Bacteriophages in Rumen Contents of Sheep

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The nutrition of ruminants is intimately connected with microbial digestion in the rumen. Because a substantial proportion of the diet ultimately utilized by the animal exists as bacterial substance for a time, the fate of the bacterial cell in the alimentary tract is of interest. We are at present investigating the possible significance of the bacterial cell wall in the nutrition of the sheep as such walls constitute from 10 to 30 % of the dry weight of the cell and are known to contain carbohydrate (1). As a part of the investigation a general survey by electron microscopy of bacterial types present in rumen contents was made. Phage particles both free and attached to cells were found to be present. Many empty bacterial cell envelopes and fragments of cell walls were also found. A brief report (2) of the occurrence of phage in the rumen liquor of bovines indicated that the phenomenon is of more than local interest and may be a constant feature of the bacterial population in the rumen.

Rumen contents were obtained from fistulated sheep fed a diet of lucerne chaff, and from freshly slaughtered sheep fed a diet of unknown composition. The contents were filtered through muslin and samples were examined in the electron microscope in negative contrast using potassium phosphotungstate. In order to exclude selective removal of large micro-organisms during the staining procedure, the agar stripping technique (3) was also used. Bacteriophages were present in samples of rumen contents from both sources and were present both free in the rumen liquor and attached to cells of different types. The most common had polyhedral heads (Pl. 1, fig. 1) and were associated with a very small coccus (< 1 μ in diameter) which was present in large numbers. Plate 2, fig. 3, shows part of a lysed curved rod shaped organism associated with phage particles. A number of full and empty bacteriophages are associated with the bacterial body. Most of the phage particles occurring free in the rumen liquor were single. Occasionally, however, we found some also with polyhedral heads, clustered together (Pl. 2, fig. 4). The tails of these particles have a common attachment to an unknown fibril. Of particular interest was the discovery of groups of spirochaetes with surfaces heavily lined with bacteriophages (Pl. 2, fig. 4). The fibrillar structure of the spirochaetes is discernible and some of the phage heads appear to have discharged their contents. The association of phages with spirochaetes seems not to have been previously described.

A further feature brought out by the survey was the large number of cell walls seen in each rumen sample (Pl. 2, fig. 4). At times these represented up to about 10 % of the total number of bacterial bodies present. Lysis of bacteria in the rumen was indicated by the presence of these cell walls. (Pl. 2, fig. 4 shows a complete cell wall and Pl. 2, fig. 3, shows isolated fragments of cell envelope possessing the "honeycomb" appearance (4).

Although it is difficult to make quantitative measurements of the numbers of phage particles in the rumen, we have observed them in all samples examined. In addition, there are several morphologically different phages in the rumen at any one time. This
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suggests that phage activity may be important in determining bacterial population relationships.

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Russell Grimwade School of Biochemistry
School of Microbiology
University of Melbourne
Parkville, N.2
Victoria, Australia

REFERENCES


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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Electron micrograph of a small coccus infected by a phage with a short, stout tail; negatively stained with 2% potassium phosphotungstate. The polyhedral nature of the viral head is evident.

Fig. 2. Electron micrograph of a curved rod negatively stained as in fig. 1. The cell appears to have been lysed by a phage with a long tail. A number of phages which have discharged their contents are still attached to the cell.

PLATE 2

Fig. 3. Electron micrograph of a cluster of phages, negatively stained as in fig. 1. The polyhedral nature of the viral head is evident and close examination of the phage reveals base-plates and a common line of attachment to some unknown fibrillar material.

Fig. 4. Electron micrograph of phage-infected spirochaetes, negatively stained as in fig. 1 and using the agar stripping technique (3). The edge of the organism is lined with many small phages. The fibrillar structure of the spirochaetes is clearly shown. Also present in the lower section of the micrograph is a bacterial cell wall typical of those found in large numbers in sheep rumen contents.