within normal limits.

Fig. 5. Faecal thiaminase I activity of sheep fed rhizome and autoclaved rhizome diets. (Expt. 3). (•), (+•) Sheep 1 and 2, on active rhizome diet. Faecal thiaminase I levels fluctuated considerably between individual animals for 20 days, and then tended to stabilize; enzymic activity of the faeces are indicative of the level throughout the alimentary tract and were sufficient to destroy available thiamine; (●), (○) sheep 3 and 4, on autoclaved rhizome diet gave faeces with negligible thiaminase I activity and the animals remained healthy.

All the other animals in this experiment have, so far, remained healthy. The relative gain in weight of the animals on the autoclaved as compared with the active rhizome diets, is marked. Faecal thiaminase, by itself, is not a sufficient indicator that thiamine deficiency will occur; wether 3 on 15 per cent. rhizome diet which had access to hay, actually consumed more of the pellets than wether 1 but, in spite of this, thiamine deficiency was not induced. Both animals on the 15 per cent. rhizome diet showed thiaminase levels 5 to 10 times less than those
on the 33 per cent. rhizome diet (cf. Fig. 5, expt. 3). Thiaminase activity of the diets were: for the active 15 per cent. rhizome diet, 12 μg. thiamine destroyed/min./g. diet, whilst for the 15 per cent. autoclaved rhizome diet it was 0.35 μg. thiamine destroyed/min./g. diet. The 5 per cent. rhizome diet destroyed 4 μg. thiamine/min./g. and the 5 per cent. autoclaved rhizome diet 0.15 μg. thiamine/min./g. These values varied slightly between different batches.

Fig. 6. Clinical symptoms exhibited by sheep with acute thiamine deficiency, induced by thiaminase I containing rhizome diets. (a) Illustrates the stance, and (b) opisthotonus shown by the animal.

Clinical and Pathological Findings

Figure 6 illustrates the opisthotonus exhibited by sheep after the induction of thiamine deficiency by the introduction of plant thiaminase I into the diet. At post-mortem, the brains were visibly abnormal. There were swollen areas in the cerebral gyri showing a yellowish discoloration. The morbid anatomy appeared to the Veterinary Investigation Officer at Bangor to be identical with those seen in field cases of CCN in sheep. The brains were fixed in 10 per cent. neutral formol saline and transported to the Central Veterinary Laboratory, Weybridge, where the diagnosis was confirmed in all cases. The histopathology of CCN has been described by Terlecki and Markson (1959). It is considered unnecessary to reproduce the evidence here, but the stained sections are available for inspection. It may be of interest to record that brains from our thiamine
deficient sheep fixed in formol-saline show a creamy coloured fluorescence when viewed under u.v. light; this phenomenon was recently described by Ziffo and Inglis (1974) who claim that necrotic areas are easily made visible this way.

The occurrence and effects of thiaminase on animals are summarized in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Thiaminase (source)</th>
<th>Animal sp. (avitaminosis B1)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyprinidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carp viscera</td>
<td>Silver fox (Chastek paralysis)</td>
<td>Evans, Carlson and Green, (1942)</td>
</tr>
<tr>
<td>Pteridophytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bracken (fronds)</td>
<td>Rat</td>
<td>Weswig et al. (1946)</td>
</tr>
<tr>
<td>Bracken (fronds)</td>
<td>Horse (Staggers)</td>
<td>Roberts, Evans and Evans (1949)</td>
</tr>
<tr>
<td>Bracken (rhizomes)</td>
<td>Pig</td>
<td>Evans, Evans and Roberts (1951)</td>
</tr>
<tr>
<td>Bracken (rhizomes)</td>
<td>Sheep</td>
<td>Evans, Humphreys, Goulden, Thomas and Evans (1963)</td>
</tr>
<tr>
<td>Horsetail</td>
<td>Horse (Staggers)</td>
<td>Present paper</td>
</tr>
<tr>
<td>Micro-organisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. thiaminolyticus</td>
<td>Human (hypothiaminosis—a form of beri-beri)</td>
<td>Matsukawa et al. (1954)</td>
</tr>
<tr>
<td>Cl. thiaminolyticum</td>
<td>(also cats, guinea pigs, rats, hens and rabbits)</td>
<td>Kimura (1965)</td>
</tr>
<tr>
<td>B. aneurinolyticus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidentified</td>
<td>Sheep and cattle (CCN)</td>
<td>Pill (1967)</td>
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<tr>
<td>Cl. sporogenes?</td>
<td>Sheep and cattle</td>
<td>Shreeve and Edwin (1974)</td>
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</tbody>
</table>

Discussion

Thiaminases are enzymes which destroy thiamine according to the mechanisms illustrated below.

*Also some R.SH and R.SO₂H compounds (e.g. cysteine and hypotaurine) can act as co-substrates. (Fujita, 1954; Wittcliff and Airth, 1970).
The bracken plant contains thiaminase I, which catalyses the nucleophilic displacement of the thiazole moiety of thiamine by another base referred to as the co-substrate. Depending on the chemical nature of the predominant co-substrate present in the feed and/or alimentary tract contents which participates in this reaction, the thiamine analogue formed could also act as a thiamine antagonist. This would have the effect of exacerbating the onset of thiamine deficiency in the animal. Thiaminase II is a hydrolase, with a very limited distribution in nature, mainly in some micro-organisms e.g. Bacillus aneurinolyticus, and need not concern us here.

Because of the synthetic activities of the rumen micro-flora, the induction of a deficiency in thiamine in sheep and cattle requires an adequate level of thiaminase I to destroy the vitamin not only in the rumen, but also at the sites of absorption after autolysis and digestion of the micro-organisms in the abomasum and small intestine. The bracken rhizome diets used provide these conditions, because of their high thiaminase I activity, and the fact that some particles of rhizome powder escape digestion and can even be seen in the faeces. This interpretation is supported by thiaminase I assays of intestinal contents throughout the alimentary tract. Autoclaving the rhizome powder inactivates the enzyme and abolishes the capacity of the rhizome diet to produce thiamine deficiency in the sheep.

The aetiology of CCN in ruminants has been summarized by Edwin and Lewis (1971); there exists a state of avitaminosis B₁ in these animals and rumen contents show thiaminase I activity. So far, the only isolate from rumen liquor of CCN cases which elaborates the enzyme is Clostridium sporogenes (Edwin and Jackman, 1974; Hayashi, Yushii, Harada, Nigeta, Tsubota, Shibutake, Sasaki, Suzaki and Tagaki, 1964). Administration per os of rumen liquor from CCN cases, or cultures of thiaminase I containing bacteria to normal sheep and cattle have, so far as we are aware, failed to precipitate the condition (Matsukawa, Misawa, Fujimiya, Kobayashi, Horikawa and Takato, 1954; Hamada, 1956; Evans et al., 1958). Roberts and Boyd (1974) surveyed the thiaminase I activity of rumen contents from healthy and affected sheep by the sensitive radiochemical method; enzyme activity was widely distributed among both groups, although CCN cases tended to show the highest values. Despite these results, it still appears probable that field cases of CCN arise because of the development of a gut micro-flora which elaborates high levels of thiaminase I activity—sufficient to deny thiamine to the ruminant. The factors which control this delicate balance have not been elucidated, but the animals respond to thiamine therapy in the early stages of the disease.

A thiamine antagonist, “amprolium” (1- (4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinum chloride hydrochloride, Merck, Sharp and Dohme, Ltd.) in massive oral doses, produces a condition indistinguishable from CCN in preruminant calves (Markson, Terlecki and Lewis, 1966), ruminant calves (Markson, Edwin, Lewis and Richardson, 1974), and in some adult sheep (Loew and Dunlop, 1972). None of the other well-known thiamine antagonists —e.g. oxythiamine or pyrithiamine, were effective at the dose level tried (Pill, Davies, Collings and Venn, 1966; Markson, Lewis, Terlecki, Edwin and Ford, 1972); neither does the attempted destruction of thiamine in the alimentary tract by sodium sulphite (Edwin, Lewis and Allcroft, 1968).
THIAMINE DEFICIENCY IN SHEEP

That thiamine deficiency, accompanied by the histopathological lesions of CCN, can be experimentally induced in sheep by the inclusion of a plant source of thiaminase I at the requisite level in the diet, supports the view that CCN is a "thiaminase disease" of ruminants. There are now many examples of the consequences to animals of ingesting feedstuffs containing an active source of thiaminase I, or those developing a gut micro-flora which produces this enzyme "in situ" to an excessive degree; (see in Table 2).

SUMMARY

Acute thiamine deficiency was induced in adult sheep by the inclusion of a potent source of thiaminase I in the form of low temperature dried, milled bracken rhizomes in the diet. Autoclaving the rhizome powder abolished its capacity to induce avitaminosis B, and also inactivated the enzyme.

The rhizome powder destroyed about 80 μg. thiamine per minute per gram, and the minimum quantity required was 15 to 25 per cent. by weight of the pelleted diet.

When the plant source of thiaminase I formed 25 to 33 per cent. of the ration, symptoms took from 25 to 40 days to develop—roughly the time required in simple-stomached animals. Decreasing the concentration to 15 per cent. prolonged this time to 3 months, whilst a 5 per cent. level did not produce the disease. Thiaminase I activity was present in the contents from all parts of the alimentary tract and faeces of sheep fed the rhizome diets. Even when the enzyme concentration was insufficient to cause the clinical syndrome, animals did not thrive as well as sheep fed on similar diets in which the thiaminase I had been inactivated.

Sheep made thiamine deficient in this way exhibited all the biochemical and clinical features characteristic of thiamine deficiency in homogastric animals; the response to thiamine therapy was dramatic in the early stages. The clinical features and histopathology resembled those of field cases of cerebrocortical necrosis in sheep. The results support the view that cerebrocortical necrosis is a "thiaminase disease" of ruminants.

ACKNOWLEDGMENTS

This work was supported by a grant from the Agricultural Research Council. Dr D. J. Humphreys and Mr W. E. J. Davies, B.Sc. are grateful to the Ministry of Agriculture, Fisheries and Food for postgraduate research scholarships. We thank the staff of the Veterinary Investigation Department, Bryn Adda, Bangor and the Veterinary Laboratory, Weybridge, for help with post-mortem and histopathology respectively.

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[Received for publication, July 1st, 1974]