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RUMINAL THIAMINASE TYPE 1 ACTIVITY ASSOCIATED WITH OUTBREAKS OF POLIOENCEPHALOMALACIA IN SHEEP

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Although the thiamin responsive disease, polioencephalomalacia (PEM), has been reported in many overseas countries (Loew 1972), the prevalence in grazing sheep is seldom high in comparison with recent outbreaks in W.A. The enzymatic destruction of thiamin in the rumen of affected animals has been reported in overseas studies but the source of thiaminase has not been established (Edwin *et al.* 1968; Roberts and Boyd 1974; Boyd and Walton 1977). Equivocal evidence exists that thiaminase synthesizing bacteria may be responsible for the increased level of enzyme in the rumen (Shreeve and Edwin 1974; Morgan and Lawson 1974). In this laboratory preliminary work has confirmed both the presence of thiaminase producing bacteria and high levels of thiaminase type 1 in the rumen fluid of PEM affected sheep. Significantly high levels of the enzyme have also been found in clinically normal animals from some affected flocks as reported by Linklater *et al.* (1977). The present study was undertaken to evaluate the significance in PEM cases of the elevated thiaminase type 1 found in rumen fluid and to attempt an identification of the source of the enzyme.

Histopathologically confirmed outbreaks of PEM were investigated, and the rumen contents and faeces from affected sheep examined. Elevated thiaminase type 1 activity was assayed in the majority of cases, indicating a high potential for thiamin degradation *in vivo*. Purification and partial characterisation of ruminal thiaminase has been carried out in this study. Sephacryl S-200 gel filtration chromatography and SDS-polyacrylamide gel electrophoresis separations showed thiaminase type 1 activity in two protein fractions of molecular size 70 000 and 140 000 daltons. The enzyme has a broad pH range (5.0-8.2) with a maximum at pH 7.2, and is stable in the range 4.0-8.5. The apparent K_m for thiamin is 95 µM. Activity of the enzyme is increased in the presence of 10 mM dithiothreitol, suggesting -SH groups may be involved in activation of thiaminase *in vivo*. Chaotrophic salt studies indicate that the enzyme may exist as a dimer.

Our data suggest that in the PEM cases studied thiamin degradation in the rumen was due to enzymatic breakdown, and that the thiaminase type 1 extracted from the rumen fluid is different from that produced by those *Cl. sporogenes* and *B. thiaminolyticus* isolates recovered from outbreaks of the disease in ruminants elsewhere.

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