curve represents degradation where all the cell wall is accessible to enzymes and the 'Within tissue' curve represents degradation where access is restricted by other tissue.

PROTEIN METABOLISM

Turnover of microbial protein during in vitro rumen fermentations.

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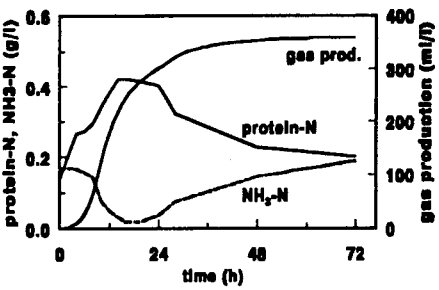
Recently developed techniques using continuous measurement of gas production in vitro are being used to study the fermentation kinetics of ruminant feeds [1]. Cumulative gas production curves can be described by a three-phasic model. The first two phases represent fermentation of soluble and insoluble carbohydrates respectively. The third phase, starting after *c.* 16h, had an unknown source. The present study indicates that the final phase is caused by degradation of microbial mass.

Strained rumen fluid was diluted with 2 volumes of buffer and incubated with glucose, starch or cellulose at 39°C for 72h. Maximal microbial protein concentrations were reached after 8 (glucose) to 16h (cellulose). After 48 and 72h incubation protein levels had decreased to, respectively, 25-50% and 10-25% of the maximal levels. No effect of the type of substrate on the maximal protein level was observed. Ammonia concentrations mirrored that of protein, demonstrating the dual role of ammonia as a precursor of protein synthesis and end-product of protein degradation. The observed protein degradation was at least partially dependent on the presence of protozoa. Rumen fluid from which protozoa were removed by centrifugation (10min 200*g*) showed a 2-3 fold reduced rate of protein degradation.



Cumulative gas production and protein-N and ammonia-N concentrations during incubation of crystalline cellulose in buffered rumen fluid.

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A role for plant enzymes in the early stages of herbage protein digestion in ruminants.

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Proteolytic enzymes in plants are intimately involved in controlled cell-death (apoptosis) during plant senescence. They also cause much of the digestion of plant proteins in the silo after cutting grass for silage. Although in ensiled herbage it is accepted that plant enzymes initiate the conversion of herbage proteins to low molecular weight peptides [1], microor-

ganisms are considered responsible for herbage protein degradation in the rumen. However, the proteolytic activity of the rumen microbial population is only moderate when compared with other proteolytic microorganisms and the animal's own gastric and pancreatic secretions [2]. Thus we conclude from this and much anecdotal evidence that plant enzyme mediated proteolysis is involved in protein breakdown in the rumen of grazing animals [3].

To test the above hypothesis, we have studied plant enzyme mediated proteolysis *in vitro* in fresh grasses incubated under anaerobic conditions. Four treatments were used: 1) herbage plus buffer, 2) herbage plus filter-sterilized rumen fluid, 3) herbage plus heat-treated rumen fluid and 4) herbage plus strained fresh rumen fluid. Herbage proteins were extracted after 0, 6, 12 and 24h of incubation and protein degradation profiles compared using SDS-PAGE. During the initial 12h of incubation, no difference was found in protein band profiles between the four treatments; the large Rubisco fraction (MW *ca.* 55kDa) was partially degraded to smaller sub-units. By 24h of incubation, the large Rubisco fraction had further been degraded into smaller bands in treatments 1, 2 and 3, whereas for the treatment involving fresh rumen fluid most bands including the large Rubisco fraction and its derivatives had disappeared completely. To monitor the presence or absence of microbial activity in each treatment, volatile fatty acids from culture supernatants were measured. With treatments 1, 2 and 3, acetate, propionate and butyrate concentrations were negligible over 24h of incubation, indicating an absence of effective microbial growth. With fresh rumen fluid, however, high levels of acetate (*ca.* 40 mM), propionate (*ca.* 40mM) and butyrate (*ca.* 8mM) did accumulate. This significant microbial activity in treatment 4 probably made a major contribution to the disappearance of peptides between 12 and 24h of incubation. Moreover, studies with up to 15 different grass species showed that plant proteolytic activity varies according to age and cultivar. In conclusion our results (a) indicate that there is scope to manipulate proteolysis in plants and (b) support the hypothesis that plant proteases are involved in initial cleavage of plant proteins in the rumen, whereas rumen micro-organisms make their contribution during the later stages of proteolysis.

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Factors affecting de novo synthesis of amino acids by mixed microorganisms from the sheep rumen in vitro and by pure cultures of rumen bacteria. C Atasoglu[1], C Valdés[2], ND Walker[1], CJ Newbold[1], RJ Wallace[1] *([1]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [2]Departamento de Producción Animal I, Facultad de Veterinaria, Universidad de León, E-24007 León, Spain)*

Ammonia has an important role in providing nitrogen for protein synthesis by rumen microorganisms. Estimates of the contribution of ammonia versus preformed amino acids to protein synthesis by the