SHORT COMMUNICATIONS

Factors Concerned in the Lysis of Bacteria in the Alimentary Tract of Sheep

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As the conversion of dietary protein to bacterial protein in the rumen is substantial (McDonald & Hall, 1957; Weller, Gray & Pilgrim, 1958) bacteria are thought to be an important source of amino acids for sheep fed on a roughage diet. Bacterial components may also be a source of glucose since the major glucogenic precursor from dietary sources, propionate, supplies little more than half the glucose requirement of sheep (Leng, Steel & Luick, 1967).

That digestion of bacteria begins in the rumen is indicated by the presence of large numbers of lysed bacteria (Hoogenraad, Hird, Holmes & Millis, 1967) and by the metabolism of ^{14}C -labelled *Bacillus subtilis* and *Escherichia coli* to volatile fatty acids in this organ (Hoogenraad, Hird, White & Leng, 1970). These experiments showed that whole organisms, walls and contents are digested and metabolized when injected into the alimentary tract of sheep (Hoogenraad *et al.* 1970). In the present paper the lysis of bacteria by rumen contents and their digestion by proteolytic and autolytic enzymes is reported.

The freeze-dried ^{14}C -labelled bacteria were prepared as previously described (Hoogenraad *et al.* 1970). The rumen fluid fractions were: 'whole rumen contents'—rumen contents filtered through muslin; 'clarified rumen liquor'—whole rumen contents centrifuged at 20,000g for 20 min. at 4° to remove micro-organisms; heat-inactivated rumen fractions obtained by heating 'whole rumen contents' or 'clarified rumen liquor' at 100° for 15 min. Each of the fluid fractions was buffered at pH 6.0 (the pH of the original rumen fluid) by the addition of 0.5 M-phosphate buffer (sodium salt) to a final concentration of 0.1 M.

To 15 mg. ^{14}C -labelled bacterial preparations was added 7.5 ml. of the various fractions of rumen fluids. These were incubated at 40° with stirring and samples (1.0 ml.) were removed at zero time, 1, 2, 4 and 6 h. The samples were centrifuged at 20,000g for 20 min. and 0.5 ml. of supernatant fluid was added to 10 ml. Bray's solution (1960) and counted in a liquid scintillation counter (Ansitron) and corrected for quenching.

The activity of autolytic enzymes was measured by incubating ^{14}C -labelled bacterial preparations in 0.1 M-phosphate buffer at pH 5.0, 6.0, 7.0 and 8.0.

Figure 1 shows an increase in the autolytic release of ^{14}C from labelled *Escherichia coli* with increased pH. At pH 6.0, approximately 20% of the ^{14}C in the *E. coli* was released in 6 h. This was similar to the amount of ^{14}C released when the bacteria were incubated in heat-inactivated rumen fractions at the same pH value. This activity therefore reflects the activity of autolytic enzymes in *E. coli* at this pH. Incubation in 'whole rumen contents' resulted in a lysis considerably above that due to autolysis.

In contrast, 'clarified rumen liquor' had little effect on the release of ^{14}C from *E. coli*, indicating that there is lytic activity towards *E. coli* associated with the microbial population of the rumen.

The release of ^{14}C from labelled *Bacillus subtilis* also increased with increase in pH. However, in addition to 'whole rumen contents', 'clarified rumen liquor' substantially increased the amount of ^{14}C released. With this organism the amount of ^{14}C released by autolysis at pH 5 and 6 was approximately half that obtained with *Escherichia coli*. Thus there was a difference between the Gram-positive and Gram-negative

Fig. 1. Incubation of ^{14}C -labelled *Escherichia coli* in 'whole rumen contents' (○), 'clarified rumen liquor' (△), heat-inactivated RC (●) and heat-inactivated RL (▲) at 40°; pH 6.0. Autolysis, measured after incubation of cells in 0.1 M-phosphate buffer for 6 h. at 40°, is given by: A, pH 8.0; B, pH 7.0; C, pH 6.0; D, pH 5.0. For preparation of rumen fluid fractions see text.

organisms. The agents responsible for the lytic activity were not further investigated. Jarvis (1968) found that clarified bovine rumen liquor contains agents which lyse *Butyrivibrio* cells which are normally present in the rumen.

After leaving the rumen, bacteria encounter the normal mammalian digestive system including pepsin, trypsin and chymotrypsin. Freeze-dried preparations of walls of rumen bacteria (100 mg.), prepared as previously described by Hoogenraad & Hird (1970), were incubated with pepsin, trypsin and chymotrypsin in a recording pH-stat (Radiometer, Copenhagen) for 18 h. at 38° under N_2 . The digestion was followed, after addition of trypsin (2%) and chymotrypsin (1%), by the titre of 0.2 M-NaOH at pH 8.0 or, when pepsin (1%) was used, by the titre of 0.2 M-HCl at pH 3.0. Control incubations, at pH 8.0 and pH 3.0, for self-digestion of the cell walls and for each enzyme were done and appropriate corrections applied. After digestion the suspension

was centrifuged at 20,000g for 20 min. and the pellet washed twice with water. The pellet and combined supernatant fluids were separately freeze-dried and analysed for amino acids (Moore & Stein, 1963). Total carbohydrates were determined using the phenol-sulphuric acid method (Dubois *et al.* 1951). The cell wall preparations contained 36.6% amino acids and 7.3% carbohydrate.

With each enzyme the digestion and autolysis were substantially complete after 18 h., as shown by titration. A part of the total amino acids had not been released and was inaccessible to the proteolytic enzymes. Pepsin released 15% of the wall mass and 45% of the total amino acids above that released in the control incubation at pH 3.0. There was no preferential release of any particular amino acid.

Trypsin and chymotrypsin released approximately 10% of the cell wall mass and 30% of the total amino acids above that released in the control incubation at pH 8.0. In the case of the tryptic digest there was a preferential release of lysine. Although carbohydrate was also released during the tryptic and chymotryptic digestion this was less than the amount released in the control incubation at pH 8.0 (40%, 46% and 54% respectively of the original carbohydrate). It is possible that trypsin and chymotrypsin destroyed bacteriolytic enzymes that would otherwise have released carbohydrates.

The bacteriolytic enzymes present in the wall preparation are themselves responsible for a considerable lysis of the walls, releasing 40% of the wall mass, 35% of the amino acids and 54% of the carbohydrates at pH 8.0, and 26% of the mass and 13% of the amino acids at pH 3.0.

It appears therefore that a combination of lytic processes derived from rumen bacteria themselves, lytic factors present in rumen digesta and the normal digestive processes of the animal are involved in the extensive digestion of rumen bacteria previously found (Hoogenraad *et al.* 1970) in the intact animal.

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